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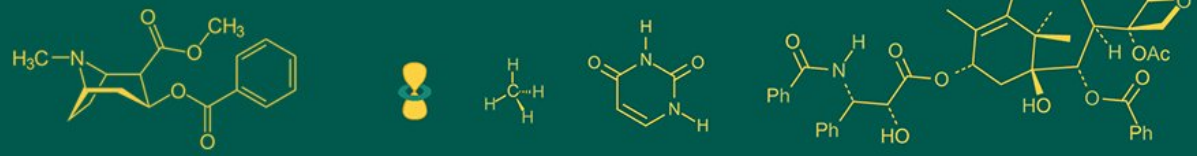

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Qualitative and quantitative determination of antioxidant activity of *Aloe vera* gel powder extracts

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Abstract
Three distinct solvent solutions, including methanol, ethanol, and water, were used to screen for antioxidant activity and analyze the overall phenolic and flavonoid profiles of *Aloe vera* gel. The AlCl3 method was used to measure the flavonoid content of *Aloe vera*'s three distinct plant extracts and the Folin-Ciocalteu method was used to evaluate the total phenolic content. The aloe gel powder aqueous extract showed the least amount of phenolic and flavonoid components. The findings indicated that the total phenolic content of ethanol and methanol extracts was significantly higher (p<0.05) at 25.28±1.95 and 23.80±0.29 mg GAE/g, respectively than that of aqueous extracts (9.77±0.83 mg GAE/g). Additionally, the flavonoid content of ethanol extract was significantly higher (p<0.05) at 28 mg QE/g, compared to methanol (18.56 mg QE/g) and aqueous extracts (12.44 mg QE/g). All extracts showed reduced antioxidant activity in comparison to ascorbic acid as standards, according to the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and FRAP assays used to measure *In vitro* antioxidant activity. It was found that all of the three extracts had flavonoids, tannins, and phenols. The antioxidant property of methanol extract was found to be 69.97% which was significantly higher (p< 0.05) than ethanolic extract (67.77%) and water extract (45.63%). The reducing power activity of the ethanolic extract was higher than that of the aqueous extract (IC_{50} = 249470.83 µg/ml & 249900.83 µg/ml) respectively at 1000 µg/ml. The presence of poly-phenols, tannins, and flavonoids are responsible for the three extracts' reducing power and DPPH free radical scavenging properties. It also implies that the solvent polarity is responsible for the various chemicals found in plants.

Keywords: *Aloe vera* gel powder, Antioxidant activity, DPPH, FRAP, Phytochemicals, TPC, TFC

Introduction
Aloe vera is a perennial succulent herb that thrives in tropical and subtropical climates and is used as a cosmetic, therapeutic, and ornamental plant. It is regarded as a mystical plant with several therapeutic uses throughout history. It is beneficial for numerous aspects of health due to its therapeutic qualities. Constipation, burns, wounds, and skin irritations have all been treated for thousands of years with the Aloe genus. Anti-inflammatory (Vijayalakshmi *et al*., 2012) [30, 9], antiviral, antioxidant (Saeed *et al*., 2022; Gorsi *et al*., 2019) [24], antibacterial, immunostimulant, antifungal, analgesic, antitumor (Zhang *et al*., 2017) [33], antidiabetic, and inhibiting tumor cell activation and proliferation are just a few of the many pharmacological qualities of aloe (Ray *et al*., 2013) [22]. The transparent and sensitive tissue found inside *Aloe vera* leaves is the primary reason for its reputation as a medicinal herb. The health advantages of *Aloe vera* extracts are attributed to their polysaccharides (Ni & Tizard, 2004) [19]. *Aloe vera* gel contains a widespread range of beneficial properties, such as antibacterial, anti-diabetic, antioxidant, anti-inflammatory, and gastrointestinal tract protection (Talmadge *et al*., 2004; Kar & Bera, 2018) [28, 13]. Numerous researchers have documented the antioxidant properties of *Aloe vera* gel, which are attributed to substances such as superoxide dismutase enzymes, glutathione peroxidase activity, and phenolic antioxidants (Hamman, 2008) [10]. For treating dry skin, *Aloe vera* is the ideal moisturizer. In addition to its anti-acne properties, it helps prevent skin wrinkles (West *et al*., 2003) [32]. The biological process by which living things make energy is oxidation, which leads to the development of reactive oxygen species. These species have been linked to numerous disorders, including cancer, inflammation, and degeneration, as well as aging (Debnath *et al*.,2011; Schäfer and Wink, 2009; Akerele *et al*., 2008) [8, 25, 2].

