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Effect of Keitt Mango (*Mangifera indica* L.) Seed Kernels Ethanolic Extract Against Two Strains of Food Pathogenic Bacteria

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Abstract: Nowadays, consumers are increasingly demanding natural food ingredients that are expected to be safe and healthy. Processing of mango fruits generates a significant amount of by-products such as peels and seeds which represent up to 60% of the fruit. Mango by-products are an important source of bioactive phenolic compounds. Phenolic characterization is an essential step for the utilization of mango by-products as food ingredients and preservative and so provides an added value to mango production in Egypt. The current study was to explore the potential of the agro-industrial waste from mango keitt seed kernel as antibacterial against pathogenic food borne .The bioactive properties of the phenolics extract was accessed by measuring their free radical scavenging activity and antioxidant effects; moreover, their safety on human were explored by MTT assay. This study is emphasized specifically on antimicrobial activity of seed kernels extract of mango (Keitt) were studied by the cup agar diffusion method on four representative test microbes used were *Staphylococcus aureus* ATCC 6538-P as (G+ve), *Escherichia coli* ATCC 25933 as (G-ve) as food- borne pathogenic bacteria. The antimicrobial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter (mm). Neomycin was use as antibacterial agents at 100µg/ml. for comparison. In addition, two different approach (high-performance liquid chromatography and FTIR a powerful techniques for the characterization of phenolic compounds of seed kernels extracts of mango (Keitt), in addition to traditional method (Folin-Ciocalteau) was used to extract the bioactive compounds of mango seed kernel and their antioxidant potential was estimated using 2, 2-diphenyl-1-picrylhydrazyl(DPPH) scavenging assays, to more understand phenolic compounds effects of antibacterial, antioxidants activities, other biological effects, moreover biosafety on human were explored by MTT assay. The results were expressed as the average diameter of bacterial inhibition zones surrounding the wells. The results showed that Keitt variety gave relatively high antibacterial activity 2.23 cm. For the known antibiotic, which was Neomycin, under the same conditions the diameter of inhibition zones were between 2.30 to 3.30. Then, the minimum inhibition concentration tests were conducted for Keitt variety. The results obtained confirmed the antibacterial potential of mango kernels extract and this would probably become an alternative source of new and natural antibacterial agents of food industries. Finally, the extract was analyzed by high-performance liquid chromatography (HPLC) and FT-IR in order to identify some phenolic compounds and relate these substances with the antibacterial, antioxidant results; about 19 bioactive phenolic compounds were identified by HPLC. The highest amounts of phenolic compounds were ferulic acid, coumaric acid, methyl gallate, naringenin, catechin, gallic acid, syringic acid, ellagic acid, hlorogenic acid, querectin, daidzein and cinnamic acid. The study shows that mango seed kernel (Keitt var) is an abundant and cost-effective source of potential natural antioxidant and an alternative source of chemical antimicrobial agents and safe on human health.

Key wards: Keitt mango seed kernel extract · Anti-bacterial properties · *Staph aureus* · *E. coli O.157* · HPLC · DPPH scavenging assays · MTT assay

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INTRODUCTION

Mango (*Mangifera indica L.*) is recognized as one of the most important tropical and subtropical fruits in the world. It is produced in large quantities and highly accepted by consumers. Nowadays, the production of mango takes place in more than 115 countries [1]. According to the Food and Agriculture Organization (FAO) of the United Nations, the global production of mango was 46.5 million metric tons in 2016.

Approximately 77% of the world's mangoes are produced in Asian countries, while 13% and 9% are produced in the Americas and African countries, respectively. The significant increases of mango consumption in domestic activity lead to the accumulation of waste. Mango seed kernel usually wasted when processing. However, with appropriate treatment and study, the kernel might be possibly used as a food ingredient and even in other purposes.

In a study for Egyptian mango by-product to proximate its compositional quality of mango seed kernel, mango seed kernels contained a considerable amount of total phenolic compounds, eight phenolic compounds were identified which tannin and vanillin were in highest amounts [2].

