Hindawi
Journal of Chemistry
Volume 2020, Article ID 7394751, 10 pages
https://doi.org/10.1155/2020/7394751

Research Article

Antioxidant, Antibacterial, and Anticancer Activities of Bitter Gourd Fruit Extracts at Three Different Cultivation Stages

Syed Ali Raza Naqvi,[1] **Shafaqat Ali**,[2,3] **Tauqir A. Sherazi**,[4] **Atta-Ul Haq**,[1] **Muhammad Saeed**,[1] **Muhammad Sulman**,[1] **Muhammad Rizwan**,[2] **Saad Alkahtani**,[5] **and Mohamed M. Abdel-Daim**[5,6]

[1]*Department of Chemistry, Government College University, Faisalabad 38000, Pakistan*
[2]*Department of Environmental Sciences, Government College University, Faisalabad 38000, Pakistan*
[3]*Department of Biological Sciences and Technology, China Medical University, Taichung 40402, Taiwan*
[4]*Department of Chemistry, COMSAT University Islamabad, Abbottabad Campus, Abbottabad, Pakistan*
[5]*Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia*
[6]*Pharmacology Department, Faculty of Veterinary Medicine, Suez Canal University, Ismailia 41522, Egypt*

Correspondence should be addressed to Syed Ali Raza Naqvi; drarnaqvi@gmail.com and Shafaqat Ali; shafaqataligill@gcuf.edu.pk

Received 2 December 2019; Revised 3 March 2020; Accepted 24 March 2020; Published 23 April 2020

Academic Editor: Angela Cardinali

In this study, we are presenting the effect of three ripening stages of air-dried bitter gourd fruit extracts on phenolic acid composition, antioxidant, antibacterial, and anticancer activities. The results showed mature bitter gourd fruit extract in 100% methanol showing 78% DPPHo scavenging activity. Immature dried fruit extract in 80% and 100% methanol showed promising antibacterial activities, i.e., >18.5 ± 0.21 mm zone-of-inhibition against *Staphylococcus aureus*, while mature dried fruit extract in 80% methanol showed 18.4 ± 0.17 mm zone-of-inhibition against *Escherichia coli*. Anticancer activity results of 100% methanol extracts of ripened fruit possess showed 6.72 ± 1.81 and 3.55 ± 0.51 mg/mL IC_{50} values with HeLa and MDBK cancer cell lines, respectively. The overall results indicate that the immature and ripen fruits of BG could be extracted in pure methanol as an antibacterial and anticancer phytomedicine.

1. Introduction

Infections and cancers are two major human diseases across the world. Recently (December 2019), novel corona virus infection (COVID-19) created graveyard threat to all nations over Earth. In a short span (~3 months) of time about 200 countries victimized with COVID-19. The most advanced states, especially USA and Italy remained fail to cope COVID-19 infection and thousands of deaths were reported in a few days. World Health Organization (WHO) in this scenario declared international emergency. Bacterial infection is another serious threat to humanity as bacterial resistance to antibiotics growing continuously. Cancer is another leading cause of deaths as the oncology field bears limited effective therapeutic strategies.

Antibiotics are the main weapons being used to fight against bacterial infection. The choice of antibiotics, however, decreased due to resistance factor which increased the threat of severe consequences [1]. Infections are estimated to contribute to 20% of all human tumors [2]. It was observed that the patients with bronchogenic carcinomas have active tuberculosis more frequently than the general population. Despite the direct effect of pathogenic organisms which is most probably misstep, the induction of inflammation and production of mutagenic compounds by bacterial metabolism are the two important reasons. Noncardia gastric carcinoma and colon carcinoma are common examples of the bacterium origin cancers [1]. Bacterial infection has attributed about 1.2 million cancer cases per year [3].

Natural products and phytochemicals are gaining the attention in global emergence of bacterial resistance and increasing cases of oxidative stress diseases scenario [4, 5]. Phytomedicines carry multiple advantages over infectious and cancerous chemotherapeutic drugs which includes less side effects and nonresistive by bacteria. Plants have long been investigated for new therapeutic agents. Over 80% of the world population and 90% of the African population rely on medicinal plant based therapy [6]. Similarly, pharmaceutical industry during the period of 1981-2002 developed about 61% new natural product based drugs that were highly effective against infectious and cancerous diseases [7]. As reported by WHO, medicinal plants would be the best and cheap source of obtaining the anti-infection and anticancer agents [8]. Therefore, screening of the flora for natural products and phytochemicals to suppress and eradicate infection and malignant diseases is one of the primary focuses.

Bitter gourd (BG) is a wild variety of *Momordica charantia* belonging to family *Cucurbitaceae*, consumed both as vegetable and folk medicines. Many biologically active compounds have been identified in *Cucurbitaceae* family including phenolics, steroidal glucosides, alkaloids [9], conjugated linolenic acid isomers [10], lysophosphatidylcholines [11], organosulfur compounds, and cucurbitane-type triterpenoids [12, 13]. Certain common diseases such as diabetic mellitus, hypoglycemia, heart disease, HIV (human immunodeficiency virus), cancer, and microbial infections have been investigated to treat with phytochemical fractions and compounds isolated from gourd family [14, 15].

The aim of presented study was to investigate the antioxidant, antibacterial, and anticancer activities of BG fruit extracts at three different cultivation stages for taking the maximum advantage of BG in functional food and phytomedicine practice.

2. Materials and Methods

2.1. Materials. The bacterial strains of *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*), *Pasteurella multocida* (*P. multocida*), and *Escherichia coli* (*E. coli*) were obtained from University of Health Sciences (Lahore, Pakistan). University of Veterinary and Animal Sciences (Lahore, Pakistan) provided MDBK cancer cell line. The bacteria were cultured in nutrient broth agar (13 g/L distilled water, Oxoid UK). The MDBK cell line was cultured in glasco-modified-eagle medium (GMEM) supplemented with heat-inactivated fetal bovine serum (FBS, 10%, Gibco), penicillin (100 units/mL) and streptomycin (100 μg/mL), and CO_2 (5%) in humidified atmosphere at 37°C. DPPH free radical (DPPH^{o}), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (DMT-DPTB) and rifampicin was purchased from Sigma-Aldrich. All reagents and chemicals were of analytical grade.