Flavonoids and polyphenols are two more phytochemicals found in *Aloe vera* that aid in reducing oxidative stress (Saeed *et al*., 2018) [23]. By inhibiting free radicals throughout their oxidation process, phytochemicals scavenge them.

Polyphenols' redox characteristics, which were crucial in helping them absorb neutralized free radicals, were primarily responsible for their antioxidant effects. Plants' polyphenolic components and antioxidant qualities are connected (Barreria *et al*., 2008) [5]. Because it contains flavonoids, *Aloe vera* gel can work as good antioxidant. Semi-polar solvents are a better substitute source of bioactive chemicals, according to a prior study that demonstrated the impact of solvent polarity on plant extraction (Ho *et al*., 2022). The current study assessed the total phenol, and total flavonoid, presence in aqueous, ethanolic and methanolic extracts of *Aloe vera* gel power as well as the reducing power and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities.

Materials and methods

Chemicals and reagents: All the chemicals including aluminium chloride, quercetin, sodium phosphate buffer, potassium ferric cyanide, methanol, and Folin-Ciocalteau reagent etc. used in the study were of analytical grade from standard companies.

Procurement of raw materials: For the current study, *Aloe vera* leaves (*Aloe barbadensis* Miller) were collected from plant sale outlet in Bareilly area. The healthy plants were selected for the purpose.

Plant material processing: The leaves were collected, and cleaned under running water to get rid of any debris, and a sharp knife was used to separate the *Aloe vera* gel from the leaves. The gel was blended and spread in trays and dried at 50 ± 5^0 Celsius in an electric oven. After drying, the dried gel was scraped out, crushed finely and then grinding was done in electric mixer to obtain a fine powder. The powder was stored in airtight container till further use.

Aloe vera gel extract preparation

With only minor adjustments, *Aloe vera* gel powder extract was made using the protocol described by Manye *et al*. (2023) [16]. A blender was used to homogenize the *Aloe vera* gel. For 48 hours, the homogenate was dissolved in 1:10 ratios of distilled water, methanol, and ethanol. After that, the solutions were filtered and further evaporated in an oven at 45 °C to produce the respective *Aloe vera* gel extracts.

Proximate composition:

Aloe vera gel was tested for moisture and dry matter content following the procedure given by AOAC (2006) [2].

In-vitro antioxidant activities

DPPH radical scavenging activity 1,1-Diphenyl-2-picrylhydrazyl: The extracts' (DPPH) radical scavenging activity was assessed using, with some changes to, Madhujith and Shahidi (2006) [15] technique. For the aqueous, ethanol, and methanol extracts, a solution of DPPH containing approximately 0.2 mmol/L was made using distilled water and methanol, respectively. The extracts were added in varying amounts (100–1000 µg/mL) to the solution (500 µL). After giving the mixture a good shake, it was let to stand at room temperature for thirty minutes. For the baseline correction, a control was made following the same process, but without the sample extracts, distilled water, or methanol. Then, using a spectrophotometer, variations in the absorbance of the various extract concentrations were determined at 517 nm. The outcomes were contrasted with ascorbic acid, a common antioxidant. The ability of DPPH radical scavenging activity was calculated as follows:

DPPH scavenging effect (% of inhibition) = (Absorbance of the control-Absorbance of the sample/Absorbance of the control) x100.

The reducing power activity: The procedure developed by Manye *et al*. (2023) [16] was utilized to ascertain the extracts' capacity to reduce. One mL of recently made 1% potassium ferric cyanide and various quantities of the extracts (100–1000 µg/mL) were combined. The sodium phosphate buffer had a pH of 6.6. The mixture was incubated for 20 min at 50 °C in a water bath. The mixture was then centrifuged for 10 minutes at 3000 rpm after adding 1 mL of 10% trichloroacetic acid. The supernatant (2 mL) was combined with 2 mL of distilled water and 500 µL of newly made 1% ferric chloride and the reading was taken at 700 nm.