The large amount of waste produced by the food industries causes serious environmental problems and also results in economic losses if not utilized effectively. Different research reports have revealed that food industry by-products can be good sources of potentially valuable bioactive compounds. As such, the mango juice industry uses only the edible portions of the mangoes and a considerable amount of peels and seeds are discarded as industrial waste. Mango by-products, especially seeds is considered to be cheap sources of valuable food and nutraceutical ingredients. The main uses of natural food ingredients derived from mango by-products are presented and discussed and the mainstream sectors of application for these by-products, such as in the food, pharmaceutical and nutraceutical industries, are highlighted.

Sandhu and Lim [3], mentioned to approximately 40% to 60% waste is generated during processing of mangoes; 12% to 15% consists of peels and 15% to 20% of kernels. According to mango varieties, the seed represents from 10% to 25% of the whole fruit weight. The kernel inside the seed represents from 45% to 75% of the seed and about 20% of the whole fruit. However, more than one million tons of mango seeds are being treated as wastes.

Soong and *Barlow* [4] suggested that mango seed kernel could be used as a potential source for functional food ingredients due to its high quality of fat and protein as well as high levels of natural antioxidants. Many of the outbreaks caused by *E. coli* O157:H7 have been associated with eating undercooked ground beef [5]. Spoilage microorganisms can readily grow in both fresh and precooked meat products. Therefore, the objective of this study was to examine the antimicrobial activity of mango kernel extracts against strains of *Escherichia coli O157:H7* and *Staph aureus*.

Sowmiya *et al*. [6] indicated antibacterial activity for seed kernel aqueous and ethanolic extracts. These extracts showed remarkable activity against the uropathogens isolated from clinical samples of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebisella pneumoniae and Streptococcus pyogens*. Zhang *et al*. [7] and Burt, [8] reported that several plant extracts have been used to inhibit the growth of or to reduce the number of, several food borne pathogens such including Salmonella spp., Escherichia coli O157:H7 and *Listeria monocytogenes*.

Chemical and synthetic preservatives are still used for preventing food spoilage in the food industry. The adverse effects of these chemical preservatives on human health necessitate the search for potentially effective, healthier, safer and natural food preservatives based on plant sources. It is a common belief that biologically active pure compounds are more effective than crude extracts.

The extracts of mango seed kernels display a broad antibacterial spectrum and are more effective against gram- positive than the gram-negative bacteria. The antibacterial activity of the mango seed kernels is stable against heat (121°C, 15 minutes), freezing (-20°C, 16 hours) and pH treatment (pH 3 to 9) normally used in food processing [9].

Consumers are now, paying closer attention to the risk of food borne pathogens as well as the presence of artificial chemical preservatives used to control food borne pathogens. Synthetic preservatives have been used in foods for decades; however, an increasing perception by consumers that synthetic compounds may lead to negative health consequences has led to a reduced acceptance of their use in foods [10]. As a result, consumers as well as the food industry are looking to use more natural food preservatives that have strong antimicrobial activity in order to ensure safe, wholesome food products. This suggests that plant products have relatively high levels of antimicrobial agents and can be used to inhibit the growth of food borne pathogens.

The present study was designed to determine the proximate composition, phenolic compounds in mango seed kernel extract (MSKE), as antibacterial effect, antioxidants activity and biosafety (non-toxicological status of kernel ethanolic extract).

This research focus on antibacterial properties of Keitt mango kernel extracts against Staphylococcus aureus, Escherichia coli as food- borne pathogenic bacteria and study cytotoxic effect on human normal fibroblast cell line to assurance of safety mango kernel extract as the natural preservative in food industries. Finally, the extract was analyzed by high-performance liquid chromatography-electrospray ionization (HPLC) and FT-IR in order to identify phenolic compounds, and relate these substances with the antibacterial and antioxidant results.

MATERIALS AND METHODS

Materials: Keitt variety, 20 kg of mango kiett was purchased from local market, seeds as by-products (waste) of mango were stored at 4°C until utilization for the next procedures and all chemicals were purchased from the El-Gamhouria Company for chemicals and medical requisites (Alexandria, Egypt).

