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Research Article

Phytochemical and Antibacterial Activity of *Aloe vera, Camellia sinensis* and *Momordica charantia*

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Abstract: Traditional medicines, largely derived from plants, contain bioactive compounds that serve as protective agents against environmental stressors (biotic and abiotic) and can also enhance human health. With the alarming rise in antimicrobial resistance, there is an urgent need to explore safe and effective plant-derived alternatives to synthetic antibiotics. The present study was therefore designed to evaluate the phytochemical composition and antibacterial potential of three commonly used medicinal plants: *Camellia sinensis*, *Aloe vera*, and *Momordica charantia*. Aqueous and ethanolic extracts were prepared and qualitatively screened for tannins, alkaloids, saponins, flavonoids, steroids, proteins, carbohydrates, phlobatannins, and terpenoids. Quantitative analysis further revealed that *M. charantia* had the highest alkaloid content (5.29%), while its ethanolic extract exhibited the greatest protein (37.9 mg/g dry extract) and carbohydrate levels (30.6 mg/g dry extract). The antibacterial activity of the extracts was tested against a Gram-positive strain (*Staphylococcus aureus*) and a Gram-negative strain (*Klebsiella pneumoniae*). The results showed that *C. sinensis* consistently demonstrated the strongest antibacterial activity in both aqueous and ethanolic extracts, producing inhibition zones of 20 ± 0.95 mm against *S. aureus* and 12 ± 2 mm against *K. pneumoniae*. In comparison, *M. charantia* exhibited selective inhibition, being effective only against *S. aureus*, while *A. vera* showed moderate antibacterial activity depending on the extraction solvent. Overall, the findings highlight the therapeutic promise of *C. sinensis* as a natural antimicrobial agent. Future studies should expand antimicrobial screening to additional pathogens and include in vivo assays to validate the clinical applicability of these extracts.

Keywords: Phytochemicals, Qualitative Analysis, Quantitative Analysis, Antibacterial Activity, Zone of Inhibition, Phytotherapy.

1. INTRODUCTION

Nature has long been recognized for its positive effects on human health, largely due to the presence of plants and their bioactive compounds. Plants produce diverse secondary metabolites, collectively known as phytochemicals, which are not essential nutrients but play critical roles in disease prevention and therapeutic applications [1-3]. These compounds help modulate oxidative stress, regulate immune responses, and exert antimicrobial effects, making them promising alternatives to synthetic antibiotics in the time of rising drug resistance [4-6].

Phytochemicals are grouped into several classes, each with distinct biological activities. Flavonoids, found in vegetables, fruits, tea, and red wine, are well-documented for their antioxidant, anti-inflammatory, and anticancer effects [7]. Alkaloids, nitrogen-containing compounds such as caffeine in coffee or theobromine in cocoa, display analgesic, antimicrobial, and anti-inflammatory properties [8]. Saponins, abundant in legumes and beans, possess immunomodulatory and anticancer activity [9, 10]. Terpenoids from aromatic plants like mint and citrus fruits display antimicrobial and antioxidant effects [11]. Phytosterols in seeds and nuts help regulate cholesterol metabolism and induce apoptosis in tumor cells [12].

In South Asian traditional medicine, plants such as Bitter gourd (*Momordica charantia*), *Aloe vera*, and Green tea (*Camellia sinensis*) are frequently used to manage infections, metabolic disorders, and inflammatory conditions.

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However, despite their well-documented ethnopharmacological value, systematic comparisons of their phytochemical content and antibacterial efficacy against clinically important pathogens remain limited [13].

Bitter gourd is widely known for its hypoglycemic effects, mediated by compounds such as charantin, momordicins, and vicine [14-16]. Beyond its metabolic role, recent studies have reported that extracts of *M. charantia* exhibit strong antibacterial activity against a range of pathogens, including *Staphylococcus aureus* and periodontal bacteria, while also displaying antioxidant and anti-inflammatory properties [17-19].