Total phenolic content: A minor modification of Singleton and Rossi (1965) method was used to determine the total phenolic content of the extracts under examination. 500 µL of the double-diluted Folin-Ciocalteu reagent was combined with 100 µL of the sample solution, which had been produced in ethanol at different concentrations and diluted with 5 mL of distilled water. The solution was diluted to 10 mL by adding 1.5 mL of a 20% sodium carbonate solution after 10 minutes. After the prepared samples were stored for two hours at room temperature, the absorbance at 765 nm was determined. The data were represented as gallic acid equivalents (GAE) mg per gram of extracts and were computed using a standard curve of gallic acid (0.5–10 µg/mL).

Total flavonoid content: The total flavonoids in the extracts under investigation were ascertained by slightly altering the technique described by Meda, *et al*. (2005) [17]. A 0.5 mL diluted extract solution and 0.5 mL of 2% aluminum chloride were combined. Following a 20-minute incubation period at room temperature, the reaction mixture's absorbance at 415 nm was determined. 0.5 milliliters of sample and 0.5 milliliters of distilled water made up a blank sample. The spectrophotometer was zeroed using a 0.5 mL sample of aluminum chloride combined with 0.5 mL of distilled water. The data were represented as quercetin equivalents (mg QE) per gram of extracts and were computed using a standard curve of quercetin (10-100 µg/mL).

Statistical analysis: One-way analysis of variance was performed to analyze the data, and Tukey's test was employed to compare means at the 5% level. Version 27.0 of SPSS was used for statistical analysis (2020) [19].

Results and discussion: From the moisture content analysis, it was observed that the fresh *Aloe vera* gel had 97.35% moisture and 2.57% dry matter content. The findings demonstrated that *Aloe vera* gel is high in water

content due to the presence of mucus, which maintains it hydrated. The current research verified the previous findings of (Saeed *et al*., 2022) [24] reporting *Aloe vera* plant leaves primarily consist of 97.40-99.50% water. This result was similar to the study done by (Ahmed and Hussain, 2013) [1], where the water and dry matter contents were 97% and 3%, respectively.

Screening of phytochemical components: *Aloe vera* gel powder contains significant phytochemical elements, according to the current research. Table 1 displays the findings of a qualitative analysis conducted on the presence of active phytochemicals. While alkaloids were lacking from the sample (aqueous and methanol, ethanol) under study, glycosides, tannins, phenols, flavonoids, and saponins were confirmed to be present. According to a recent study (Gorsi *et al*., 2019; Bista *et al*., 2020) [9, 6], *Aloe vera* contains different polysaccharides, saponins, tannins, enzymes, and a variety of vitamins and minerals.

Table 1: Phytochemicals components of *Aloe vera* gel powder

Components	Test Name	Observations	*Aloe vera* gel powder extract		
			Aqueous	methanol	ethanol
Reducing sugars	Fehling's test	Brown to red color	+	+	+
Alkaloids	Wagner's test	Absence of red color precipitation	-	-	-
Glycosides	Killer-killiani test	Blue-green colour	+	+	+
Flavonoids	Shinoda test	Pinkish colour	+	+	+
Tannins	Ferric chloride test	Bluish black	+	+	+
Phenols	Lead acetate test	White precipitation	+	+	+
Saponins	Foam test	Occurrence of foam	+	+	+
Proteins	Biuret's test	Violet to purple in colour	+	+	+
Triterpeniods	Salkowaski test	Greenish-blue	+	+	+

+ = presence of phytochemical, - = absence of phytochemical

In-Vitro antioxidant assay

DPPH radical scavenging Assay: As extract concentration increased, it was observed that the DPPH radical's scavenging activity increased as well. Moreover, research has shown that the primary cause of the antioxidant action of plant products is the phenolic compounds' capacity to scavenge free radicals, including flavonoids, polyphenols, and tannins (Gorci *et al*., 2019). As per Hasan *et al*. (2008), the primary cause of phenolic compounds' antioxidant activity is their ability to reduce oxygen or break down peroxides. These qualities are also known to absorb and neutralize free radicals. *Aloe vera* extracts' capacity to scavenge radicals is responsible for the drop in DPPH radical absorbance. Ethanolic extracts of *Aloe vera* gel powder showed the greatest rate of scavenging among all the extracts, and aqueous extracts of *Aloe vera* showed the slowest rate.

