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Original Research

The Antioxidant, Anticancer, and Antimicrobial Properties of Bioactive Compounds in Bitter Apple Seed Extract and Their Application in Preserving Minced Beef

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Abstract

The current study prepared, characterized, and evaluated a bitter apple seed ethanolic extract (BASEE) for its antioxidant, anticancer, antibacterial, and antifungal properties, as well as its use in preserving minced beef. The bitter apple seed ethanolic extract had a total phenolic content of 32 mg GAE g^{-1} extract. BASEE contains dominant compounds, including ellagic (0.9 mg g^{-1}), cinnamic (0.8 mg g^{-1}), or gallic (0.6 mg g^{-1}) acids. The bitter apple seed ethanolic extract exhibited antioxidant activity by inhibiting DPPH free radicals. It exhibited activity towards *Staphylococcus aureus* and *Escherichia coli*, with effectiveness depending on the concentration. BASEE (400 µg mL^{-1}) significantly inhibited *F. oxysporum* mycelial growth, resulting in a 94% reduction in development. HCT116 cells treated with BASEE for 48 h stopped their growth, with IC_{50} values of 55 µg mL^{-1} for HCT116 cells and 100 µg mL^{-1} for A549 cells. However, IC_{50} values were significantly higher than those of Vero normal cells (1000 µg mL^{-1}). Results indicate that HCT116 cells showed greater

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sensitivity to the bitter apple seed ethanolic extract compared to the A549 cells and normal cells (Vero). The findings indicate that BASEE may function as an alternative antioxidant, antibacterial, antifungal, and anticancer agent. Bitter apple seed ethanolic extract can be effectively used as a natural preservative and a better alternative to synthetic compounds.

Keywords: antibacterial, antifungal, anticancer, *Citrullus colocynthis*, ethanol extract, minced beef

Introduction

Infectious diseases are well recognized as a major contributor to global mortality [1, 2]. Although numerous antimicrobial agents have been developed, the misuse of these treatments has led to severe resistance among many microorganisms, resulting in the emergence of multidrug-resistant pathogens [3]. Mortality rate associated with resistant strains is twice as high as that of non-resistant strains, resulting in a significant burden on the medical and public sectors [4]. Environmentally friendly bactericides are gradually supplanting antibiotics as a viable alternative [5, 6]. Microbial contamination is a significant problem that can affect food's longevity and potentially result in consumer illness [7]. Consequently, numerous substances function as preservatives, augmenting the safety and longevity of food products [8]. Due to heightened consumer awareness of the harmful effects of chemical preservatives and a growing preference for natural ingredients, researchers have been concentrating on developing natural additives with antimicrobial properties for use in the food industry [9, 10]. Contemporary farming methods depend on artificial fungicides to manage plant diseases [11]. Nevertheless, there are apprehensions regarding the repercussions of these fungicides on human and environmental well-being [12]. Eco-friendly fungicides are gradually replacing synthetic fungicides as an alternative [13-18]. Using herbal medications is one of the most ancient and secure approaches to treating various diseases [19-21]. Bitter apple (*Citrullus colocynthis*) is a widely used medicinal plant in the pharmaceutical industry worldwide because of its antibacterial, anticancer, antioxidant, antifungal, and anti-inflammatory properties [22]. Bitter apples are a plant native to arid regions. It has a significant historical significance as a valuable medicinal plant and a reservoir of bioactive substances [23]. The medicinal benefits of this plant have been documented in traditional medical systems across India, Pakistan, China, Asia, and Africa. These benefits include treating diabetes, the common cold, cough, toothaches, wounds, and gastrointestinal diseases like indigestion, dysentery, and colic pain [24]. *Citrullus colocynthis* fruit contains several bioactive substances that have been documented in the literature. They are divided into six categories: fatty acids, carbohydrates, flavonoids, alkaloids, glycosides, and phenolics [25]. Therefore, the current study prepared, characterized, and evaluated a bitter apple seed ethanolic extract (BASEE) for its antioxidant, anticancer, antibacterial, and antifungal activities and their use in preserving minced beef.

Materials and Methods

Plant Materials

Fruits of bitter apple were purchased from a local herbal market in Zagazig City, Egypt, and the collected fruits were separated from any mixed contaminants. The seeds were isolated, cleansed, dehydrated at ambient temperature, and then ground using an electric blender.

Chemical Analysis

The moisture, fat, protein, fiber, and ash content of bitter apple seeds was determined utilizing AOAC procedures as described by Helrich [26]. For moisture estimation, seeds were desiccated at 105ºC until the weight remained constant. The Soxhlet apparatus was used to extract and estimate the fat content with petroleum ether. The Kjeldahl method was utilized to evaluate protein content (%N × 6.25). Carbohydrate content was calculated as a percentage by subtracting ash, fat, fiber, and protein amounts from the total dry weight. The moisture content was presented as a percentage of fresh weight and other contents as a percentage of dry weight.

Preparation of Extract

Fifty grams of ground seeds were extracted with 1 L of 70% ethanol at 2ºC±3ºC for 24 h with the assistance of a magnetic stirrer. Then, the resulting mixture was filtered using a Whatman No. 1 filter paper. A rotary evaporator was utilized to separate ethanol from the extract, followed by lyophilization to separate the remaining water. The specimen was stored at -20ºC for subsequent examination [27].