Aloe vera contains polysaccharides, anthraquinones, glycoproteins, and antioxidants that contribute to its anti-inflammatory, wound-healing, and skin-protective activities [20-22]. Importantly, *A. vera* extracts have also demonstrated antimicrobial activity against a variety of clinical isolates, suggesting its potential as a complementary therapeutic agent [22].

Green tea (*Camellia sinensis*) is particularly rich in catechins. These compounds are potent antioxidants and have been associated with cardiovascular and metabolic health benefits [23-25]. In addition, in vitro studies have revealed that green tea extracts exhibit antibacterial activity against pathogens including *S. aureus* and MRSA [26, 27], highlighting its potential dual role in both preventive and therapeutic health strategies.

Despite such promising findings, there is still limited research that directly compares these plants' phytochemical composition with their antibacterial efficacy against clinically relevant pathogens. Therefore, the present study was designed to evaluate and compare the antibacterial activity of bitter gourd, *Aloe vera*, and green tea extracts against *Staphylococcus aureus* and *Klebsiella pneumoniae*, and to correlate these activities with the presence of key phytochemicals (alkaloids, saponins, etc.). By employing both aqueous and ethanol extracts, this work highlights the novelty of a comparative phytochemical–antibacterial approach in the Pakistani context, addressing an urgent need for safer plant-based alternatives to conventional antibiotics.

2. MATERIALS AND METHODS

2.1. Sample Preparation

Sample plants (*Momordica charantia* (leaves), *Aloe vera* (skin) and *Camellia sinensis* (leaves and buds) were collected from the local markets in Gujranwala, Pakistan and then washed with tap water, dried under open air and sunlight till completely dry and ground into fine powder by using electric grinder.

2.1.1. Extraction

Solvents (ethanol and distilled water) were used for extraction. Powdered samples (3 g) were soaked in each of the solvent separately. The samples were kept for 4 hours in the water bath to ensure complete dissolution of chemicals and then kept on orbital shaker for 12 hours; filtered and stored at 4ºC for further analysis [23].

2.2. Qualitative Phytochemical Analysis

2.2.1. Test for tannins

4-6 drops of 5% ferric chloride were added to 2 ml of the sample extract. The hydrolyzed tannins can be detected by observing the color changes to blue-black [28].

2.2.2. Test for flavonoids

2% NaOH solution was added to test tubes containing the sample (1 ml). 3-4 drops of dilute HCl were added. Deep yellow color that disappears within seconds indicates a positive test [29].

2.2.3. Test for alkaloids

Sample extract (1 ml) was taken in tube and mixed with 2 ml of conc. HCl and boiled at 100 ºC in a water bath for 5 minutes. 4-6 drops of Wagner's reagent were added to the sample. Reddish-brown precipitate indicates positive result [28].

2.2.4. Test for saponins

Sample extract (2 ml) was tested for foam formation by shaking the tube vigorously for almost 30 seconds. Foam formation confirms the presence of Saponins [30].

2.2.5. Test for steroids

Chloroform (2 ml) was added to a 1 ml sample extract. A few (3-4) drops of concentrated H_2SO_4 were added alongside the walls of the test tube. The red ring at inter-phase indicates positive test [29].

2.2.6. Test for protein

Sample extract (2 ml) was boiled with few drops of ninhydrin solution (1%). Violet-blue colour (known as Ruhemann's purple) confirms the presence of protein [31].

2.2.7. Qualitative test for carbohydrates

a) Molisch test: Ten drops of Molisch reagent were added to 2 ml of the extract. Later, concentrated H_2SO_4 was added along the sides of the test tube. The violet ring at the inter-sectional plane indicates positive test [28].
b) Benedict test: Crude plant extract (1 ml) was added to Benedict's reagent (1 ml) and boiled. Red precipitates demonstrate the occurrence of reducing carbohydrates [28].

2.2.8. Test for phylobatannins

Liquid gelatin was added to sample extract (2 ml). Creamy texture indicates the presence of phylobatannins [28].

2.2.9. Test for terpenoids

Crude extract (1 ml) was added to 2.0 ml of chloroform in a test tube. Concentrated H_2SO_4 (2 ml) was added to the solution and the solution was boiled for 2 minutes. Reddish brown color indicates presence of terpenoids [32].

