

Research Article

Evaluation of antibacterial and antioxidant activity of *Aloe vera* (*Aloe barbadensis* Miller) gel powder using different solvents

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Abstract

The present study was planned to investigate the phytochemical compounds present in *Aloe vera* and antimicrobial and antioxidant activity of *Aloe vera* gel powder with different solvents. The phytochemical compounds were screened qualitatively and glycosides, flavonoids, tannins, phenols, saponins, proteins, triterpenoids gave positive results while alkaloids and phlobatannin gave negative results. The polyphenol and flavonoid contents and antioxidant activity of *Aloe vera* gel powder extract using three different solvents (ethanol, methanol, acetone) were analyzed. The ethanolic extract showed maximum polyphenol (28.44 ± 2.67 mg GAE/g), flavonoid (63.90 ± 2.39 mg CE/g) and DPPH radical scavenging activity ($51.09 \pm 0.33\%$). Antibacterial activity of extracts was examined by agar diffusion method with four microbial species (*Salmonella typhi*, *Escherchia coli*, *Bacillus subtilis* and *Staphylococcus aureus*). Maximum antibacterial activity was observed in ethanol extract of *Aloe vera* gel powder. Hence, *Aloe vera* gel powder can be used as an antimicrobial and antioxidant agent in food preservation and to prevent diseases related to oxidative stress.

Keywords: *Aloe vera* gel powder; Antibacterial activity; Antioxidant capacity; Phytochemicals; Solvents; Total polyphenols

Introduction

Aloe vera has been used in ancient cultures of India, Egypt, Greece, Rome and China for its medicinal value for several thousand years. It was known as plant of immortality in biblical times by the Egyptians and believed to be effective for wound healing, for anti-inflammatory effect, for radiation injury, to treat gastrointestinal problems, skin diseases and as an anti-ulcer and anti-diabetes. The

Aloe vera is derived from the Arabic word "Alloeh" which mean bitter substance and also known as first aid plant or burn plant [1]. In the food industry, it has been considered as an important ingredient in functional foods and health drinks, beverages and curd [2, 3]. *Aloe vera* is considered as curative agent mainly due to presence of transparent and soft tissue inside the leaf. The polysaccharides present in *Aloe vera* extracts

are responsible for health benefits [4, 5]. *Aloe vera* gel has many biological activities including its antimicrobial activity, anti-diabetic effects, antioxidant activity, anti-inflammatory and protection of gastrointestinal tract [2, 4, 6-8]. Antioxidant effect of *Aloe vera* gel has been reported by several researchers. In *Aloe vera* gel, compounds like phenolic antioxidants, glutathione peroxidase activity and superoxide dismutase enzymes are responsible for its antioxidant effects [2]. Anthraquinones responsible for antimicrobial activity were [9] isolated from the exudates of *Aloe vera*. Due to its important constituents, *Aloe vera* has been used as main and important ingredient for soaps, shampoos, creams, lotions and other products in the toiletry and cosmetic industry. In the pharmaceutical industry, it has been utilized in production of ointments and gel production as well as in manufacturing of tablets and capsules [10, 11]. The present study was planned to screen phytochemical compounds (qualitative method) and to analyze antibacterial activity and antioxidant capacity of *Aloe vera* gel powder (extracts of ethanol, methanol and acetone).

Materials and methods

Procurement of raw materials

Aloe vera leaves (*Aloe barbadensis* Miller) were purchased from local nurseries of Sargodha and selected on the basis of size, maturity, color, freshness for the present study. Other chemicals, required for this study were purchased from Scientific Store, Faisalabad.

Preparation of extract

Aloe vera gel was separated from leaves by using sharp knife. *Aloe vera* rind and gel were dried in conventional oven at $50 \pm 5^\circ\text{C}$ [12]. The dried samples were crushed, powdered and packed in air tight container for further studies. For preparation of extract, 0.5 g powdered material was mixed with 50 ml of 80% aqueous solution of solvents (methanol,

ethanol, acetone) and placed in an ultrasonic bath for 20 min.

Phytochemical Screening

Analysis of phytochemical constituents of *Aloe vera* gel powder was done qualitatively [13, 14].

Total phenolic assay

The reagent Folin-Ciocalteu was used to measure total phenolic content of the extracts [15]. In, 0.5 ml of extracts, 2.5 ml of Folin-Ciocalteu's phenolic reagent was added. After 5 min at room temperature 2.5 ml of 7.5% sodium carbonate solution was added and allowed to incubate for 90 min at room temperature and absorbance was determined at 765 nm with UV-VIS spectrophotometer. Total phenolic content of samples was expressed as milligram of gallic acid equivalents (GAE)/g dry weight.