2.2. Preparation of Extracts. Fresh bitter gourds were plucked from the fields located around the Faisalabad, Pakistan, at three different stages, that is, immature stage (light green, 8–12-day fruit age, 4–6 cm in length with soft white seeds), mature stage (full green, 16–22-day fruit age, 10–14 cm in length with yellowish white seeds), and ripen stage (full yellow, 30-day-fruit age, 10–14 cm in length with red seeds) (Figure 1). Fruits were properly washed with distilled water, dried under shade with mechanical air blowing, chopped, and then ground to fine powder having 80–150 mesh size. The phytochemicals from BG fruit powder were extracted in 60%, 80%, and 100% methanol solvent using 1:10 (w/v) powder to solvent ratio at room temperature (RT) using orbital shaker (220 rpm) for 24 h. The extraction mixture was then filtered using filter paper (Whatman No.1). The filtrate was concentrated with rotary evaporator till thick paste at 65°C followed by refrigerating at 4°C for further analysis. Percent yield of the extracts was calculated using the following equation;

$$\text{Yield}\,(\%) = \frac{\text{Weight of solvent free extract}\,(g) \times 100}{\text{Dried extract weight}\,(g)}. \quad (1)$$

2.3. Determination of Total Phenolics. TPC in BG extracts were investigated with Folin-Ciocalteu reagent (FCR) assay [16]. The assay was carried out by adding 0.5 mL FCR to 50 mg dry extract followed by diluting the mixture with 7.5 mL deionized water (DW), vigorously shaking, and incubation at RT for 10 min. Then after adding 1.5 mL sodium carbonate solution (20 %), the mixture was heated for 20 min at 40°C followed by ice-bath cooling. The absorbance of the cooled solution was than measured at 755 nm. The results are presented as mg gallic acid equivalent (GAE) per g dry matter.

2.4. Determination of Antioxidant Potential. Antioxidant potential of bitter gourd was studied from the different solvent extracts using DPPH^{o} scavenging, reducing power, linoleic acid, and ferric reducing power assays.

2.4.1. DPPH Free Radical Scavenging Activity. DPPH^{o} scavenging assay was performed following the protocol described by Iqbal and coworkers [17]. Briefly, freshly prepared 5 mL methanol solution of DPPH free radical (0.025 g/L) was mixed with 1 mL ethanol solution of extract (25 μg/mL). The contents were then vortexed for 1 min, allowed to stand at RT for 20 min, and then recorded the absorbance at 510 nm. DPPH^{o} scavenging activity was calculated with the following expression.

Percent inhibition of DPPH^{o} = $(1 - As/A_c) \times 100$.

A_s = absorbance of sample.

A_c = absorbance of control.

2.4.2. Determination of Reducing Power Activity. The reducing power of bitter gourd extracts was determined using procedure described by Iqbal and coworkers with slight modification [17]. Methanol extract (0, 10, 20, 30, 40, or 50 mg) was added to 5 mL of sodium-phosphate buffer (pH 6.6). Then 5 mL aqueous solution of potassium-ferricyanide

Figure 1: Different growth levels of BG: (a) immature stage, (b) mature stage, and (c) ripen stage.

(1%) was added and the mixture was heated at 50°C for 20 min. Then, 5 mL aqueous solution of trichloroacetic acid (10%) was added to the mixture, centrifuged at 1 krpm for 10 min at 4°C. First supernatant layer was separated and diluted with 5 mL DW, and then 1 mL ferric chloride solution (0.1%) was added for recording absorbance at 700 nm. The experiment was repeated in triplicate and results were expressed mean absorbance.

2.4.3. Percent Inhibition of Linoleic Acid Peroxidation. Inhibition of linoleic acid peroxidation (LAP) chemical test reported previously was followed to conduct antioxidant activity of methanol extracts [18]. Briefly, to 5 mg dry BG extract subsequent addition of linoleic acid (0.13 mL), absolute ethanol (10 mL), and sodium-phosphate buffer (10 mL, 0.2 M, pH 7.2) was done. The solution mixture was diluted up to 25 mL with distilled water followed by incubation at 37°C for 172 h. The incubated solution mixture (0.2 mL) and 0.2 mL ferrous chloride solution ($FeCl_2$) (20 mM in 3.5 % HCl) were then added into 10 mL ethanol (75%), mixed sequentially and stirred for 3 min and finally recorded the absorbance at 500 nm. A solution without sample used as negative control and with BHT was taken as standard. The percent inhibition of linoleic acid peroxidation was calculated by the following equation.

Inhibition of LAP (%) = [(Ac/As)] × 100.

A_c = absorbance of control.

A_s = absorbance of sample.

2.4.4. Ferric Reducing Antioxidant Power (FRAP). FRAP assay was carried out as described earlier [19]. Briefly, FRAP reagent was prepared by preparing three different solutions and then mixing them with specific ratio: solution 1: acetate buffer (pH 6; a mixture of sodium acetate (3.1 g) and acetic acid per 20 mL per liter, solution 2: TPTZ (10 mM) in HCL (40 mM), and solution 3: $FeCl_3$ solution (20 mM). These three solutions were mixed at 10 : 1:1 (v/v/v) and the mixture was incubated at 37°C for 10 min. Then to the 1 mL of methanol samples (0, 1, 2, 3, 4, or 5 mg/mL), 1.0 mL FRAP reagent was added to test tube, incubated for 30 min at 25°C followed by recording the absorbance at 593 nm. The results were expressed in terms of mean absorbance (directly related to antioxidant potential) of triplicate values ± standard deviation.

2.5. Determination of Antibacterial Activities. Antibacterial activities were evaluated by using disc diffusion method reported in literature [20]. Nutrient agar 13 g/L was suspended homogenously in distilled water and sterilized by autoclaving followed by the addition of 100 μL/100 mL of the inoculums in sterilized culture medium. This medium was then transferred to sterilized Petri plate and allowed to solidify. Small filter paper discs of about 1.5 mm diameter were placed on an aluminum foil and 100 μL of the BGE sample (0.5 mg/μL in DMSO) was dropped on them. These discs were allowed to dry and then laid flat on growth medium in Petri plates. The Petri plates were then incubated at 37°C for 24 h. At the end of incubation period zone-of-inhibition was measured with the help of zone reader to assess antibacterial potential. The results were compared with antimicrobial agent rifampicin, taken as standard.

2.6. In Vitro Anticancer Activities. The *in vitro* anticancer activity was performed with HeLa cancer cell line belonging to human cervical cancer and Madin-Darby bovine kidney (MDBK) cancer cell line belonging to animal cancer following the procedure described by Mosmann [21]. The extracts were diluted at five different concentrations, that is, 0.312 mg/ml, 0.625 mg/mL, 1.25 mg/mL, 2.5 mg/mL, and 5 mg/mL, in labeled wells; already having 2000 cancer cells/well. DMEM, supplemented with 5% FBS as a cell line growth medium was incubated at 37°C under 5% CO_2 pressure for 48 h. Following the incubation, 20 μL of DMT-DPTB stock solution (5 mg/mL) was added to each well and further incubated for 3 h to form purple formazan crystals. The crystals were then dissolved in DMSO (100 μL/well) to record OD value at 570 nm with reference cisplatin (control) using the following expression:

$$\text{Percentage inhibition (\%)} = [(\text{ODcontrol} - \text{ODsample/ODcontrol})] \times 100. \quad (2)$$

2.7. Statistical Analysis. The experiments were conducted in three replicates and the generated data was analyzed using CoSTAT V 6.3 software (Cohort software, Berkeley, California, USA).