2.3. Quantitative Phytochemical Analysis

2.3.1. Extract preparation

Plant samples (4 grams) were extracted individually with ethanol and water (40 ml). The extraction was carried out in a water bath at 25 °C for one hour. The samples were filtered, and the resulting extract was dried at 60 °C in a hot air oven. Dried extracts were then stored in amber-coloured glass bottles at 4 °C to prevent oxidative damage. Percent yield of the extracts was calculated using the following formula:

$$\text{Percent Yield (\%)} = \frac{W1}{W2} \times 100$$

Where,
W1 = Weight of the extract residue obtained after solvent removal
W2 = Weight of the plant powder used

2.3.2. Total alkaloids determination

The total alkaloid content was determined by adding 20 ml of 10% acetic acid in ethanol to 1 gram of the sample. The resulting solution was covered with foil and allowed to settle for four hours. The solution was filtered after 4 hours and the filtrate was boiled down in a water bath to reach 1/4 of the original volume. Concentrated ammonium hydroxide solution was then carefully added until the precipitation process was completed. The precipitates were then collected, rinsed with dilute NH_4OH (ammonium hydroxide), and then filtered. The residue was dried up to constant weight to determine total alkaloid content. In this study, alkaloids were determined from the plants leaves/ buds samples; total alkaloids were determined using the following formula:

$$\text{Alkaloids (\%)} = \frac{\text{mass of residue}}{\text{mass of sample}} \times 100$$

2.3.3. Total protein determination

Bradford reagent (2.5 ml) was added to a test tube containing 0.1 ml of extract. The test tube was allowed to stand for 5 minutes. Absorbance was noted at 595 nm. Bovine serum albumin (BSA) was used as standard and blank tube contained distilled water in place of sample [33].

2.3.4. Total carbohydrate determination

Sample extract (100 µl) was added to 1900 µl distilled water to make the total volume 2 ml. DNS (2 ml) was added, and the tubes were placed in boiling water for 5 minutes. Then 6 ml distilled water was added, and absorbance was recorded at 550 nm. In blank tube, sample extract was replaced with distilled water [34].

2.4. Antibacterial Activity

Antibacterial activity of *Momordica charantia, Camellia sinensis* and *Aloe vera* was observed against a Gram-positive (*Staphylococcus aureus*

ATCC 25923) and Gram-negative bacterium (*Klebsiella pneumoniae ATCC 13883*).

2.4.1. Inoculum preparation

Sterilized nutrient broth medium was used for inoculum development. A single bacterial colony of *Klebsiella pneumoniae* and *Staphylococcus aureus,* was separately inoculated into sterilized nutrient broth. Flasks were kept in the shaking incubator for ~24 hours. Flasks were kept in the shaking incubator at 37 °C, 150 rpm for 24 hours until OD600 = 0.5, corresponding to 1×10^8 CFU/ml. This inoculum was diluted to 1×10^6 CFU/ml before testing.

2.4.2. Determination of antibacterial activity

To check the antibacterial activity, wells were created in the petri plates with the help of sterilized tips. These wells were then loaded with the sample extracts (150 µl). The final concentration of the extract was standardized at 10 mg/150 µl (66.6 mg/ml), for antibacterial testing, based on preliminary assays and literature reports [35]. All experiments were performed in triplicate (n = 3) for each extract and control. Streptomycin (10 µg/ml) was used as a positive control, while distilled water and ethanol were used as negative controls. The inclusion of solvent controls was to account for any inhibitory effect of extraction solvents themselves. The plates were kept in the incubator at 37 °C for 24 hours. Zones of inhibition were measured in millimeters using a vernier caliper [36].