Table 2: Antioxidant activity by DPPH radical scavenging of *Aloe vera* gel powder extracts

Concentrations (µg/ml)	Aqueous extract (%)	Methanol extract (%)	Ethanol extract (%)	Ascorbic acid
100	25.04 ± 0.28^{Aa}	39.82 ± 0.19^{Ab}	42.58 ± 0.22^{Ac}	52.92 ± 0.25^{Ad}
250	27.60 ± 0.18^{Ba}	47.86 ± 0.12^{Bb}	51.15 ± 0.44^{Bc}	60.96 ± 0.33^{Bd}
500	31.62 ± 0.14^{Ca}	58.65 ± 0.14^{Cb}	55.62 ± 0.23^{Cc}	76.12 ± 0.26^{Cd}
750	40.71 ± 0.18^{Da}	65.82 ± 0.16^{Db}	63.32 ± 0.28^{Dc}	91.05 ± 0.17^{Dd}
1000	45.63 ± 0.30^{Ea}	69.97 ± 0.25^{Eb}	67.77 ± 0.24^{Ec}	96.31 ± 0.22^{Ed}
IC 50	1190.39 ± 4.31^{d}	330.58 ± 0.98^{c}	292.29 ± 0.56^{b}	17.40 ± 0.61^{a}

All values are expressed as Mean±SE (n=6). Different superscripts in the column (capital letter) and row (small letter) indicate a significant difference ($p<0.05$)

Compounds with the capacity to contribute electrons that neutralize DPPH free radicals and avert oxidative stress are said to possess DPPH radical scavenging activity (Saeed *et al*., 2022) [24]. When an antioxidant is present, DPPH reacts to form 1,1-Diphenyl-2-picrylhydrazyl-hydrogen (DPPH-H), which has a lower absorbance at 517 nm and a yellow hue (Takshak and Agrawal, 2019). In contrast, DPPH reacts with odd electrons to yield high absorbance at 517 nm (purple color). Table 2 and Fig.2 display the maximal antioxidant activity of 67.77±0.24% for *Aloe vera* gel powder in ethanol solvent, 69.97±0.25% for *Aloe vera* gel methanol extract, and 45.63±0.30% for aqueous extract at 1000 µg/ml concentration. The study's measured antioxidant percentage at 100 µg/ml concentration was higher than that of Waris *et al*. (2018) [31] and lower than that of Saeed *et al*. (2022) [24]. The age of *Aloe vera* leaf affects its antioxidant activity. According to a study (Hu *et al*., 2003), plants have several active chemicals with varying antioxidant activity depending on their developmental stage. A chemical's IC_{50} value, or the concentration at which 50% of the compound is inhibited, indicates its DPPH free radical scavenging activity. Higher antioxidant activity is indicated by a lower IC_{50}. It is the quantity of antioxidants needed to reduce free radicals by 50% and is negatively correlated with scavenging power.

The higher the antioxidant activity of the extract, the lower its IC_{50}. The ethanol extract of *Aloe vera* gel powder had a lower IC_{50} (292.29 µg/ml) in the current study's results than the methanol extract (IC_{50} = 330.58 µg/ml) and aqueous extract (1190.39 µg/ml) indicating that when measured against methanol and water extract, the ethanol extract has much higher antioxidant activity. The standard ascorbic acid's IC_{50} was determined to be 17.40 µg/ml. The standard (quercetin) had IC_{50} values of 36.8 µg/ml, 73.00 µg/ml, and 572.14 µg/ml for Aloe gel and for the methanol and aqueous extracts of *Aloe vera*, respectively, while the IC_{50} of the standard (AV) was reported to be higher at 103.4 µg/ml and 54.0 µg/ml in some earlier studies by (Manye *et al*., 2023)

[16]. According to Vidić *et al*. (2014), the concentration of gel extract was much greater for 50% inhibition, with values of 80.2±4.2 mg/mL for ultrasonic extract and 558.9±55.2 mg/mL for Soxhlet extract. An extract with higher antioxidant activities can be produced by certain solvents than by others, according to a prior study that examined the impact of solvent polarity on biological activity (Ng *et al*., 2020). It was suggested that the ethanol extract of *Aloe vera* gel powder has a higher antioxidant capacity than the methanol and aqueous extract based on the IC_{50} found in the current investigation. However, the findings of earlier research suggest that different sections of the same plant contain diverse phytochemical elements that are thought to activate antioxidant activities (Manye *et al*., 2023) [16].