Chemical Characterization of Extract

Total phenolic content (TPCs) of BASEE was determined by the Folin-Ciocalteau method [28]. To 1 mL of the extract solution (1000 µg/mL), add 1 mL of 0.25 N Folin-Ciocalteu's reagent, which has been diluted with water at a ratio of 1:10 (V/V), 1 mL of Na_2CO_3 (1N), and 7 mL of distilled water. The tubes were vortexed and subsequently incubated at room temperature for 2 h.

The spectrophotometer measured the solution's absorption at 726 nm. The TPCs were expressed as gallic acid equivalents (GAE) in mg/g extract and calculated as follows: $y = 0.001x + 0.0563$ ($R^2 = 0.9792$), where y is absorbance and x is concentration of gallic acid.

To quantify phenolic compounds in BASEE, HPLC was utilized as described previously [29]. Phenolics were identified by HPLC-Agilent 1100 apparatus with a UV/Vis detector, and absorbance was recorded at 254 nm. C18 column (125 mm × 4.60 mm, 5 µm particle size) was used. Phenolic components were extracted utilizing a gradient mobile phase consisting of two solvents: solvent A (methyl alcohol) and solvent B (acetic acid) in water at a ratio of 1:25. Flow rate was regulated to 1.0 mL/min, and column temperature was consistently kept at 37ºC for the whole test duration.

Antioxidant Activity Estimation

The antioxidant activity of BASEE (100-500 µg mL^{-1}) was measured using the DPPH assay [30]. A 50 µL sample solution was added to 5 mL of 0.004% ethanolic DPPH and incubated for 30 min at room temperature in the dark. Absorbance was measured at 517 nm, and the antioxidant capacity of DPPH radicals (%) was calculated using the following formula:

$$\text{Inhibition (\%)} = [(\text{Abs control} - \text{Abs sample})/\text{abs control}] \times 100$$

DPPH assay (IC_{50}) values showed the concentration of compounds to scavenge 50% of DPPH.

Reducing power for BASEE (100-500 µg mL^{-1}) was estimated by measuring absorption of Perl's Prussian blue complex resulting from reducing Fe^{+3} to Fe^{+2} at 700 nm, following [31].

Antibacterial Activity Estimation

Antibacterial activity of BASEE (25-500 µg mL^{-1}) was assessed using the disk diffusion method toward *S. aureus* and *E. coli* [32]. The bacterial suspension was swabbed onto the surface of brain heart infusion agar (Oxoid) plates. Disks (6 mm) were soaked in various concentrations of BASEE. They were then placed onto an agar surface. Plates were placed in an incubator at 37ºC for 24 h, and the diameter of inhibition zones (mm) was estimated [33].

Antifungal Activity Estimation

The effect of BASEE (100-400 µg mL^{-1}) was examined on the growth rate of *Fusarium oxysporum* utilizing potato dextrose agar (PDA) medium. Plates were incubated at 25ºC. Diameters of colonies were measured on a regular basis until fungal growth completely covered the control of Petri plates. The subsequent equation was employed to compute linear growth reduction (LGR).

$$\text{LGR (\%)} = \text{CG-TG/CG} \times 100$$

Where LGR: linear growth reduction; CG: control growth; TG: treatment growth.

Scanning electron microscope (SEM) analysis of *Fusarium oxysporum* treated with BASEE (200 µg/mL) compared to a control for 4 h at 25ºC was conducted according to [34].

Anticancer Activity

MTT-assay

Cell viability was estimated utilizing 3-[4,5-dimethylthiazol]-2,5-diphenyltetrazolium bromide (MTT) assay to evaluate efficacy of BASEE (50-1000 µg mL^{-1}) against both normal (Vero) and cancer (A549 and HCT-116) cell lines, following the methods outlined before [35] and [36]. The cells were grown in DEME media with heat-inactivated fetal bovine serum (10%), penicillin (10 U mL^{-1}, Sigma-Aldrich), and streptomycin (10 µg mL^{-1}, Sigma-Aldrich). Cells (1.0×10^4) were incubated in sterile 96-well microplates. After incubation, the monolayer sheet of cells was separated, and the growth media was decanted. The cells were exposed to BASEE at various concentrations in a volume of 150 µl per well. The control was treated with an equal volume of saline. All plates were placed in a 5% CO_2 incubator and incubated at 37ºC for 48 h. After removing media, cells were washed with phosphate-buffered saline (PBS). Following this, 50 µL $well^{-1}$ of MTT solution (Sigma-Aldrich, 0.5 mg mL^{-1}) was added to the plates and incubated for 4-5 h. Subsequently, 50 µL per well of DMSO solution was added. The absorbance of each well was measured at 590 nm using an ELISA reader. The test was conducted in triplicate for all cells. The viability percentage was calculated as follows:

$$\text{Cell viability (\%)} = (\text{Ab sample/Ab control}) \times 100$$

The anti-cancer activity of bitter apple seed ethanolic extract was assessed by determining the IC_{50} values 48 hours after treatment.

Morphological Analysis

The impact of BASEE on cell morphology was examined using HCT116 cells as a model among the tested cell lines. The cells were initially seeded in 12-well plates with DMEM supplemented with 10% fetal bovine serum at a density of 5×10^5 cells/well, and then they were cultured for 48 h. After that, the media were aspirated, and cells were treated with varying concentrations (50-1000 µg mL^{-1}) of BASEE. Changes in cell morphology were documented using a normal inverted microscope (Nikon) at 200x magnification compared to untreated cells.