2.4.3. Statistical analysis

All data were analyzed using Costat statistical software (Snedecor and Cochran, 1980). Descriptive statistics (mean ± standard deviation) were calculated, One-way ANOVA was performed to assess significant differences among treatments, and means were separated using Tukey's HSD post-hoc test at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Physical Properties of Plant Extracts

The aqueous and ethanolic extracts of *Camellia sinensis*, *Aloe vera*, and *Momordica charantia* exhibited distinct colors and viscosities, reflecting differences in solvent polarity and phytochemical solubility (Table 1). Aqueous extracts generally appeared yellow to orange, while ethanolic extracts were green to olive in color, consistent with earlier reports that extraction solvents influence pigment and metabolite profiles [17, 37]. Ethanol and water were selected as extraction solvents due to their safety, availability, and complementary polarity ranges. Ethanol favors the extraction of relatively non-polar compounds such as alkaloids and flavonoids, while water is more efficient for polar constituents like tannins, carbohydrates, and proteins. This dual approach ensured a broad coverage of phytochemicals and reflects commonly used practices in pharmacognosy studies [38, 39].

3.2. Qualitative and Quantitative Phytochemical Analysis

Momordica charantia showed positive results for proteins, tannins, phylobatannins, steroids and alkaloids with both the extracts. Saponins and flavonoids were present in aqueous extract only and were absent in ethanol extract. Benedict's test for carbohydrates was negative for both extracts. Jia *et al.* [15] carried out the phytochemical screening of *Momordica charantia* and reported the presence of triterpenes, polysaccharides, saponins, proteins and flavonoids.

Table 1. Physical properties of different plant extracts.

Sample botanical name	Sample common name	Physical properties	Aqueous extract	Ethanol extract
Camellia sinensis	Green tea	Colour	Yellow brown	Dark green
		Viscosity	Viscous	Non-Viscous
Aloe vera	Aloe vera	Colour	Yellow	Dark green
		Viscosity	Viscous	Non-Viscous
Momordica charantia	Bitter gourd	Colour	Orange	Olive green
		Viscosity	Non-Viscous	Non-Viscous

Camellia sinensis showed positive results for carbohydrates, tannins, phylobatannins and alkaloids with both extracts and showed positive results for proteins, steroids and terpenoids with ethanol extract only and gave positive results for saponins with aqueous extract only. Both extracts gave negative results for flavonoids. The aqueous extract of *Aloe vera* gave negative results for proteins, carbohydrates, steroids, and terpenoids, while it gave positive results for tannins, flavonoids, phylobatannins, alkaloids, and saponins (Table 2). The variability of phytochemical content depends on the extraction method [40].

Qualitative screening confirmed the presence or absence of key phytochemicals, whereas quantitative assays provided numerical validation, thereby strengthening the reliability of the findings. Qualitative and quantitative analyses revealed variation in secondary metabolite content across species and solvents. Ethanol extract of *M. charantia* showed the least percentage yield of 4% while aqueous extract of *Aloe vera* showed the highest percentage yield (32.5%) as shown in Figure 1. *M. charantia* was particularly rich in alkaloids (5.29%), whereas *Aloe vera* gave the lowest (4.38%) alkaloid content (Figure 2). The ethanol extract of *M. charantia* gave the maximum carbohydrate concentration (30.6 mg/g dry extract), while the aqueous extract of *A. vera* contained the minimum protein (2.14 mg/g dry extract) (Figure 3). Similarly, protein analysis showed that the ethanol extract of *M. charantia* had the highest protein content (37.9 mg/g dry extract), in contrast to the aqueous extract of *A. vera*, which recorded the lowest protein content (1.45 mg/g dry extract) (Figure 4). These findings are comparable with earlier reports highlighting *M. charantia* as a source of bioactive triterpenoids and alkaloids [15]. The differences observed between aqueous and ethanol extracts align with solvent polarity: ethanol more efficiently extracts phenolics, terpenoids, and alkaloids, while water favors hydrophilic compounds such as polysaccharides [41].

3.3. Antibacterial Activity

Ethanol extract of *C. sinensis* produced the strongest inhibition against *S. aureus* (20 ± 0.95 mm), which can be attributed to its higher tannin and alkaloid contents (Table 3). Tannins from green tea are well-documented for disrupting bacterial membranes and enzyme activity [42]. In contrast, *A. vera* showed weaker activity, consistent with its lower alkaloid and protein levels. In the present study, negative controls (sterile water and ethanol) did not produce any inhibition zones, confirming that the observed antibacterial effects were solely due to the plant extracts.