Total flavonoids assay

The method used to determine the total flavonoids in *Aloe vera* sample was Aluminium Chloride (AlCl_3) colorimetric assay [16]. In 10 ml volumetric flask which have 4 ml of H_2O , 1 ml of extract was added and after 5 min 10% AlCl_3 and after one min, 2 ml of 1M NaOH was mixed and total volume was made upto 10 ml with H_2O . The absorbance of mixture was measured using UV-VIS spectrophotometer at 510 nm. Total flavonoid content of samples was expressed as mg catechin equivalents (CE)/g dry sample.

Antioxidant activity determination

Antioxidant activity of methanolic, ethanolic and acetone extracts were analyzed by DPPH (2,2-diphenyl-1-picrylhydrozyl) method [17]. In 3 ml of extract, 0.1 mM of DPPH solution was added and kept in dark place for 30 min at room temperature and absorbance was measured at 517 nm on a UV-VIS spectrophotometer.

The antioxidant activity was calculated by using the following formula:

$$\text{Antioxidant activity (\%)} = \frac{[(\text{AC} - \text{AE}) / \text{AC}] \times 100}{100}$$

Where

AC = absorbance of a DPPH solution without extract

AE = absorbance of the tested extract

Antibacterial activity of Aloe vera

The antibacterial activity of *Aloe vera* was determined by disc diffusion technique [18]. The bacterial test organisms i-e gram positive (*Bacillus Subtilis*, *Staphylococcus Aureus*) and gram negative (*Salmonella Typhi*, *Eschersia Coli*) were inoculated at the nutrient agar medium using sterile cotton buds and incubated at 37°C for 24 hrs. The diameter of inhibition zones was measured in millimetre.

Results and discussion

Screening of phytochemical components

The present studies revealed that *Aloe vera* gel powder contain important phytochemical compounds. The presences of active phytochemicals were qualitatively analyzed and results are shown in (Table 1). Glycosides, flavonoids, tannins, phenols and spanions were found to be present in the studied sample while phlobatannins and alkaloids were found absent. Presence of mono- and polysaccharides, saponins, tannins, organic acids, sterols, enzymes, various vitamins and minerals in *Aloe vera* were confirmed in a previous study [19].

Total phenolic, total flavonoid and antioxidant capacity of Aloe vera gel powder

Polyphenols are the group of compounds containing phenolic hydroxyl attached to ring structures, due to which they function as antioxidant. These compounds have many functions such as reducing agents, singlet oxygen quenchers and hydrogen donating antioxidants [20].

Total phenolic and flavonoid contents of *Aloe vera* gel powder were extracted by using methanol, ethanol and acetone solvents. Results showed that ethanol extract had highest total phenolic content (28.44±2.673 mg GAE/g) and total flavonoids

(63.90±2.396 mgCE/g) as compared to methanol (27.15±2.244 mgGAE/g, 16.76±3.048 mgCE/g) and acetone (11.48±0.238 mgGAE/g, 5.21±0.478 mgCE/g) extract, respectively (Table 2).

The *Aloe vera* gel powder in ethanol solvent showed maximum antioxidant activity 51.09 % while acetone extract of *Aloe vera gel* showed minimum antioxidant activity 24.37% (Table 3). *Aloe vera* possesses antioxidant properties as showing great amount of polyphenols. As over past many years, the food manufacturers and scientists showed great interest in importance of polyphenols. Antioxidants play a vital role in prevention of many diseases linked with oxidative stress like cancer, cardiovascular disease, nerve degeneration [21] and diabetes [22].

The antioxidant activity observed by *Aloe vera* gel may be comparable to amount of ascorbic acid, since both functions as scavenger of free radicals which are responsible cellular damage, aging and cardiovascular diseases [23, 24]. The antioxidant activity of *Aloe vera* depends upon the maturity. It was [25] reported that at different stages of development, the plant has different active compounds showing different antioxidant activity.

Antibacterial activity of Aloe vera

Antibacterial activity of *Aloe vera* gel powder in methanol, ethanol and acetone was analyzed against two gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) and two gram negative (*Salmonella typhi*, *Eschersia coli*). The ethanolic extract of *Aloe vera gel* powder showed antibacterial activity against all test organisms (gram positive and gram negative) with highest activity on *Bacillus subtilis*, and *Staphylococcus aureus* i-e 20.33± 0.073 mm and 18.63± 0.101mm of inhibition zone, respectively (Table 4). Acetone extract showed maximum antibacterial activity (13.60±0.141 mm) against *Salmonella typhi*. In a previous study,

it was observed that acetone exhibit maximum antibacterial activities against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* other than aqueous extract and ethanol extract [26]. Some plant compounds

like saponins [27] have been reported to have direct antimicrobial activity. In (Figure 1) compares antibacterial activity of different extracts (ethanol, methanol, and acetone) of *Aloe vera* gel powder with positive control (ciprofloxacin).