3. Results and Discussion

3.1. Extraction Yield and Total Phenolics. Extraction of air-dried BG fruit at three ripening stages was performed in methanol using three different concentrations (60, 80, and 100%). The extraction process was carried out under controlled set of mechanical agitation, humidity, pressure, and temperature conditions. Extraction yield of bitter gourd fruits at different stages with different methanol concentrations is shown in Table 1. The immature fruit contained slightly higher % yield as compared to the mature and ripen

Table 1: Extract yield and total PC of different percent methanol extract of BG fruit at different growth stages.

Methanol (%)	Immature fruit		Mature fruit		Ripen fruit	
	%yield	%TPC	%yield	%TPC	%yield	%TPC
100	9.81 ± 0.78^{a}	4.33 ± 0.24^{a}	9.83 ± 0.43^{a}	8.17 ± 0.13^{a}	7.17 ± 0.53^{a}	5.09 ± 0.10^{a}
80	14.06 ± 0.65^{b}	2.18 ± 0.12^{b}	12.66 ± 0.98^{b}	7.46 ± 0.18^{b}	9.46 ± 0.77^{b}	4.11 ± 0.09^{b}
60	15.98 ± 1.15^{c}	1.91 ± 0.11^{c}	15.23 ± 1.25^{c}	5.36 ± 0.12^{c}	13.62 ± 0.89^{c}	3.69 ± 0.15^{c}

Values with the same letters in superscript do not differ significantly; values are significant at level $P < 0.001$.

fruits. The amount of total PC (TPC) expressed as percent weight of dried extract ranged from $1.91 \pm 0.11\%$ to $8.17 \pm 0.13\%$ (Table 1). The lowest TPC was noted for immature BG fruit extract in 60% methanol while the highest TPC was found for mature fruit extract in 100% methanol. A significant difference between %yield of extracts and %TPC of immature BG fruit extract indicated that immature fruit contained more nonphenolic compounds which gradually metabolized to secondary metabolites. However, after certain level of maturity variety of other compounds also formed which contribute in biological activities.

3.2. Scavenging of DPPH Free Radicals. The antioxidant activity of BG fruit extract was assessed using DPPH^{o} as it shows neutral behavior with superoxide anion and hydroxyl radicals [22]. Our results showed the highest free radical scavenging potential $78.12 \pm 2.64\%$ in case of mature BG fruit extracts in 100% methanol. Both immature and ripen fruit extracts showed less than 60% radical scavenging potential. The results are shown in Figure 2(a). Previously, antioxidant activity of different parts of BG extracts in water was reported showing leaf, stem, mature fruit, and ripen fruit able to scavenge 83%, 80%, 81%, and 53% DPPH free radical [23]. Rezaeizadeh et al. reported 80% DPPH^{o} scavenging action of methanol extract of whole fruit including seeds at concentration of 500 μg/mL [24]. When compared to BHT, known to be the strong synthetic antioxidant, $79.13 \pm 2.43\%$ free radical scavenging activity was found which was slightly greater than 100% methanol extract of mature BG fruit ($78.12 \pm 2.64\%$). Not only did the higher value of DPPH^{o} scavenging of fruit extract attribute to PC but also other compounds also effect the antioxidant activity which is commonly known as synergism.

3.3. Percent Inhibition of Linoleic Acid Peroxidation (LAP). Oxidation of lipids, carried out by ROS, paved the way to pathogens to initiate infectious diseases. Generally, ROS attack polyunsaturated fatty acid chains of cell membrane which initiates self-propagated chain reaction. Results of inhibition potential of LAP by different stages of BG fruit are shown in Figure 2(b). Extraction in 60% methanol solution showed poor inhibition of LAP (21%–31%), while 80% methanol solution extracted good level of antioxidants from BG fruit. The promising extraction was carried out by 100% methanol solvent. This was reflected by favorable inhibition of LAP (34% to 57%). Inhibition of linoleic acid peroxidation largely shown by mature stage of BG fruit ($56.78 \pm 1.64\%$) when extracted in 100% methanol solvent-other stages, that is, immature stage, showed inhibition of LAP showed $34.21 \pm 3.52\%$ while ripen stage showed $49.56 \pm 1.23\%$. The standard antioxidant compound (BHT) showed $72.45 \pm 2.66\%$ inhibition of LAP which is more than crude fruit extract. Butylated hydroxyl toluene is the most widely used synthetic antioxidant but it has been restricted to use as an antioxidant because of its carcinogenic potential [24, 25].

3.4. Reducing Power Activity. Published researches have demonstrated that the electron donating potential of naturally extracted compounds is associated mainly with its reducing power [26]. Figures 3(a)–3(c) demonstrate the reducing power of different BG fruit extracts. In general results showed concentration dependent reducing power ability which directly related to absorbance. The highest reducing power (absorbance = 1.05) was noted with 100% methanol extract sample followed by mature BG fruit extract sample (absorbance = 0.97) extracted in 80% methanol solution. Ripen fruit extracts also showed satisfactory reducing ability; extraction in 100% methanol showed absorbance 0.69 while immature fruit showed poor reductive ability. The reducing power of standard ascorbic acid showed 1.8 absorbance at 700 nm which was higher than all extracts. The assay is based on the ability of natural extract to change the yellow color of the test solution to various shades of green and blue depending on reduction of $Fe3^{+}$ to $Fe2^{+}$ which is then compared to reduction ability of ascorbic acid, which is known as best reducing agent.

3.5. Ferric Reducing Antioxidant Power. The FRAP assay is commonly used for the antioxidant studies of plant extracts which is based on the electron-transfer reaction. The mechanism is carried out as the colorless [$Fe3^{+}$]-TPTZ accepts an electron from antioxidants to generate the colored [$Fe2^{+}$]-TPTZ adduct. The absorbance of visible light directly related to the electron donating potential of antioxidants. The ferric reducing antioxidant power of immature, mature, and ripen BG fruit extracts in 60%, 80%, and 100% was measured and the results are shown in Figures 3(d)–3(f). Generally, the FRAP activity of all samples was found concentration dependent. The maximum absorbance of sample solutions was noted with 5 mg extract at 593 nm. Mature BG fruit extract in 100% methanol showed 0.73 absorbance whereas 80% and 60% methanol extract showed absorbance of 0.67 and 0.53, respectively. Immature and ripen fruit extracts, however, showed comparatively low absorbance. It was also noted that pure methanol extract appeared to be more active than 80% and 60% methanol extracts.