Table 2. Qualitative phytochemical analysis.

Tests		***Momordica charantia***		***Camellia sinensis***		***Aloe vera***	
		Aqueous extract	**Ethanol extract**	**Aqueous extract**	**Ethanol extract**	**Aqueous extract**	**Ethanol extract**
Proteins		+++	++	-	++	-	+
Carbohydrates	Molisch Test	+	+++	+	+++	-	+
	Benedict's Test	-	-	+	+	-	+
Tannins		+	+++	+	+	+	+++
Flavonoids		+	-	-	-	++	-
Phylobatannins		+++	+	++	+	+	+
Steroids		+	+++	-	+++	-	++
Terpenoids		-	+++	-	+++	-	+
Alkaloids		+++	++	+++	+++	++	+++
Saponins		+++	-	+	-	+	-

Absent (-), Slightly Present (+), Moderately Present (++), High concentration (+++)

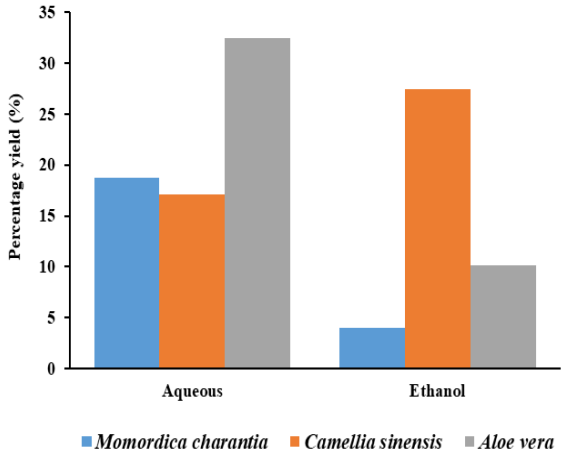

Fig. 1. Percentage yield of selected plants in aqueous and ethanol extracts.

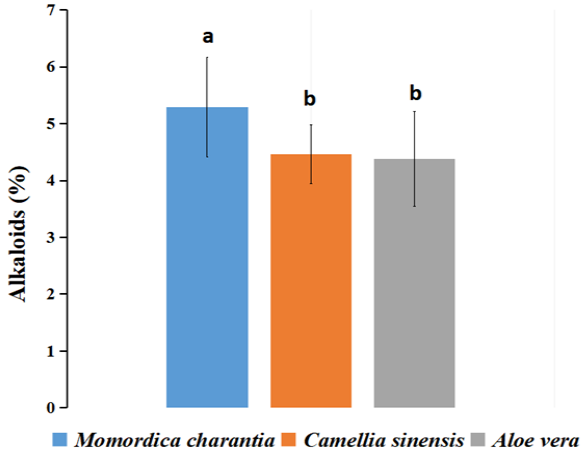

Fig. 2. Total alkaloid content in selected plants.
Error bars represent the standard deviation (± SD) among triplicate. Values with different superscript letters differ significantly ($p \leq 0.05$).

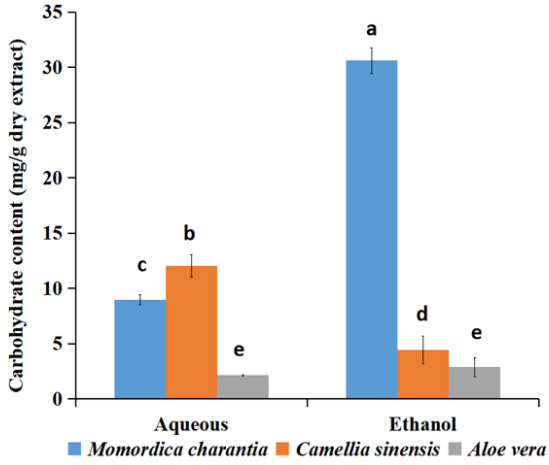

Fig. 3. Total carbohydrate content of selected plants in aqueous and ethanol extracts.
Error bars represent the standard deviation (± SD) among triplicate. Values with different superscript letters differ significantly ($p \leq 0.05$).