Using BASEE for Preserving Minced Beef

Raw beef was obtained from a local market in Zagazig, Sharkia Governorate, Egypt. Beef from the right thigh muscle was uniformly minced with a sanitized meat grinder. One hundred grams of minced beef samples were deposited in autoclaved polyethylene bags and mixed in a stomacher for 2 min at 300 rpm. All samples, except for the negative control, were treated with BASEE at 100 µg $mL^{-1}$and 200 µg mL^{-1}. The stomach bags containing the samples were wrapped and stored under aerobic conditions at 4ºC for 12 days. Chemical and microbiological analyses of both treated samples and controls were performed at designated intervals throughout the storage period (0-2 days). For microbiological analyses, total viable count (TVC) and psychrotrophic bacterial count (Psy) were measured every 3 d of preservation at 4ºC during the storage period, according to APHA [37]. Ten grams from each sample were placed in the stomacher bag with 90 mL of phosphate-buffered saline diluent at room temperature. Following homogenization of the samples for 60 seconds, serial two-fold dilutions were prepared. Total viable count was evaluated on nutrient Agar (NA) at 25ºC after 72 h, and Psy on NA at 7ºC after 10 d. Microbiological data were transformed into logarithms of the number of colonies forming units (CFU g^{-1}).

A comparison was made between untreated minced beef and minced beef supplemented with BASEE at 100 and 200 µg mL^{-1} to observe the conversion of myoglobin from its reduced form (oxymyoglobin) to its oxidized form (metmyoglobin). This estimation was carried out following the method described by Osman et al. [38].

Statistical Analysis

Data analysis was performed using SPSS Statistics for Windows, version 22 (IBM Corp., Armonk, NY, USA). Unless otherwise stated, a significance level of P≤0.05 was considered statistically significant.

Results and Discussion

Proximate Analysis

The findings in Fig. 1 present the percentage of moisture content and proximate composition (fat, protein, ash, fiber, and carbohydrates) for the bitter apple seeds. The bitter apple seeds' moisture content was 6.25%. Our findings align with previous research [39]. Based on a prior study [40], the moisture content of *Citrullus colocynthis* and *Citrullus vulgaris* seeds has been reported as 3.08% and 2.75%, respectively; values that are notably lower than those observed in the present study. The fat content of bitter apple seeds (18.36%) was lower than (23.16%) that studied [41]. The protein concentration was 11.99%. The literature reports a relatively higher protein content compared to the findings of the present study [39]. The percentage of fiber and ash is 6.45% and 9.57%, respectively.

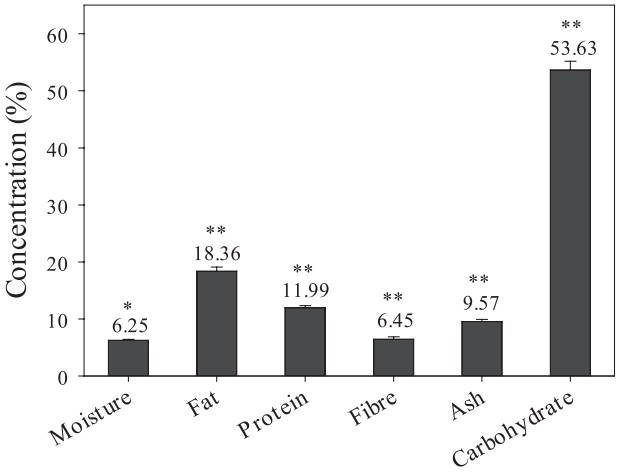

Fig. 1. Proximate analysis of bitter apple seeds. * The weight of the fresh sample was used to calculate the moisture content. ** The dry weight was used to calculate the content. Means±SD followed by different letters indicate significant differences based on Tukey's HSD test (P<0.05).

These findings are higher than the ones that [40] reported. Carbohydrates made up 53.63% of the bitter apple seeds' content.

Bitter Apple Seed Ethanolic Extract Characterization

Bitter apple seed ethanolic extract was prepared from bitter apple seeds, and data for extract yield and TPCs are presented in Table 1. The ethanolic extract yield was measured to be 15 g 100 g^{-1} of seeds. The TPC of BASEE was determined to be 32 mg GAE g^{-1} of dry extract. Our findings align with those reported [42]. According to [43], Yemeni bitter apple seeds have the highest TPC (6.1 g GAE 100 g^{-1} extract) compared to bitter apples obtained from Pune, India (3.2 g GAE 100 g^{-1} extract).

Ellagic acid, cinnamic acid, and gallic acid are the primary compounds found in the analyzed extract (Table 2). Phenolic component concentrations exhibited significant variation, ranging from 0.051 to 0.9 mg g^{-1} extract. According to results, gallic acid, syringic acid, chlorogenic acid, ellagic acid, pyrocatechol, vanillin, ferulic acid, daidzein, quercetin, naringenin, cinnamic acid, or hesperetin had 0.60±0.018, 0.230±0.002,

Table 1. Yield of extract (g), and TPCs (GAE g^{-1} dry extract)

Parameters	Concentration
Extraction yield	15 g 100g^{-1}
TPCs	32 mg GAE g^{-1} dry extract

Note: TPCs: total phenolic compounds.

Table 2. Major phenolic compounds concentration (mg g^{-1} dry extract).

Component	Concentration (mg/g)
Gallic acid	0.60±0.018
Chlorogenic acid	0.230±0.002
Syringic acid	0.370±0.007
Pyro catechol	0.260±0.002
Ellagic acid	0.900±0.027
Vanillin	0.050±0.001
Ferulic acid	0.051±0.001
Naringenin	0.085±0.001
Daidzein	0.059±0.001
Quercetin	0.130±0.002
Cinnamic acid	0.800±0.008
Hesperetin	0.073±0.001

Values are presented as mean±SD.