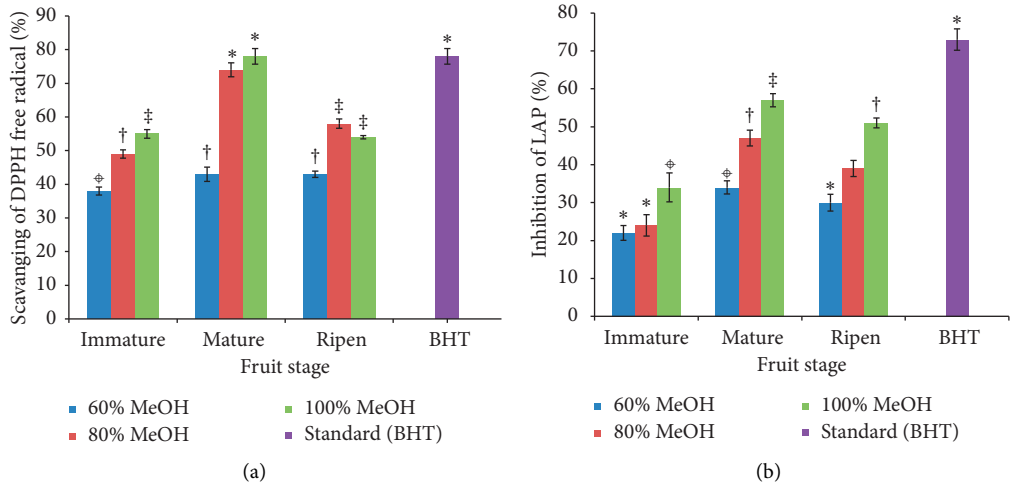


Figure 2: Antioxidant activities of immature, mature, and ripen BG extracts in 60%, 80%, and 100% methanol solvents on DPPH free radicals (a) and linoleic acid peroxidation (b). Each experiment was conducted in triplicate; similar signs show nonsignificant difference while nonsimilar signs show significant difference at level $P < 0.001$.

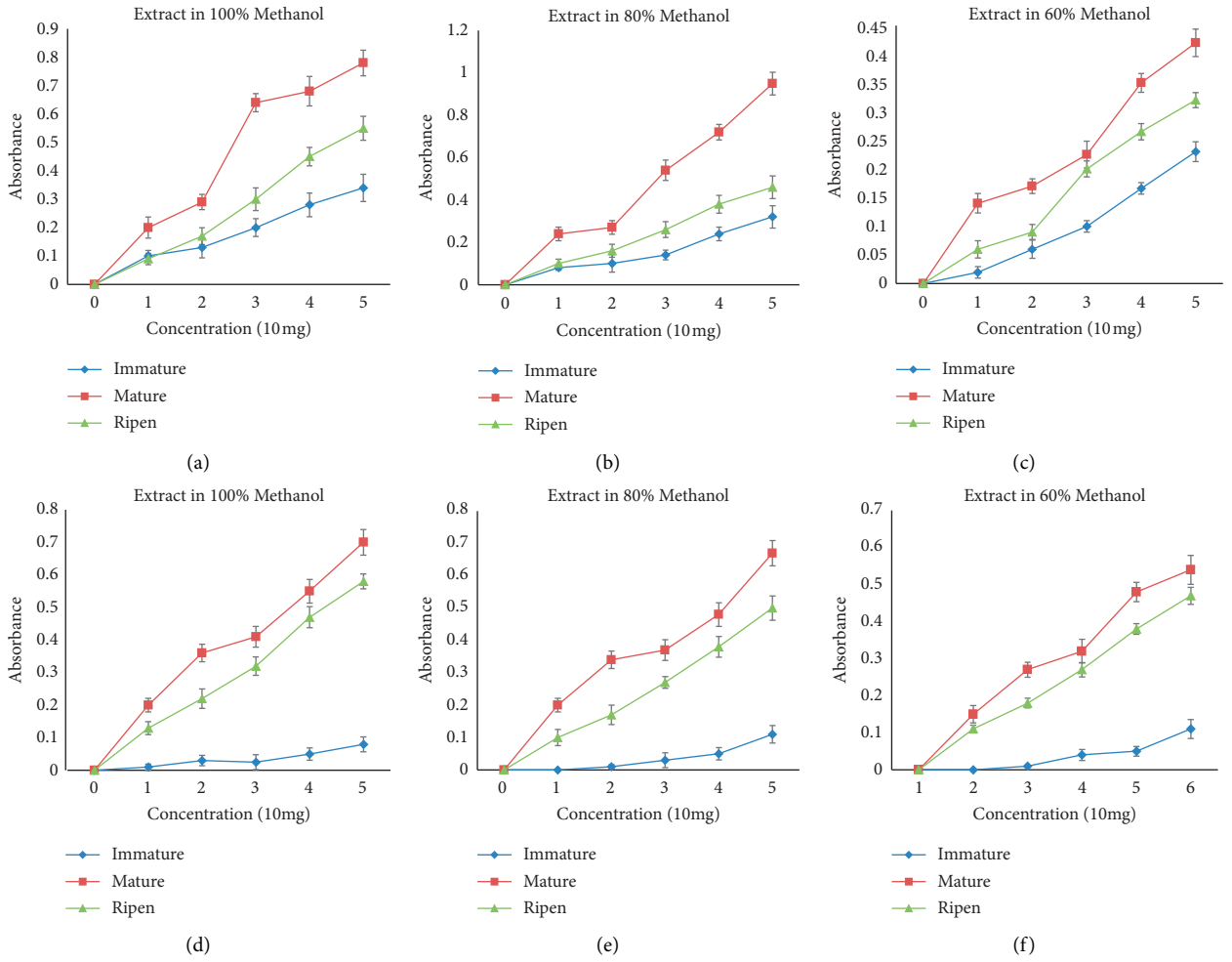


Figure 3: Antioxidant activities of immature, mature, and ripen BG extracts in 60%, 80%, and 100% methanol solvents on ferricyanide reducing power (a, b, c) and ferric reducing antioxidant power (d, e, f). Each value is expressed in terms of mean absorbance ($n = 3$) ± standard deviation.

Table 2: Antibacterial activity of immature, mature, and ripen BG extracts in 60%, 80%, and 100% methanol using food-born and MDR bacterial strains.

Solvent	Fruit	Zone-of-inhibition (mm)			
		S. aureus	*B. subtilis*	*P. multocida*	*E. coli*
Extract in 60% methanol	Immature	12.4 ± 0.32^{c}	18.8 ± 0.06^{b}	8.1 ± 0.71^{d}	12.2 ± 0.44^{c}
	Mature	17.8 ± 0.12^{b}	15.9 ± 0.26^{c}	14.1 ± 0.46^{d}	16.2 ± 0.41^{c}
	Ripen	11.8 ± 0.12^{c}	14.9 ± 0.75^{c}	13.3 ± 0.26^{c}	14.9 ± 0.36^{c}
Extract in 80% methanol	Immature	18.2 ± 0.12^{b}	17.2 ± 0.06^{d}	16.2 ± 0.45^{c}	16.3 ± 1.61^{b}
	Mature	15.9 ± 0.41^{c}	17.5 ± 0.31^{b}	16.3 ± 0.15^{c}	18.4 ± 0.17^{b}
	Ripen	16.1 ± 0.52^{b}	16.3 ± 0.17^{b}	13.7 ± 0.47^{c}	16.1 ± 0.29^{b}
Extract in 100% methanol	Immature	18.5 ± 0.21^{b}	18.4 ± 0.06^{c}	17.7 ± 0.15^{b}	17.4 ± 0.72^{b}
	Mature	15.9 ± 0.56^{c}	17.2 ± 0.36^{b}	17.5 ± 0.33^{b}	16.1 ± 0.38^{c}
	Ripen	15.9 ± 0.31^{b}	16.4 ± 0.44^{b}	16.2 ± 0.35^{b}	14.1 ± 0.38^{d}
Control	Rifampicin	26.8 ± 0.11^{a}	26.5 ± 0.06^{a}	24.5 ± 0.17^{a}	23.9 ± 0.09^{a}

Values with the same letters in superscript in similar colored shade cells do not differ significantly. Values are significant at level $P < 0.001$.