Methods

Agar Well Diffusion Method: The antibacterial properties of mango seed kernel extract can be investigated by using the agar well diffusion method, the wider of zone surrounding a well, the more susceptible the pathogen microorganisms. Also, through this method, the minimum inhibitory concentration (MIC) of the mango seed kernels extract against food- borne pathogenic bacteria can be determined. The MIC is the lowest concentration of a drug or antibiotic that prevents growth of a particular pathogen [11].

Preparation of Mango Seed Kernels: The seeds were washed and air-dried and the kernels were removed manually from the seeds. The kernels were milled into fine powder by using grinder and then were frozen at -80°C and transferred to freeze dryer to remove the excessive moisture for several days. Finally, the samples were stored at 4°C until utilization for the next procedures.

Preparation of Mango Kernel Ethanolic Extract: 100g powder kernels plus absolute 95% ethanol was added at a ratio of 3:1 (v/w) and kept 24 hrs with gentle shaking at 40°C, the crude extracts were filtered under vacuum through Whatman No. 1 filter paper . Filtered extracts were dried using a rotary evaporator at 35°C, the concentrated extracts were subsequently dried by lyophilization and stored at 4°C for further use, Mahmood, [12] and measurement of their total phenolic content (TPC), antioxidant activity (AA), antibacterial and cytotoxicity activity.

Physicochemical Analysis

Determination of Total Phenolic Content: Total phenolic content of the extract was determined colorimetrically, using the Folin-Ciocalteu method, as described by Singleton *et al.* [13].

The total phenolic contents were determined on the basis of the calibration curve of gallic acid and expressed as gallic acid equivalents (GAE), in milligrams per gram of the sample.

Gallic acid was used as a standard solution because it is one of the natural and stable phenols and is relatively cheap compared to others.

Determination and Identification of Phenolics Compounds of Mongo Kernels Extract by HPLC and FTIR

Determination and Identification of Phenolic Compounds Using HPLC: HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using Eclipse C18 column (4.6 mm x 250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate 0.9 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (82% A); 15-16 min (82% A) and 16-20 (82%A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 5 µl for each of the sample solutions. The column temperature was maintained at 40°C [14].

Determination and Identification of Phenolic Compounds by Using FTIR: ATR-FTRI was measured (FTRI Lab, National Research Center, Giza, Egypt) by using Bruker VERTEX 80 (Germany) combined Platinum Diamond disk as that of an internal reflector in the range 4000- 400 cm^{-1} with resolution 4 cm^{-1}, refractive index 2.4. [15].

Measurement of Antioxidant Activity of Mango Keitt Extract (DPPH Free Radical Scavenge Assay): The free radical scavenging properties of extract was evaluated by the 2, 2-diphenyl-1- picrylhydrazyl (DPPH)assay method in (Bioassay-Cell Culture Laboratory, National Research Centre, El-Tahrir St., Dokki, Cairo 12622, Egypt)

(a) (b) (c)

Fig. 1: Egyptian Keitt mango cultivar: " kernel ethanolic extract (a), mango seeds (b)" and "Keitt mango fruit (c)

according to Shimada *et al*. [16] and Abeer *et al*. [17], the absorbance was measured at 517 nm. Sample concentration range between (100 to 0.78 µM), each assay was carried out in triplicate. The percentage of radial scavenging activity was calculated using the below mentioned formula, according of the method of Oktay *et al.* [18]:

$$Scavenging\ activity\ \% = 100 * \frac{A\ Blank - A\ Sample}{A\ Blank}$$

The IC50 value for each sample, defined as the concentration of the test sample leading to 50% reduction of the initial DPPH concentration, was calculated from the non linear regression curve of Log concentration of the test extract (µg/ml) against the mean percentage of the radical scavenging activity.

The Antibacterial Activities

Sample Preparation: 10mg of Keitt kernel ethanolic extract sample was dissolved in 2ml of DMSO.