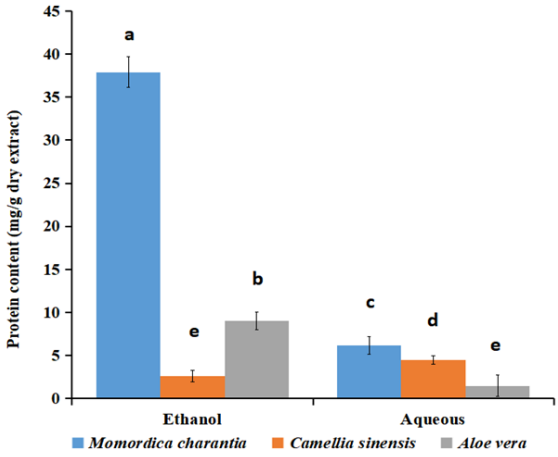

Fig. 4. Total protein content of selected plants in aqueous and ethanol extracts.
Error bars represent the standard deviation (± SD) among triplicate. Values with different superscript letters differ significantly ($p \leq 0.05$).

Table 3. Antibacterial activity.

Sample	Extract	Zone of Inhibition (mm)	
		Staphylococcus aureus	*Klebsiella pneumoniae*
Momordica charantia	Ethanol	15 ± 0.45^{c}	0.0 ± 0^{d}
	Aqueous	0.0 ± 0^{d}	0.0 ± 0^{d}
Camellia Sinensis	Ethanol	20 ± 0.95^{b}	12 ± 0.2^{b}
	Aqueous	10 ± 0.57^{c}	11 ± 0.14^{c}
Aloe vera	Ethanol	11 ± 0.1^{c}	12 ± 0.28^{b}
	Aqueous	0.0 ± 0^{d}	0.0 ± 0^{d}
Streptomycin		29 ± 0.36^{a}	24 ± 1.2^{a}

Anita *et al.* [26] reported a zone of inhibition (12.66 mm) for the ethanolic extract of *C. sinensis* against *L. acidophilus*. Sowjanya *et al.* [27] studied the antimicrobial activity of *C. sinensis* against *K. pneumoniae* and *S. aureus*, and determined the minimum bactericidal concentration (MBC) as 5 mg/ml for both strains. Ethanol extracts of *C. sinensis* and *Aloe vera* gave a zone of inhibition (12 ± 0.2 mm) against *K. pneumoniae* while aqueous extract of *C. sinensis* gave a zone of inhibition (11 ± 0.14 mm). Ethanolic extract of *M. charantia* exhibited low activity against *K. pneumoniae* (MIC = 625 µg/ml) [18]. Khalid *et al.* [19] tested the antibacterial activity of leaves of *M. charantia,* and the results showed that 80% methanolic extract exhibited the highest antibacterial potential against with inhibition zone diameters of 30 mm against *P. multocida* and 28 mm against *A. parasiticus,* respectively. Aqueous control showed no zone of inhibition, whereas ethanolic control showed a very minimum zone (3-4 mm).

4. CONCLUSIONS

This study demonstrated that the phytochemical composition of *Momordica charantia, Aloe vera,* and *Camellia sinensis* influenced their antibacterial activity, with *C. sinensis* showing the strongest and most consistent effects. *M. charantia* was effective mainly against *S. aureus* and *A. vera* showed moderate activity; whereas *C. sinensis* exhibited broad antibacterial potential, highlighting its promise as a natural therapeutic agent. However, the study was limited to two bacterial strains; future work should include additional Gram-positive, Gram-negative, and fungal pathogens, along with MIC/MBC, antioxidant, and phenolic assays to better elucidate antimicrobial mechanisms and therapeutic relevance.

5. CONFLICT OF INTEREST

Authors declare no conflict of interest.

6. ACKNOWLEDGMENTS

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