Table 1. Qualitative analysis of phytochemical components of *Aloe vera* gel powder

Components	Tests	Observations	<i>Aloe vera</i> gel powder
Reducing sugars	Fehling's test	Reddish brown ppt	+
Alkaloids	Wagner's test	No reddish brown ppt	-
Glycosides	Killer-killiani test	Bluish green in upper layer	+
Flavonoids	Shinoda test	Pink colour	+
Tannins	Ferric chloride test	Blue-black colour	+
Phenols	Lead acetate test	White ppt	+
Saponins	Foam test	Frothing takes place	+
Proteins	Biuret's test / Ninhydrin test	Violet solution / purple solution	+
Triterpenoids	Salkowaski test	Greenish blue color	+
Phlobatannin	1% Hydrochloric acid	Red ppt	-

(+) presence (-) absence

Table 2. Total polyphenols and total flavonoid contents of *Aloe vera* gel powder

Compounds	Ethanol Extract	Methanol Extract	Acetone Extract
Total polyphenols content (mg GAE/g)	28.44 ± 2.673	27.15 ± 2.244	11.48 ± 0.238
Total flavonoids content (mg CE/g)	63.90 ± 2.396	16.76 ± 3.048	5.21 ± 0.478

Values are mean ± standard deviation

Table 3. Antioxidant activity by DPPH radical scavenging of *Aloe vera* gel extracts measured as percentage inhibition

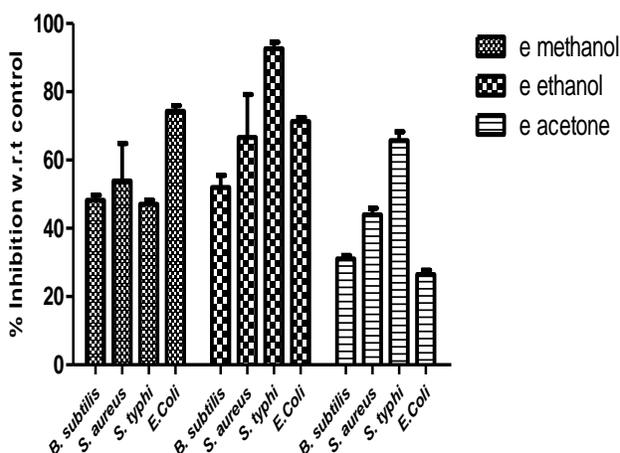
Concentrations	Ethanol extract (%)	Methanol extract (%)	Acetone extract (%)
400µg/ml	51.09 ± 0.338	49.90 ± 0.665	24.37 ± 0.624
200µg/ml	44.94 ± 1.110	47.95 ± 0.893	21.60 ± 1.225
100µg/ml	40.19 ± 0.544	43.20 ± 0.435	19.82 ± 0.617
50µg/ml	36.76 ± 0.637	39.81 ± 0.737	17.36 ± 0.765
25µg/ml	29.14 ± 0.382	30.97 ± 0.705	11.29 ± 0.666

Values are means ± standard deviation

Table 4. Antibacterial activity of different *Aloe vera* gel extracts measured as zone of inhibition (mm)

Organisms	Methanol extract	Ethanol extract	Acetone extract
<i>Bacillus subtilis</i>	17.30 ± 0.024	20.30 ± 0.073	11.30 ± 0.106
<i>Staphylococcus aureus</i>	11.33 ± 0.047	18.63 ± 0.101	10.60 ± 0.082
<i>Salmonella typhi</i>	10.93 ± 0.262	12.73 ± 0.227	13.60 ± 0.141
<i>Escherichia coli</i>	14.63 ± 0.267	12.87 ± 0.019	10.20 ± 0.188

Values are mean ± standard deviation



Graph 1. Antibacterial activity of *Aloe vera* gel powder extract using ethanol, methanol and acetone solvent w.r.t control

Conclusion

From the present study, it was concluded that *Aloe vera* gel powder contain some important medicinal compounds and ethanolic extract showed more total polyphenol, total flavonoid, antioxidant capacity and antibacterial activity as compared to methanolic and acetone extract. The ethanolic extract of *Aloe gel* powder showed highest antibacterial activity on *Bacillus subtilis* and *Staphylococcus aureus* while acetone extract showed maximum antibacterial activity against *Salmonella typhi*. So, *Aloe vera* gel powder is valuable to be used in food, cosmetics and medicines.

Authors' contributions

Conceived and designed the experiments: T Kausar & FI Gorski, Performed the experiments: FI Gorski, Analyzed the data: T Kausar & FI Gorski, Contributed reagents/materials/ analysis tools: T Kausar, FI Gorski, MA Murtaza, Wrote the paper: T Kausar.

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