0.370±0.007, 0.260±0.002, 0.900±0.027, 0.050±0.001, 0.051±0.001, 0.085±0.00, 0.059±0.001, 0.130±0.002, 0.800±0.008, 0.073±0.001 mg g^{-1} extract, respectively. Our findings align with previous research [44].

Antioxidant Activity

Findings in Fig. 2 show the DPPH assay's percentage inhibition of bitter apple seed ethanolic extract's antioxidant activity. The ethanolic extract of bitter apple seed demonstrates antioxidant activity. As the concentration increases, the ethanolic extract of bitter apple seed exhibits increased antioxidant activity. As BASEE concentration increases from 100 to 500 µg mL^{-1}, the ability of the extract to scavenge DPPH radicals correspondingly improves from 32.09% to 86.96%.

The FRAP of the bitter apple seed ethanolic extract was measured using various concentrations (100-500 µg mL^{-1}). An increase in absorbance indicates an elevation in ferric-reducing activity. Findings in Fig. 3 display the outcomes of the FRAP analysis conducted on the ethanolic extract of bitter apple seeds. The results demonstrate that all tested concentrations exhibited a substantial impact on lowering the ferric ion in a manner that is dependent on the concentration. At 100 µg mL^{-1}, the absorbance value of 1.3 was lowest, but at 500 µg mL^{-1}, the highest value of 2.31 was recorded. Bitter apple seed ethanolic extract recorded an IC_{50} value of 192 µg mL^{-1}. Antioxidant properties of bitter apple were examined by analyzing its ethanolic extract. It was determined that gallic acid, a phenolic compound, is responsible for its strong free radical scavenging activity [45]. Bioactive compounds' abundance, specifically phenolics, in bitter apples is responsible for their notable antioxidant ability [46].

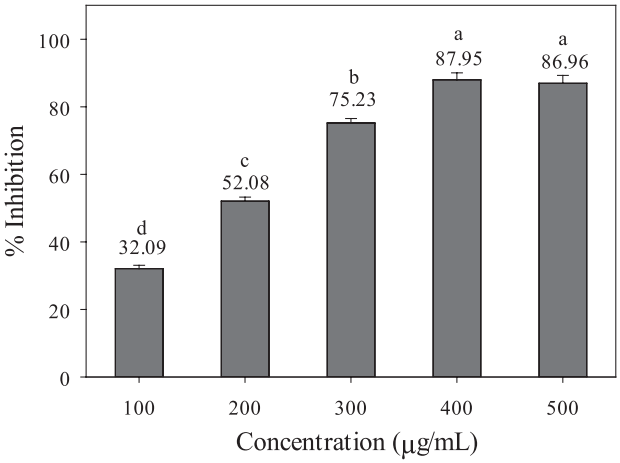

Fig. 2. Antioxidant activity (% inhibition) of bitter apple ethanolic extract at different concentrations (100-500 µg mL^{-1}). Means±SD followed by different letters indicate significant differences based on Tukey's HSD test ($P<0.05$).

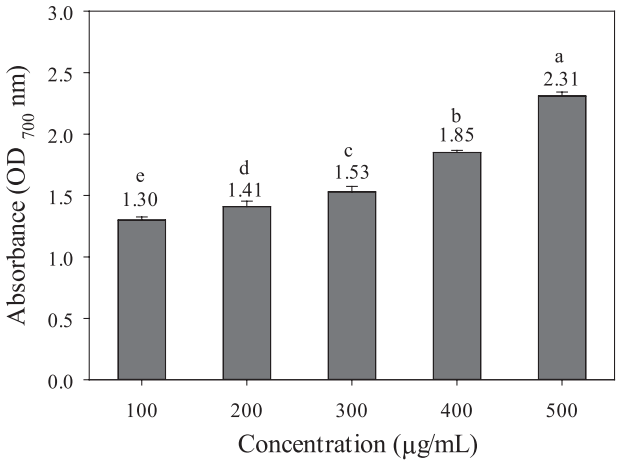

Fig. 3. Ferric reducing antioxidant power (FRAP) of bitter apple ethanolic extract at different concentrations (100-500 µg mL^{-1}). Means±SD followed by different letters indicate significant differences based on Tukey's HSD test ($P<0.05$).

Phytochemical screening of bitter apple extracts has revealed the presence of natural compounds that have good antioxidant properties [22]. Bitter apple seed ethanolic extract has antioxidant activity, with maximal percentage inhibitions of 79.4% and 72.4%, respectively [47], which is consistent with our results. *In vitro* antioxidant studies revealed that the ethanolic extract of bitter apple fruits had a maximum percentage inhibition of DPPH radicals of 62% at 800 µg mL^{-1} [48].

Antibacterial Activity

Antibacterial activity results for BASEE are depicted in Fig. 4. Bitter apple seed ethanolic extract demonstrated activity against the tested bacteria, depending on the concentration. At 25 and 50 µg mL^{-1}, no antibacterial