Antimicrobial Activity Test: The antimicrobial activity of seed kernels extract of mango (Keitt) was studied by the cup agar diffusion method. The two representative test microbes used were *Staphylococcus aureus* ATCC 6538-P as G +ve bacteria, *Escherichia coli* ATCC 25933 as G-ve bacteria. Nutrient agar plates were heavily inoculated regularly with 0.1ml of 10^5-10^6 cells/ml in case of bacteria. Czapek-Dox agar plates seeded by 0.1ml (10^6 cells/ml) the fungal inoculum was used to evaluate the antifungal activities. Three holes were initiated in each inoculated plate. 100microleter from each sample were dispensed in each cup. Then plates were kept at low temperature (4°C) for 2-4 hours to allow maximum diffusion. The plates were then incubated at 37°C for 24 hours for bacteria and at 30°C for 48 hours in upright position to allow maximum growth of the organisms. The antimicrobial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter (mm). The experiment was carried out more than once and mean of reading was recorded. Neomycin was used as antibacterial agent at 100µg/ml.

The bacterial cultures were diluted with distilled water and collected in sterile centrifuge tubes and standardized by obtaining absorbance to 0.5493 Abs at 610 nm spectrophotometrically.

The commercially available antibiotic streptomycin (1 mg/ml) was used as positive control). The antimicrobial activity was evaluated by measuring diameter of the inhibition zone formed around the well using ruler in centimeters.

Test Microbes Used: *Staphylococcus aureus* ATCC 6538 (G +ve bacteria) and *Escherichia coli* ATCC 25933 (G-ve bacteria) were used and grown on Mueller Hinton medium.

Preparation of Bacterial Culture: Bacterial cultures were prepared under sterile conditions by inculating 100ml bottle with each test microbe, capped and incubated at 35°C for 24h. clean bacterial cells were prepared by centrigugating the growth culture, under sterile condition, in cooling centrifuge at 400rpm for 15min.The bacterial cells were resuspended using 20 mL of sterile normal saline and centrifuged again at 4000 rpm for 5 min. This step was repeated until the supernatant was clear. The pellet was then suspended in 20 mL of sterile normal saline. The optical density of the bacterial suspension was recorded at 500nm and serial dilutions were carried out with appropriate aseptic techniques until the optical density was in the range of 0.5-1.0. The actual number of colony-forming units was carried out to obtain a concentration of $5x10^6$cfu/mL.

Preparation of Resazurin Solution: The resazurin solution was prepared by dissolving a 675 mg in 100 mL of sterile distilled water and shake well with vortex mixer and sterilized by filtration through membrane filter (pore size of 0.22-0.45µm).

Preparation of the Plates: Microplates, 96well, were prepared and labelled under aseptic conditions. A volume of 500µL of test material in DMSO (a stock concentration of 5mg/mL for purified compounds) was pipetted into the

first row of the plate. To all other wells 50 µL of broth medium was added. Serial dilutions were performed. To each well 10µL of resazurin indicator solution was added, 10µL of bacterial suspension (5 x10^6cfu/mL) was added to each well. Each plate was wrapped loosely with parafilm to ensure that bacteria did not become dehydrated. The plates were prepared in duplicate and placed in an incubator set at 37°C for 18–24 h. The color change was then assessed visually. Any color changes from purple to pink or colorless were recorded as positive. The lowest concentration at which color change occurred was taken as the MIC value.

Determination of Minimum Bactericidal Concentrations (MBC's) of the Effective Plants Extract: Streaks were taken from the two lowest concentrations of the plant extract plates exhibiting invisible growth (from inhibition zone of MIC plates) and subcultures onto sterile nutrient agar plates. The plates were incubated at 35°C for 24 h. then examined for bacterial growth in corresponding to plant extract concentration. MBC was taken as the concentration of plant extract that did not exhibiting any bacterial growth on the freshly inoculated agar plates.

Cytotoxic Effect on Human Normal Fibroblast Cell Line (BJ1):

Methodology of MTT Assay: Cell viability was assessed by (Bioassay-cell culture laboratory; in vitro bioassay on human tumor cell line for drug discovery (National Research Center, El-Tahrir St., Dokki, Cairo 12622, Egypt) by the mitochondrial dependent reduction of yellow MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) to purple formazan [19].