The reducing power activity: All three *Aloe vera* gel powder extracts exhibited an increase in absorbance as the concentration was raised from 100–1000 µg/ml. Nonetheless, in every dosage, the absorbance rate of the ethanol extract was greater (p<0.05) than that of the methanol and aqueous extracts (Fig. 3 & Table 3). When all three extracts (ethanol, methanol, and aqueous) were evaluated at a concentration of 1000 µg/ml the absorbance of the extracts increased to 0.273 nm, 0.245 nm, and 0.202 nm, respectively (Table 3). At 100 µg/ml, the absorbance of the ethanol, methanol, and aqueous extracts was 0.121 nm, 0.103 nm, and 0.034, respectively.

Table 3: Antioxidant activity by FRAP radical scavenging of *Aloe vera* gel powder extracts

Concentrations (µg/ml)	Aqueous extract (%)	Methanol extract (%)	Ethanol extract (%)
100	0.034 ± 0.001^{Aa}	0.103 ± 0.001^{Ab}	0.121 ± 0.002^{Ac}
250	0.068 ± 0.003^{Ba}	0.127 ± 0.002^{Bb}	0.149 ± 0.002^{Bc}
500	0.125 ± 0.002^{Ca}	0.183 ± 0.002^{Cb}	0.197 ± 0.002^{Cc}
750	0.162 ± 0.003^{Da}	0.218 ± 0.003^{Db}	0.234 ± 0.002^{Dc}
1000	0.202 ± 0.003^{Ea}	0.245 ± 0.003^{Eb}	0.273 ± 0.005^{Ec}
IC 50	249900.83 ± 3.34^{c}	249542.83 ± 3.16^{b}	249470.83 ± 6.33^{a}

All values are expressed as Mean±SE (n=6). Different superscripts in the column (capital letter) and row (small letter) indicate a significant difference (p<0.05)

Table 3 shows the IC_{50} values for ethanol, methanol, and *Aloe vera* gel powder aqueous extracts at dosages ranging from 100 to 1000 µg/ml, which were 249900.83 µg/ml, 249542.83 µg/ml, and 249470.83 µg/ml, respectively. The results are consistent with those of Mayne *et al*. (2023) [16], who observed higher absorbance values in methanol extract as opposed to aqueous extract and higher values when the concentration of *Aloe vera* extract was increased.

Quantitative phytochemical screening

Total phenolic content and Total flavonoid content

Aloe vera gel powder's total phenolic and flavonoid content was extracted using ethanol, methanol, and aqueous solvents. When compared to the aqueous solution, which had a TPC of 9.77±0.83 mg GAE/g, the total phenolic content (TPC) of the ethanol and methanol extracts was found to be significantly higher (p<0.05), with the ethanolic extract having a TPC of 25.28±1.95 and the methanolic extract having a TPC of 23.80±0.29 mg GAE/g. In comparison to methanol (18.56±1.02mg QE/g) and aqueous extracts (12.44±1.11mg QE/g), the flavonoid concentration (TFC) of ethanol extract (28.00±1.21 mg QE/g) was significantly greater ($p < 0.01$) (Table 4 & Figure 4). These findings in TFC content were found to be lower than Bista *et al*. (2020) [6], who reported TFC of ethanol extract of *Aloe vera* as 54.59 mg RE/g and 73.26 mg RE/g in methanol extract. These changes may be caused by the solvents' polarity variations because they extract different hydrophobic and hydrophilic phenolic chemicals from the sample (Chin *et al*., 2021) [7].

Aloe vera leaf extract had a total phenolic content of 28.44 mg GAE/g, 14.29 mg GAE/g, and 27.15mg GAE/g, 30.53 mg GAE/gin ethanol and methanol respectively, according to earlier investigations (Gorsi *et al*., 2019; Bista *et al*., 2020) [9, 6, 7]. Compared to the present levels, these outcomes are lower. The total phenol, flavonoid, and tannin potential values found in this study may not match those found in other studies. Research on how different components under study, solvents, extraction methods, and drying conditions affect the concentration of phytochemicals crucial to their beneficial activities may be the cause of this (Andleeb *et al*., 2021; Kaur and Kalia, 2017; Orphanides *et al*., 2013) [3, 13, 20].