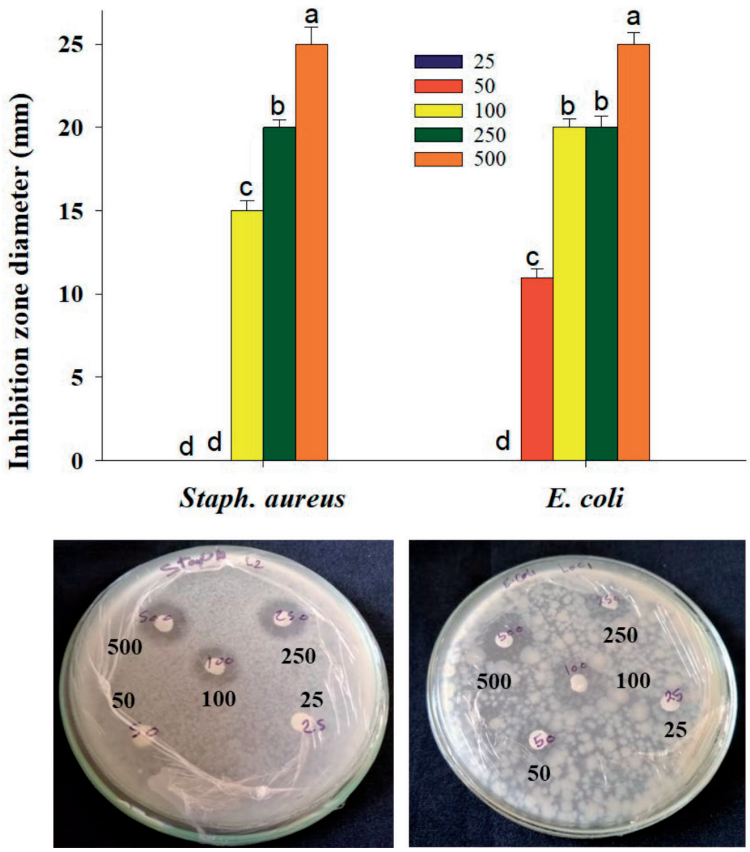

Fig. 4. Antibacterial activity of bitter apple seed ethanolic extract against *Staph. aureus* and *E. coli* at 25, 50, 100, 250, and 500 µg mL^{-1}. Means±SD followed by different letters indicate significant differences based on Tukey's HSD test (P<0.05).

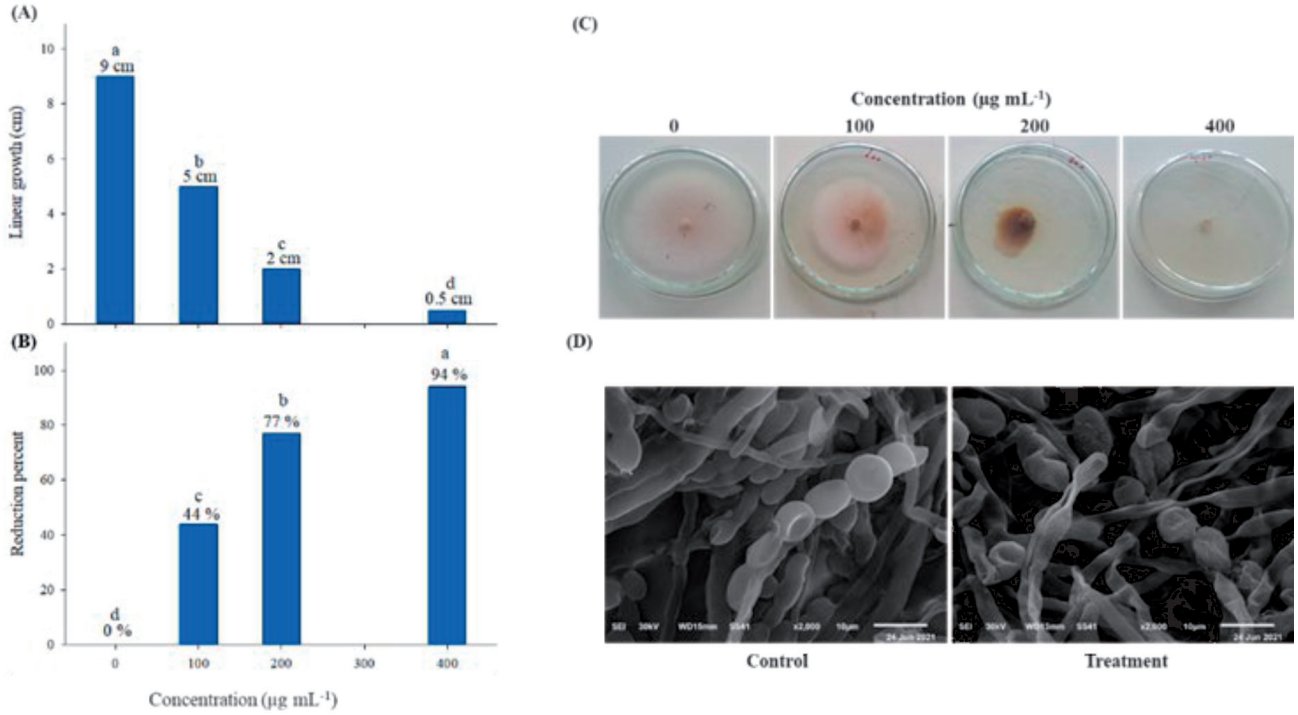

Fig. 5. a) Linear growth, b) growth reduction percent, and c) fungal growth on solid medium of *Fusarium oxysporum* as subjected to bitter apple ethanolic extract at different concentrations (0, 100, 200, and 400 µg mL^{-1}). d) Scanning electron microscopy of Fusarium oxysporum as subjected to bitter apple ethanolic extract (200 µg/mL) compared to control (0 µg mL^{-1}). For a) Means±SD followed by different letters, indicate significant differences based on Tukey's HSD test (P<0.05).