3.6. Antimicrobial Activity. Variety of *Momordica* species have been reported to bear good antibacterial activities against both Gram-positive and Gram-negative bacteria [27]. It has been suggested that phenolics containing polar isopropyl functionality are responsible for antibacterial activity of natural extracts [28]. The results indicate mature and immature BG fruit extracts are more antibacterial as compared to ripen fruit extracts. The extracts were found promisingly active against both Gram-positive (*S. aureus* and *B. subtilis*) and Gram-negative bacteria (*E. coli* and *P. multocida*). The extracts in 60% methanol were also found less active against bacterial inhibition irrespective of the maturity level. The differences in inhibition activities were found significant at $P < 0.001$ as shown in Table 2. According to the previously reported data, it has been suggested that phenolic compounds damage bacterial membrane, for example, *S. aureus* on treating with BHA (bone-like hydroxyapatite), releases nucleotide into culture medium [29], whereas exposure to *P. fragi* and *P. fluorescens* increased the leakage of protein material [30]. In the present study, the yield of BG fruit extract with 60% methanol is comparable with 80% and 100% methanol extracts but it is not warranted that their bacteriostatic activity will also be similar. However, by increasing the methanol concentration in extracted solvent the antibacterial activity also increased [29, 31]. Previously, Ozusaglam and Karakoca investigated the antibacterial activity of unripen and ripen ethanol extracts of BG seeds and fruits and found unripen BG fruit extracts are more active against microorganisms [32].

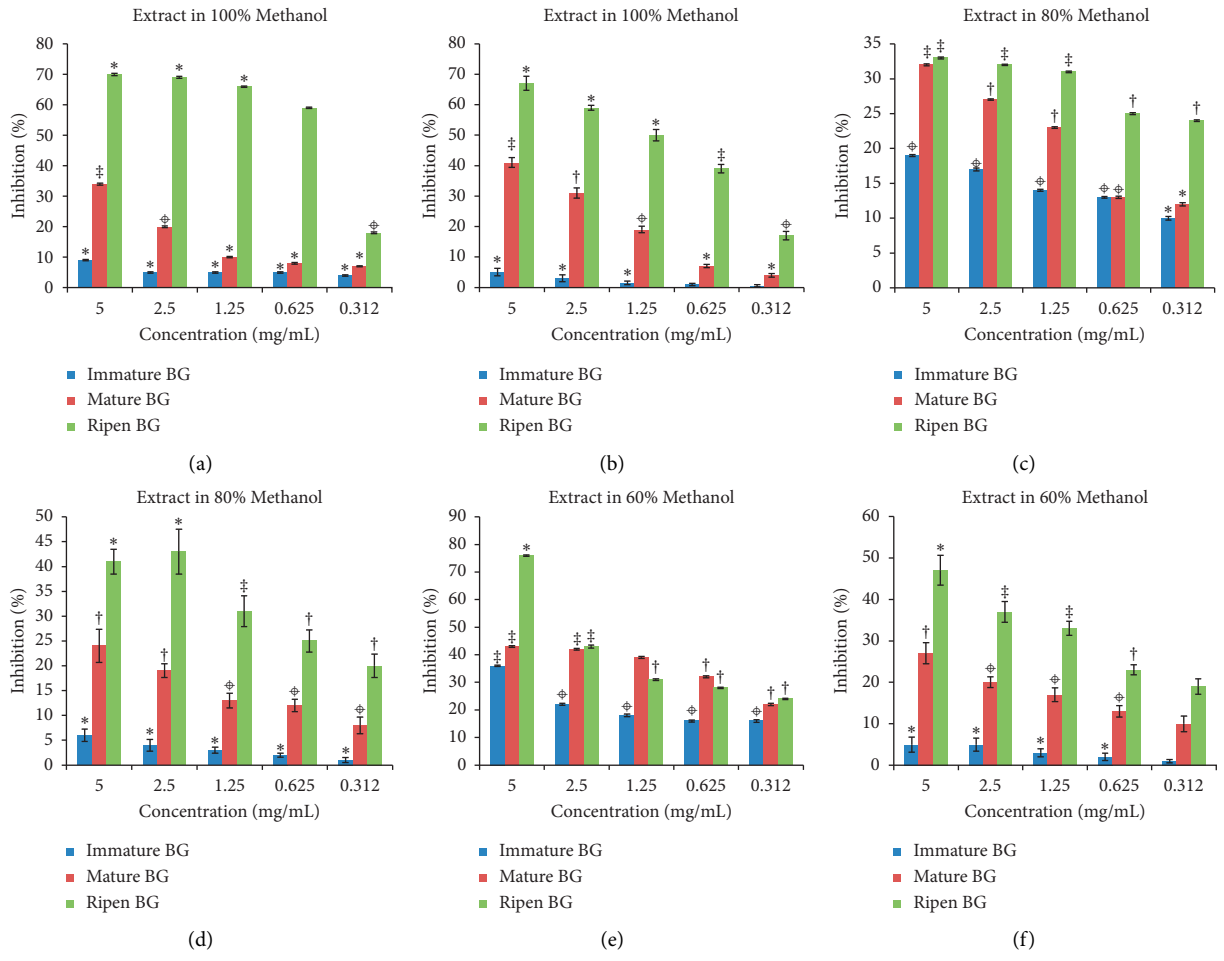
Alkaloids, glycosides, volatile oils, or tannins in addition to phenolics and flavonoids are known to present in BG fruits which largely attribute to antimicrobial activities [33]. These are best evaluated for antibacterial activities when extraction was performed with methanol solvent [33–35]. It is worth taking to mention that combined antibacterial effect of all compounds present in fruit extracts not only hacks the microbial activity and growth but also makes microbes unable to produce resistance mechanism. IC_{50} values (calculated using *S. aureus* bugs) indicated immature BG fruit extract in 80% methanol showed lowest IC_{50} (513 μg/mL) while in same solvent ripen BG fruit extract showed IC_{50} 789 μg/mL which is greater than any other extract as shown in Table 3. Our study clearly showed two main facts: immature and mature fruit extracts in concentrated methanol exhibited great antibacterial activity as compared to more aqueous and ripen fruit extracts.