Table 4: Total polyphenols and total flavonoid contents of *Aloe vera* gel powder Compounds

***Aloe vera* gel powder extract**	**Aqueous**	**Methanol**	**Ethanol**
Total polyphenols content (mg GAE/g)	9.77 ± 0.83^{a}	23.80 ± 0.29^{b}	25.28 ± 1.95^{b}
Total flavonoids content (mg QE/g)	12.44 ± 1.11^{a}	18.56 ± 1.02^{b}	28.00 ± 1.21^{c}

All values are expressed as Mean± SE (n=6). Different superscripts indicate a significant difference (p<0.05)

Fig 1: Preparation of aloe vera powder

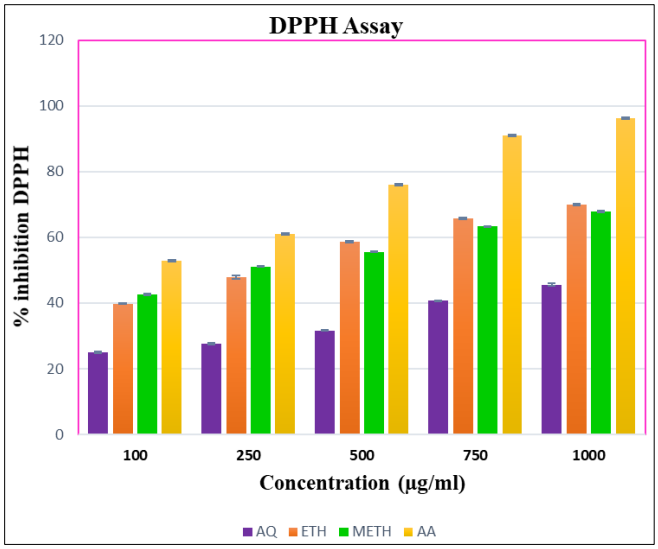
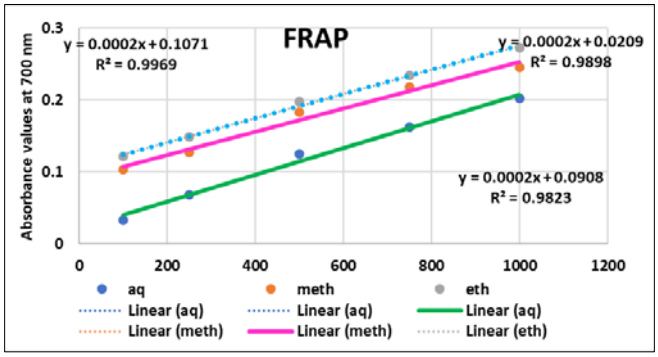


Fig 2: DPPH activity of different extracts of aloe vera gel powder at different concentrations



Methanol extract, eth-Ethanol extract, Aq- Aqueous extract

Fig 3: Correlation between FRAP reducing assay property of three extracts of Aloe vera gel powder extracts

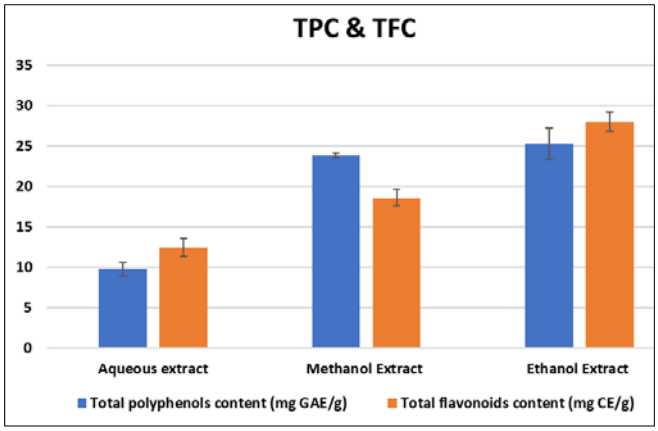


Fig 4: Total phenolic and flavonoid content of different aloe vera gel powder extracts

Conclusion

Based on the study's findings, extracts with various quantities of distinct chemicals might be made using various solvents. The ethanol and methanol extracts of *Aloe vera* gel powder showed a superior reducing power activity in addition to a larger total flavonoid and total phenol content when compared to the aqueous extract. It is thought that the polyphenols in the three extracts contribute to their reducing power and DPPH free radical scavenging properties. This shows that many chemicals present work together to give *Aloe vera* its antioxidant properties. Additionally, according to the study, solvent polarity is responsible for the occurrence of various substances in plants.

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