effect was noticed against *Staphy. aureus*. At 100, 250, and 500 µg mL^{-1}, inhibition zones measured 15, 20, and 25 mm, respectively. At 25 µg mL^{-1}, no antibacterial effect was observed against *E. coli*. At 50, 100, 250, and 500 µg mL^{-1}, inhibition zones measured 11, 20, 20, and 25 mm, respectively. No significant difference was observed between the inhibition zones at 100 µg mL^{-1} and 250 µg mL^{-1}. Numerous investigations have demonstrated that bitter apple extracts prepared from different parts are effective against *E. coli*, *P. aeruginosa*, *Staph. aureus*, and *Enterococcus faecalis* [49]. Different extracts of bitter apple, prepared using various solvents, were examined for their ability to fight harmful bacteria, such as *Salmonella*, S. *aureus, Bacillus* spp., *Proteus vulgaris*, and *Pseudomonas* spp. The findings indicated that most of the extracts had minimum inhibitor concentrations (MICs) ranging from 20 to 100 µg/mL toward all tested bacteria [50-53].

Antifungal Activity

The antifungal activity of BASEE was assessed at 100, 200, and 400 µg mL^{-1} compared to the control against *F. oxysporum*. The data is presented as linear growth (Fig. 5a)), linear growth reduction (Fig. 5b)), and fungal growth in a solid medium (Fig. 5c)). The bitter apple ethanolic extract's inhibitory effect on *F. oxysporum* demonstrated dose-dependent activity. A 400 µg mL^{-1} concentration of bitter apple ethanolic extract strongly inhibited *F. oxysporum* mycelial growth, with a 94% reduction in development and linear growth of 0.5 cm. The maximum growth reduction was observed at 400 µg mL^{-1} (94%), followed by 77% at 200 µg mL^{-1}, and 44% at 100 µg mL^{-1}.

Morphological changes in *F. oxysporum* grown treated with bitter apple ethanolic extract (200 µg/mL) were examined by SEM, and the significant changes are presented in Fig. 5d). The hyphae in *F. oxysporum* grown on PDA with 200 µg mL^{-1} of bitter apple ethanolic extract had very different shapes from those in the control group when examined with an SEM. Previous research [49] also found that water and acetone extracts can kill *Candida*. Bitter apple ethanolic extract was tested on various fungal species *in vitro* and showed promising results against all strains. The extract's effectiveness increased with higher concentrations. The findings indicated that bitter apple extracts from various parts were all effective in inhibiting the growth of fungal strains [50, 54].

Anticancer Activity

The MTT assay showed that the ethanolic extract of bitter apple seeds stopped the growth of cancer cells (HCT116 and A549) in a way that depended on the concentration, which meant that fewer cells were alive than when cells were not treated. Findings in Fig. 6 show that treating HCT116 cells with bitter apple seed ethanolic extract for 48 h stopped their growth, with IC_{50} values of 55 µg mL^{-1} for HCT116 cells or 100 µg mL^{-1} for A549 cells. However, the IC_{50} values were significantly higher than those of Vero cells (1000 µg mL^{-1}). The results indicate that the HCT116 cells showed greater sensitivity to the bitter apple seed ethanolic extract compared to the A549 cells and normal cells (Vero). The effect of bitter apple seed ethanolic extract on cell morphology was investigated using the HCT116 cell line, with the results presented in Fig. 7. The ethanolic extract from bitter apple seeds inhibited the growth of HCT116 cells in a dose-dependent manner,

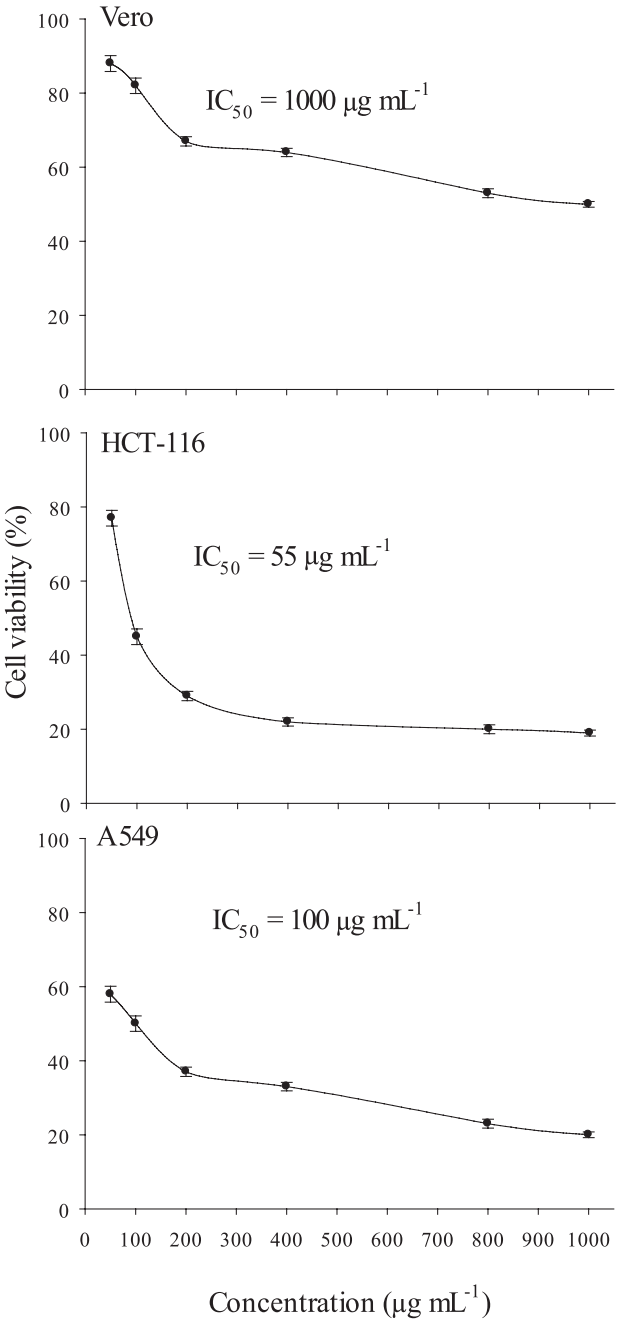

Fig. 6. Effect of bitter apple seed ethanolic extract on the cell viability of the human normal (Vero) cells, human cancer (HCT116), and A549 cell lines after treatment for 48 h compared to untreated cells.