3.7. Antiproliferative Activity. A multitude of plants and their different parts particularly fruits have been extensively investigated for the therapy of cancer [36]. Proliferation of cell mainly associated with malignant cells. Cancer is the most leading cause of mortality throughout the world beyond the discrimination of developing and developed countries. Chemotherapy, surgery, and radiotherapy are first line options to treat cancer. Chemotherapy mechanism mainly involves targeting the infected cells either to destroy the malignant cells or to stop the proliferation of cells by interfering into one or more different steps of cell division mechanisms [37]. It has been investigated that BG plant extracts showed broad-spectrum antitumor and anticancer activities and have great ability to slow down the cell proliferation rate. The results revealed that mature BG fruit extract in 100% methanol showed 72% MDBK cancer cell death followed by extracts in 80% methanol (40% inhibition) of ripen BG fruit. The 60% methanol extracts also showed high depletion of MDBK cancer cells. However, the cell line depletion activity using HeLa cancer cell line was recorded comparatively low as shown in Figure 4.

In case of MDBK cancer cell line, the lowest IC_{50} value (3.56 mg/mL) was calculated for 100% methanol extract of ripen BG followed by 100% methanol extract of mature BG (7.95 mg/mL), 80% methanol extract of ripen fruit (9.02 mg/mL), and 80% methanol extract of mature fruit (9.08 mg/mL) (Table 4). In contrast to MDBK cancer cell line, HeLa cancer cell line showed slightly less sensitivity to BG fruit extracts. The results showed that at 5 mg/mL concentration, the 100% methanol extract of ripen fruit showed maximum inhibition of cancer cell growth ($65.80 \pm 3.10\%$), followed by 60% methanol extract ($46.64 \pm 3.74\%$) and 80% methanol extract ($41.60 \pm 4.9\%$ at 2.5 mg/mL). Other methanol extracts (in 80% and 60% methanol) also showed similar activities that were observed in case of 100% methanol extract but significantly less. The IC_{50} value was also

Table 3: IC_{50} values of immature, mature, and ripen BG extracts in 60%, 80%, and 100% methanol against MDR *S. aureus* microorganism.

Fruit	IC_{50} (μg/mL)		
	60% methanol extract	80% methanol extract	100% methanol extract
Immature	548 ± 07	513 ± 12	617 ± 13
Mature	589 ± 08	524 ± 10	576 ± 17
Ripen	709 ± 18	789 ± 16	750 ± 19



BG fruit stage	LSD 5% MDBK cancer cell line			LSD 5% HeLa cancer cell line		
	100% ME	80% ME	60% ME	100% ME	80% ME	60% ME
Immature	0.569***	0.576***	0.478***	0.639***	0.609***	0.751***
Mature	0.566***	0.467***	0.640***	0.592***	0.499***	0.549***
Ripen	0.472***	0.426***	0.503***	0.469***	0.519***	0.499***

(g)

Similar signs show no significant difference while nonsimilar signs show significant difference at level $P < 0.001$

Figure 4: Anticancer activity of immature, mature, and ripen BG fruit extracts against MDBK and HeLa cancer cell line: (a) 100% methanol anticancer activity against MDBK cancer cell line; (b) 100% methanol extract anticancer activity against HeLa cancer cell line; (c) 80% methanol extract anticancer activity against MDBK cancer cell line; (d) 80% methanol extract anticancer activity against HeLa cancer cell line; (e) 60% methanol extract anticancer activity against MDBK cancer. cell line and (f) 60% methanol extract anticancer activity against HeLa cancer cell line. The LSD 5% values are represented in the table showing level of significant difference ($P < 0.001$).

calculated that was greater than MDBK cell line (Table 4). The viability of the MDBK cancer cells treated with 60%, 80%, and 100% methanol ripen BG fruit extracts was imaged with trinocular microscope as shown in Figure 5. The control drug (cisplatin) showed maximum cell death (82.45 ± 2.31%) followed by 80% and 100% methanol extracts of BG.

Different groups have reported that BG extracts play active role against number of common cancers such as

Table 4: IC_{50} values calculated for anticancer activity of immature, mature, and ripen BG extracts in 60%, 80%, and 100% methanol using HeLa and MBDK cancer cell lines.

Fruit stage	IC_{50} (mg/mL)					
	60% methanol extract		80% methanol extract		100% methanol extract	
	HeLa	MDBK	HeLa	MBBK	HeLa	MDBK
Immature	67.34 ± 2.48	48.07 ± 1.08	54.65 ± 4.05	21.28 ± 2.32	25.87 ± 3.23	19.33 ± 2.14
Mature	31.31 ± 3.09	25.88 ± 1.69	21.56 ± 2.18	9.08 ± 1.57	13.56 ± 2.12	7.95 ± 1.39
Ripen	18.52 ± 1.56	12.88 ± 1.11	15.21 ± 1.37	9.02 ± 1.23	6.72 ± 1.81	3.55 ± 0.51

*Results are mean ± S. D. of bitter gourd fruit extracts analyzed in triplicate.

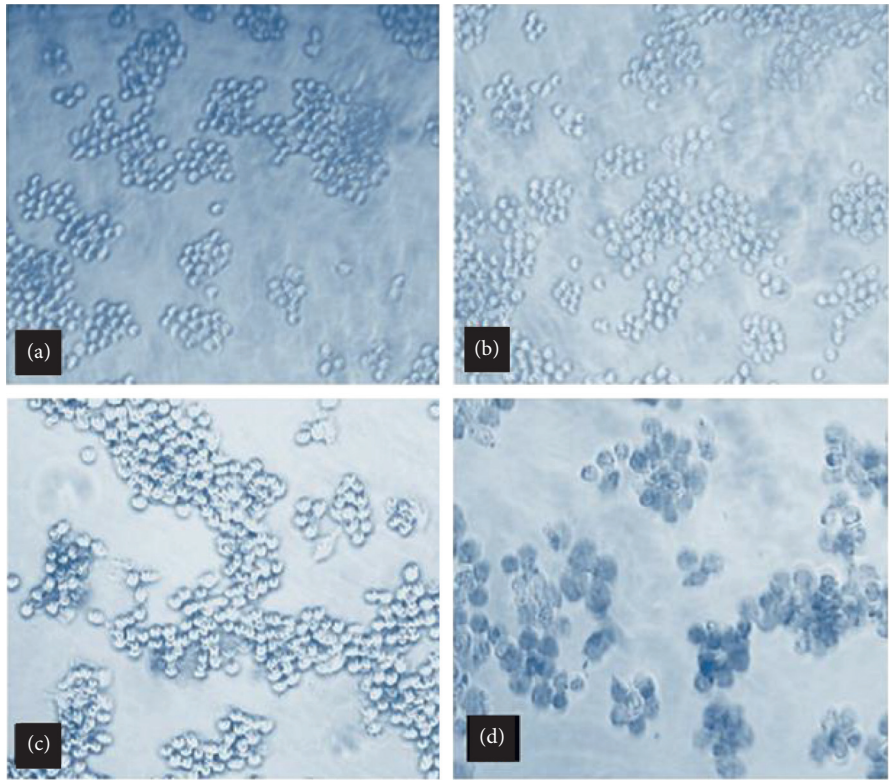


Figure 5: The effect of the ripen BG extracts (incubation with 5 mg/mL extract) on the viability of the MDBK cancer cells. Most of the cancer cells remained healthy and viable when treated with 60% methanol extract (a), treating with 80% ripen BG fruit extract showed cells depletion effect but not prominently (b), treating with ripen BG extract in 100% methanol showed strong deformation of cell structure (c), and control methotrexate anticancer drug showed very strong cell death effect (d).

lymphoma [38], skin tumour [39], breast cancer [40], lymphoid leukemia [41], prostatic cancer [38, 42], melanoma, and choriocarcinoma [27]. Furthermore, it has also been reported that aqueous extract of BG more actively inhibits the growth of prostatic adenocarcinoma [43], breast cancer, and human bladder carcinomas. Various compounds such as MAP30 (Momordica anti-HIV protein), momordin, and alpha-momorcharin isolated from BG extracts have shown anticancer activities. MAP30, a protein based molecule of 30 kDa mass in addition to topological inactivation of HIV-LTR-DNA, also induce cell damaging of breast cancer cells xenograft into mice [44]. Our results are also in good agreement with reported studies.