Procedure: All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA). Cells were suspended in DMEM-F12 medium, 1% antibiotic-antimycotic mixture (10, 000U/ml Potassium Penicillin, 10, 000µg/ml Streptomycin Sulfate and 25µg/ml Amphotericin B) and 1% L-glutamine at 37 ºC under 5% CO_2.

Cells were batch cultured for 10 days, then seeded at concentration of 10x10^3 cells/well in fresh complete growth medium in 96-well micro-titer plastic plates at 37 ºC for 24 h under 5% CO_2 using a water jacketed Carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations of sample to give a final concentration of (100, 50, 25, 12.5, 6.25, 3.125, 1.56 and 0.78 ug/ml). After 48 h of incubation, medium was aspirated, 40ul MTT salt (2.5µg/ml) were added to each well and incubated for further four hours at 37ºC under 5% CO_2. To stop the reaction and dissolving the formed crystals, 200µL of 10% Sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37ºC. DOX were used as positive control at 100µg/ml gives 100% lethality under the same conditions [20].

The absorbance was then measured using a micro-plate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595nm and a reference wavelength of 620 nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent t-test by SPSS 11 program. DMSO is the vehicle used for dissolution of plant extracts and its final concentration on the cells was less than 0.2%. The percentage of change in viability was calculated according to the formula:

(Reading of extract / Reading of negative control) -1) x 100

Statistical Analysis: A probit analysis was carried for IC_{50} and IC_{90} determination using SPSS 11 program.

RESULTS AND DISCUSSION

Total Phenolic and Scavenging Activity of KMKE: Total phenolics and scavenging activity of mango kernels extract (KMKE) are presented in Table 1a, whereas, DPPH radical scavenging method is broadly used to evaluate antioxidant activity, based on hydrogen donating ability or radical scavenging ability of any extract in alcoholic medium that results in color change from purple to yellow [21].

KMKE characterized by significantly higher amount of phenolics and greater free radical scavenging activity 23.90 ± 0.33, 93.08 ± 0.10 for DPPH activity. It means that the higher phenolic content and more scavenging activity. These findings are in harmony with those obtained by Ribeiro *et al*. [22] and Mutua *et al.* [23] reported that extract of mango seed kernel ethanolic extract at the concentration of 20 mg/ml showed 92.22% DPPH scavenging activity. Similar to this, Ashoush and Gadallah [24] have reported 95.08% ± 0.10 DPPH scavenging ability of mango seed kernel extract.

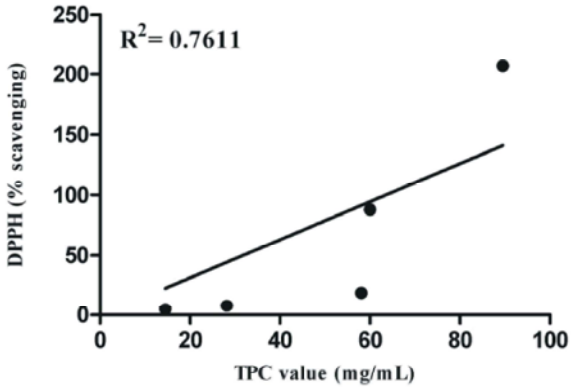


Fig. 2: Correlation between TPC and DPPH scavenging percentage

Table 1: Total phenolic, DPPH scavenging activity of KMKE:

Total phenolics (mg GAE/g)	23.90 ± 0.33
DPPH activity (%)	93.08 ± 0.10

The DPPH free radical scavenging results of the positive control and mango kernel ethanolic extract are expressed as a percentage of inhibition.

Based on the values calculated from the linearity curves, our findings showed a positive correlation between TPC and DPPH for ethanolic extract, R2 = 0.7611 (Figure 1).

As reported previously reported by Gorinstein and coworkers [25], a positive correlation between DPPH and TPC was shown. The results also indicated that there is no significant difference between the DPPH free radical scavenging capacity of mango kernel ethanolic extract and the positive control (gallic acid). Hence, we can conclude that mango kernel ethanolic extract act as a unique source of antioxidants.