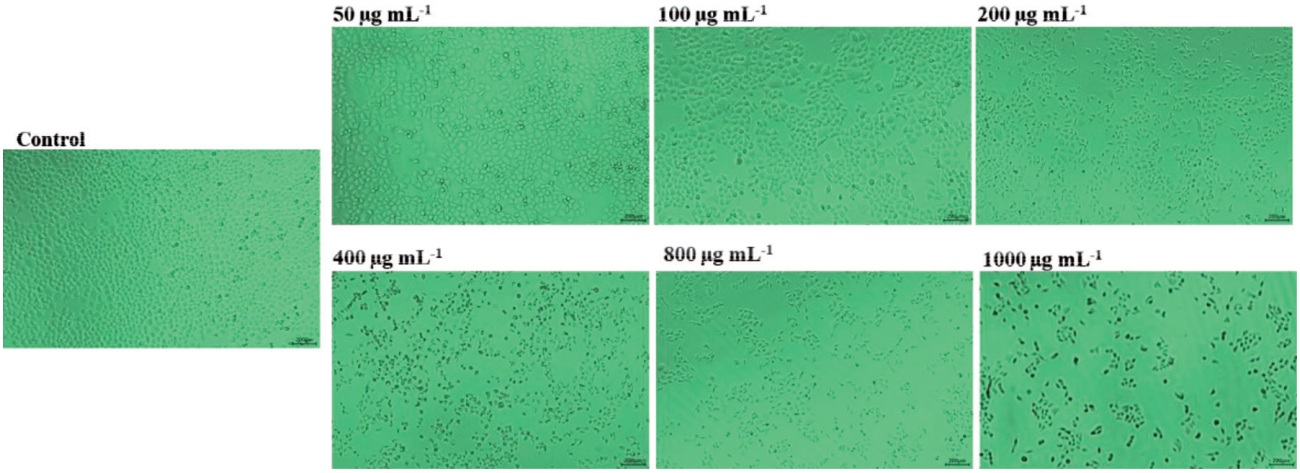

Fig. 7. Morphological alterations of HCT116 cells treated with bitter apple seed ethanolic extract (50, 100, 200, 400, 800, and 1000 µg/mL) compared to control (untreated cells) as observed under normal inverted microscope at 200x magnification.

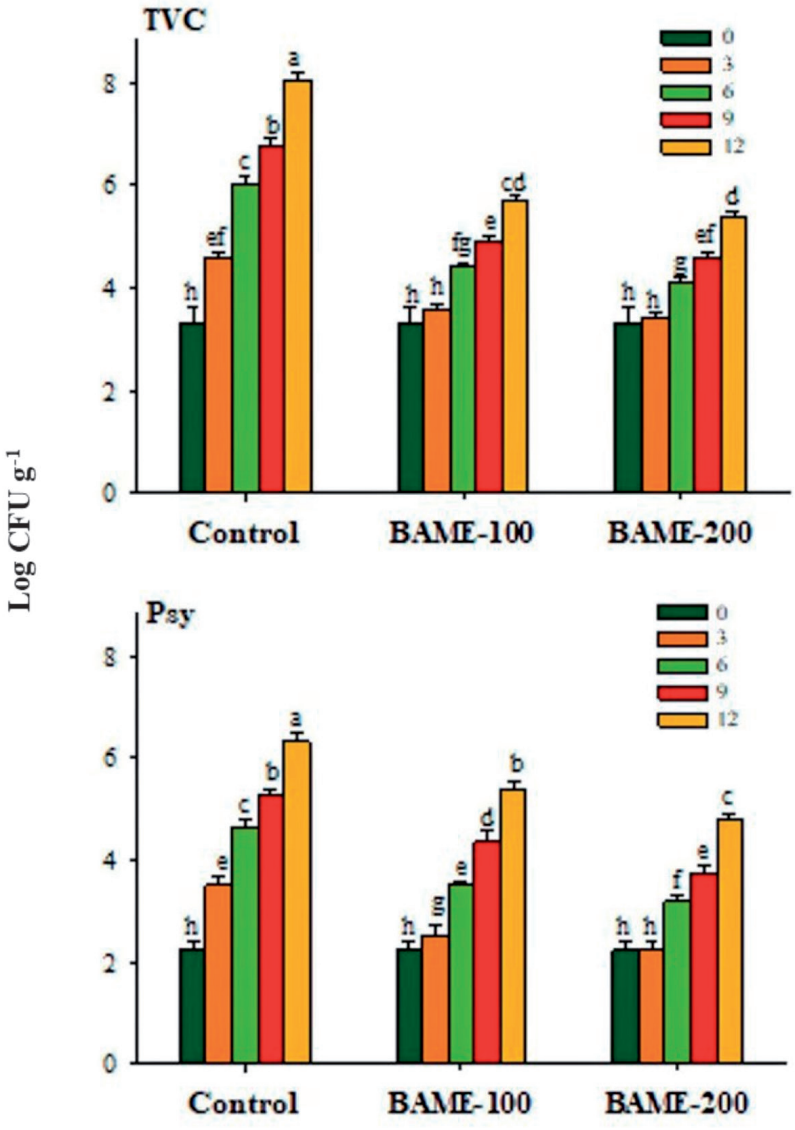

Fig. 8. Total viable count (TVC) and psychrotrophic bacterial count (Psy) in minced beef were measured during a 12-day storage period at 4ºC. The minced beef was supplemented with bitter apple ethanolic extract (BASEE) at different levels (100 and 200 µg g^{-1}). Means±SD followed by different letters indicate significant differences based on Tukey's HSD test (P<0.05).