4. Conclusion and Future Perspective

Metabolic products of plants play paramount role in the eradication or slowdown the number of disease processes. BG is well known for its incredible medicinal activities against numerous diseases, especially diabetes mellitus. In this study, our investigations to see the effect of three different ripening stages of air-dried BG extracts on antioxidant, antibacterial, and anticancer activities reveal promising results. The mature BG fruit extract in absolute methanol showed strong antioxidant activity than immature and ripen fruit extract, while immature and mature fruit were found to show promising antibacterial activity and ripen fruit extract in 100% methanol was found to have

anticancer activity. On the bases of the results, we can conclude that the immature and ripen fruits that commonly wasted at the end of BG cultivation season could be collected and air-dried for obtaining the extracts as an antibacterial and anticancer activities by including in functional food as an ingredient or as an Ayurveda medicine.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

All authors declare that no conflicts of interest exist.

Acknowledgments

This work was funded by Researchers Supporting Project number RSP 2019/26, King Saud University, Riyadh, Saudi Arabia. The authors are also grateful to Higher Education Commission (HEC) Islamabad for its support.

References

[1] S. A. R. Naqvi, S. M. A. Shah, L. Kanwal et al., "Antimicrobial and antihypercholesterolemic activities of *Pulicaria gnaphalodes*," *Dose-Response*, vol. 18, no. 1, 2020.

[2] D. van Elsland and J. Neefjes, "Bacterial infections and cancer," *EMBO Reports*, vol. 19, no. 11, Article ID e46632, 2018.

[3] A. H. Chang and J. Parsonnet, "Role of bacteria in oncogenesis," *Clinical Microbiology Reviews*, vol. 23, no. 4, pp. 837–857, 2010.

[4] S. B. Patel, U. A. Attar, D. M. Sakate, and S. G. Ghane, "Efficient extraction of cucurbitacins from *Diplocyclos palmatus* (L.) C. Jeffrey: optimization using response surface methodology, extraction methods and study of some important bioactivities," *Scientific Reports*, vol. 10, no. 1, p. 2109, 2020.

[5] S. G. Ghane, U. A. Attar, P. B. Yadav, and M. M. Lekhak, "Antioxidant, anti-diabetic, acetylcholinesterase inhibitory potential and estimation of alkaloids (lycorine and galanthamine) from *Crinum* species: an important source of anticancer and anti-Alzheimer drug," *Industrial Crops and Products*, vol. 125, pp. 168–177, 2018.

[6] O. O. Adewale, O. I. Oduyemi, and O. Ayokunle, "Oral administration of leaf extracts of *Momordica charantia* affect reproductive hormones of adult female Wistar rats," *Asian Pacific Journal of Tropical Biomedicine*, vol. 4, pp. S521–S524, 2014.

[7] G. M. Cragg and D. J. Newman, "Biodiversity: a continuing source of novel drug leads," *Pure and Applied Chemistry*, vol. 77, no. 1, pp. 7–24, 2005.

[8] R. Khan, B. Islam, M. Akram et al., "Antimicrobial activity of five herbal extracts against multi drug resistant (MDR) strains of bacteria and fungus of clinical origin," *Molecules*, vol. 14, no. 2, pp. 586–597, 2009.

[9] M. B. Krawinkel and G. B. Keding, "Bitter gourd (*Momordica charantia*): a dietary approach to hyperglycemia," *Nutrition Reviews*, vol. 64, no. 7, pp. 331–337, 2006.

[10] C.-Y. Chuang, C. Hsu, C.-Y. Chao, Y.-S. Wein, Y.-H. Kuo, and C.-j. Huang, "Fractionation and identification of 9c, 11t, 13t-conjugated linolenic acid as an activator of PPARα in bitter gourd (Momordica charantia L.)," *Journal of Biomedical Science*, vol. 13, no. 6, pp. 763–772, 2006.

[11] M. Kobori, H. Nakayama, K. Fukushima et al., "Bitter gourd suppresses lipopolysaccharide-induced inflammatory responses," *Journal of Agricultural and Food Chemistry*, vol. 56, no. 11, pp. 4004–4011, 2008.

[12] C.-I. Chang, H.-I. Tseng, Y.-W. Liao et al., "In vivo and in vitro studies to identify the hypoglycaemic constituents of *Momordica charantia* wild variant WB24," *Food Chemistry*, vol. 125, no. 2, pp. 521–528, 2011.

[13] C. Hsu, C.-L. Hsieh, Y.-H. Kuo, and C.-j. Huang, "Isolation and identification of cucurbitane-type triterpenoids with partial agonist/antagonist potential for estrogen receptors fromMomordica charantia," *Journal of Agricultural and Food Chemistry*, vol. 59, no. 9, pp. 4553–4561, 2011.

[14] S. S. Rathi, J. K. Grover, V. Vikrant, and N. R. Biswas, "Prevention of experimental diabetic cataract by Indian Ayurvedic plant extracts," *Phytotherapy Research*, vol. 16, no. 8, pp. 774–777, 2002.

[15] U. A. Attar and S. G. Ghane, "In vitro antioxidant, antidiabetic, antiacetylcholine esterase, anticancer activities and RP-HPLC analysis of phenolics from the wild bottle gourd (*Lagenaria siceraria* (Molina) Standl.)," *South African Journal of Botany*, vol. 125, pp. 360–370, 2019.

[16] K. Slinkard and V. L. Singleton, "Total phenol analysis: automation and comparison with manual methods," *American Journal of Enology and Viticulture*, vol. 28, no. 1, pp. 49–55, 1977.

[17] S. Iqbal, M. I. Bhanger, and F. Anwar, "Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan," *Food Chemistry*, vol. 93, no. 2, pp. 265–272, 2005.

[18] N. Asghar, S. A. R. Naqvi, Z. Hussain et al., "Compositional difference in antioxidant and antibacterial activity of all parts of the *Carica papaya* using different solvents," *Chemistry Central Journal*, vol. 10, no. 1, p. 5, 2016.