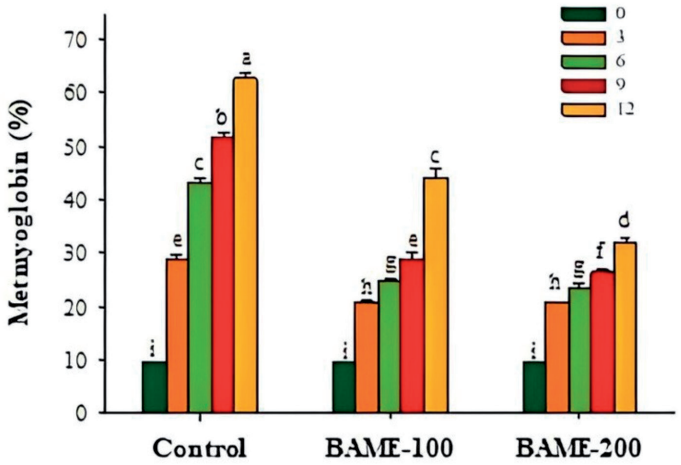

Fig. 9. Metmyoglobin (%) in minced beef was measured during a 12-day storage period at 4ºC. The minced beef was supplemented with bitter apple ethanolic extract (BASEE) at different levels (100 and 200 µg g^{-1}). Means±SD followed by different letters indicate significant differences based on Tukey's HSD test (P<0.05).

leading to noticeable changes in cell morphology. The study findings align with previous research [55], which indicates that the ethanol extract of bitter apple exhibited considerable suppression of carrageenan and serotonin, with the greatest inhibition observed in prostaglandin E1-induced paw edema. Bitter apple anticancer activity can be due to diverse mechanisms and characteristics. These encompass apoptotic pathways, antioxidant or anti-inflammatory properties, suppression of the Wnt/ß-catenin signaling pathway, and anti-metastatic properties [56-58].

Using BASEE for Preserving Minced Beef

Total viable count and Psy counts were estimated in minced beef over 12 d of storage at 4ºC after supplementation with BASEE at two levels (100 and 200 µg g^{-1}). The data is presented in Fig. 8. Generally, it was observed that both TVC and Psy counts increased over time in both control and treated samples. However, BASEE supplementation caused concentration-dependent reductions in cell counts at all storage time points. The untreated sample reached a TVC of 8.03±0.152 log CFU g^{-1} after 12 d of cold storage at 4ºC, exceeding the microbial specification of 7 log CFU g^{-1} for fresh meat [59]. The samples treated with 100 and 200 µg g^{-1} BASEE showed significantly lower TVC (5.70±0.1 and 5.36±0.115, respectively), corresponding to reductions of 29% and 33.25% compared to the control, respectively. The study findings align with previous research [60] that indicates TVC in minced beef supplemented with carrot peel extract at 40 and 100 g mL^{-1} decreased by 26.2 and 37.6% of the control, respectively.

The psy count in control samples increased from 2.23±0.152 to 6.33±0.158 log CFU g^{-1} after 12 storage days. The meat samples supplemented with 100 and 200 µg g^{-1} BASEE registered only 5.40±0.173 and 4.8±0.1, respectively. The higher preservation action of BASEE is likely due to its superior antibacterial and antioxidant properties. In conclusion, BASEE can be effectively used as a natural preservative and a better alternative to synthetic compounds.

The data in Fig. 9 indicate that the level of metmyoglobin increased in the control samples as storage time increased, suggesting a faster oxidation process. However, adding BASEE to the minced beef slowed down this process, and the extent of the slowdown depended on the concentration of BASEE. The untreated sample reached 62% metmyoglobin after 12 d of cold storage at 4ºC. The samples treated with 100 and 200 µg g^{-1} BASEE showed significantly lower metmyoglobin levels (44% and 32%, respectively), corresponding to reductions of 29 % and 48 %, respectively, compared to the control. The study findings align with previous research [38], working on minced beef supplemented with chickpea legumin.

Conclusions

In conclusion, the bitter apple seed ethanolic extract (BASEE) demonstrated significant antioxidant, antibacterial, antifungal, and anticancer properties. Rich in phenolic compounds such as ellagic, cinnamic, and gallic acids, BASEE effectively inhibited DPPH free radicals and showed concentration-dependent antimicrobial activity against *S. aureus*, *E. coli*, and *F. oxysporum*. Cytotoxicity assays revealed selective anticancer effects, particularly against HCT116 cells, while displaying minimal toxicity to normal Vero cells. Additionally, BASEE proved effective in preserving minced beef, suggesting its potential as a natural preservative. Overall, these findings support the use of BASEE as a promising alternative to synthetic additives in food preservation and biomedical applications,

due to its multifunctional bioactive properties and safety profile.

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Conflict of Interest

The authors declare no conflict of interest.

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