[19] I. F. F. Benzie and J. J. Strain, "The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay," *Analytical Biochemistry*, vol. 239, no. 1, pp. 70–76, 1996.

[20] A. Sahar, S. Naqvi, Z. Khan et al., "Screening of phytoconstituents, investigation of antioxidant and antibacterial activity of methanolic and aqueous extracts of *Cucumis sativus*," *Journal of the Chemical Society of Pakistan*, vol. 35, no. 2, pp. 457–463, 2013.

[21] T. Mosmann, "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays," *Journal of Immunological Methods*, vol. 65, no. 1-2, pp. 55–63, 1983.

[22] R. Amarowicz, I. Estrella, T. Hernández et al., "Free radical-scavenging capacity, antioxidant activity, and phenolic composition of green lentil (*Lens culinaris*)," *Food Chemistry*, vol. 121, no. 3, pp. 705–711, 2010.

[23] J. Kubola and S. Siriamornpun, "Phenolic contents and antioxidant activities of bitter gourd (Momordica charantia L.) leaf, stem and fruit fraction extracts in vitro," *Food Chemistry*, vol. 110, no. 4, pp. 881–890, 2008.

[24] A. Rezaeizadeh, A. Zuki, M. Abdollahi et al., "Determination of antioxidant activity in methanolic and chloroformic extracts of *Momordica charantia*," *African Journal of Biotechnology*, vol. 10, no. 24, pp. 4932–4940, 2011.

[25] B. Sun and M. Fukuhara, "Effects of co-administration of butylated hydroxytoluene, butylated hydroxyanisole and flavonoids on the activation of mutagens and drug-

metabolizing enzymes in mice," *Toxicology*, vol. 122, no. 1-2, pp. 61–72, 1997.

[26] A. Yıldırım, A. Mavi, and A. A. Kara, "Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts," *Journal of Agricultural and Food Chemistry*, vol. 49, no. 8, pp. 4083–4089, 2001.

[27] J. K. Grover and S. P. Yadav, "Pharmacological actions and potential uses of *Momordica charantia*: a review," *Journal of Ethnopharmacology*, vol. 93, no. 1, pp. 123–132, 2004.

[28] R. S. Farag, Z. Y. Daw, and S. H. ABO-RAYA, "Influence of some spice essential oils on *Aspergillus parasiticus* growth and production of aflatoxins in a synthetic medium," *Journal of Food Science*, vol. 54, no. 1, pp. 74–76, 1989.

[29] R. Degré and M. Sylvestre, "Effect of butylated hydroxyanisole on the cytoplasmic membrane of *Staphylococcus aureus* Wood 46," *Journal of Food Protection*, vol. 46, no. 3, pp. 206–209, 1983.

[30] P. M. Davidson and A. L. Branden, "Antimicrobial activity of non-halogenated phenolic compounds," *Journal of Food Protection*, vol. 44, no. 8, pp. 623–632, 1981.

[31] P. Wong and D. Kitts, "Studies on the dual antioxidant and antibacterial properties of parsley (*Petroselinum crispum*) and cilantro (*Coriandrum sativum*) extracts," *Food Chemistry*, vol. 97, no. 3, pp. 505–515, 2006.

[32] M. A. Ozusaglam and K. Karakoca, "Antimicrobial and antioxidant activities of *Momordica charantia* from Turkey," *African Journal of Biotechnology*, vol. 12, pp. 1548–1558, 2013.

[33] K. Mwambete, "The in vitro antimicrobial activity of fruit and leaf crude extracts of *Momordica charantia*: a Tanzania medicinal plant," *African Health Sciences*, vol. 9, no. 1, pp. 34–39, 2009.

[34] H. S. Elshafie, L. Aliberti, M. Amato, V. De Feo, and I. Camele, "Chemical composition and antimicrobial activity of chia (*Salvia hispanica* L.) essential oil," *European Food Research and Technology*, vol. 244, no. 9, pp. 1675–1682, 2018.

[35] T. A. Mohamed, M.-E. F. Hegazy, A. A. Abd El Aty et al., "Antimicrobial sesquiterpene lactones fromArtemisia sieberi," *Journal of Asian Natural Products Research*, vol. 19, no. 11, pp. 1093–1101, 2017.

[36] U. A. Attar and S. G. Ghane, "Optimized extraction of anticancer compound - cucurbitacin I and LC-MS identification of major metabolites from wild Bottle gourd (Lagenaria siceraria (Molina) Standl.)," *South African Journal of Botany*, vol. 119, pp. 181–187, 2018.

[37] N. Gautam, A. K. Mantha, and S. Mittal, "Essential oils and their constituents as anticancer agents: a mechanistic view," *BioMed Research International*, vol. 2014, Article ID 154106, 23 pages, 2014.

[38] Y. Sun, P. L. Huang, J. J. Li et al., "Anti-HIV agent MAP30 modulates the expression profile of viral and cellular genes for proliferation and apoptosis in AIDS-related lymphoma cells infected with Kaposi's sarcoma-associated virus," *Biochemical and Biophysical Research Communications*, vol. 287, no. 4, pp. 983–994, 2001.

[39] C. Ganguly, S. De, and S. Das, "Prevention of carcinogen-induced mouse skin papilloma by whole fruit aqueous extract of *Momordica charantia*," *European Journal of Cancer Prevention*, vol. 9, no. 4, pp. 283–288, 2000.

[40] T. B. Ng, W. K. Liu, S. F. Sze, and H. W. Yeung, "Action of α-momorcharin, a ribosome inactivating protein, on cultured tumor cell lines," *General Pharmacology: The Vascular System*, vol. 25, no. 1, pp. 75–77, 1994.

[41] F. Licastro, C. Franceschi, L. Barbieri, and F. Stirpe, "Toxicity ofMomordica charantia lectin and inhibitor for human normal and leukaemic lymphocytes," *Virchows Archiv B Cell Pathology Including Molecular Pathology*, vol. 33, no. 1, pp. 257–265, 1980.

[42] P. Ru, R. Steele, P. V. Nerurkar, N. Phillips, and R. B. Ray, "Bitter melon extract impairs prostate cancer cell-cycle progression and delays prostatic intraepithelial neoplasia in TRAMP model," *Cancer Prevention Research*, vol. 4, no. 12, pp. 2122–2130, 2011.

[43] A. J. Claflin, D. L. Vesely, J. L. Hudson et al., "Inhibition of growth and guanylate cyclase activity of an undifferentiated prostate adenocarcinoma by an extract of the balsam pear (Momordica charantia abbreviata)," *Proceedings of the National Academy of Sciences*, vol. 75, no. 2, pp. 989–993, 1978.

[44] S. Lee-Huang, P. L. Huang, P. L. Huang, A. S. Bourinbaiar, H. C. Chen, and H. F. Kung, "Inhibition of the integrase of human immunodeficiency virus (HIV) type 1 by anti-HIV plant proteins MAP30 and GAP31," *Proceedings of the National Academy of Sciences*, vol. 92, no. 19, pp. 8818–8822, 1995.