Differentiation and Ratios of Phenolic Compounds by HPLC and FTIR of Mango Kernel Extract: The HPLC analysis of the phenolic compounds in mango seed kernel extracts were compiled in Table 2. Results revealed that the extract of MSkE contained 19 identified phenolic compounds. The highest amount of phenolic compounds were ferulic acid, coumaric acid, methyl gallate, naringenin, catechin, gallic acid, syringic acid, ellagic acid, hlorogenic acid, querectin, daidzein and cinnamic acid (82452.02,44336.62,42191.10,35628.49,10258.01,5076.08, 3245.64, 1222.18, 107.04, 68.82, 50.09 and 10.99 µg/ml on a dry wt basis, respectively. HPLC is the preferred technique for separation and quantification both of phenolic compounds, Identified content of the phenolic compounds, Naczk and Shahidi [26]. Puravankara *et al*. [27] identified six phenolic compounds in mango seed extracts, mainly gallic acid, ellagic and gallates. The chromatogram corresponding for the ethanolic extract of mango seed showed that three compounds were found, of which one was identified, corresponding to gallic acid as the main component of the extract, in a concentration of 586.68 mg/g dw [28].The results described above indicate that the waste generated from the agro-industrial processing of Egyptian mango cultivar, 'Keitt mango', is source of bioactive phenolic compounds with antioxidant and antibacterial properties. 'keitt mango' waste provided extracts with free radical scavenging activity, a preservative effect against lipid oxidation in food products and antibacterial properties . SKS supplies a phenolic extract with the best antioxidant and antibacterial activities. Considering the aforementioned, the SKS extract was selected and analyzed by HPLC; some phenolic compounds were identified and these were related with the antioxidant and antibacterial results.

Identified in the SKS extract; furthermore, Figures 3-A of sample and 3-B of standard show the HPLC profile of extract and mass spectra of detected compounds, respectively. Twenty-two signals were considered in the chromatographic profile of the SK.

The phenolic compounds observed in SKS extracts can be related with their antioxidant and antibacterial properties, by transfer electrons and hydrogen atoms to free radical and reactive oxygen species; moreover, their final products after the reduction-oxidation reaction are frequently chemically stable. Several studies suggest that mangiferin helps to delay the lipid oxidation induced by pro-oxidant ions such as iron (Fe2+/Fe3+) in biological systems and food products (e.g., meat products) [29].

Recently, Silva da Veiga *et al*. [30] described the structure–antioxidant relationship of mangiferin using quantum chemistry calculations; they conclude that mangiferin has greater antioxidant capacity by hydrogen transfer than by electron transfer. Imran *et al*. [31] used the agro-industrial mango waste as source of mangiferin to prepare functional drinks, while, Boonnattakorn *et al*. [32] added mangiferin to ethylene vinyl acetate matrix in order to obtain an antioxidant packaging for food products.

Interestingly, Syringic acid, Coumaric acid and Naringenin were only reported in our research. Nevertheless, other phenolic acids reported in previous literature but not in our report (even though the standards for HPLC analysis were present in our study), included Rutin, Pyro- catechol, Vanillin and 4-hydroxybenzoic acid.

Table 2: Phenolic compounds quantitatively identified by HPLC in the extract obtained from 'Keitt mango 'seed kernel compared with standard

	Phenolic Standard		Mango seed kernel extract	
Phenolic compounds	Area	Conc. (µg/ml)	Area	Conc. (µg/ml)
Gallic acid	176.31	15	147.37	5076.08
Chlorogenic acid	365.67	50	1.93	107.04
Catechin	300.91	75	101.66	10258.01
Methyl gallate	270.63	15	1880.19	42191.10
Coffeic acid	218.47	18	0.00	0.00
Syringic acid	234.16	17.2	109.14	3245.64
Pyro catechol	273.77	40	0.00	0.00
Rutin	219.40	26	0.00	0.00
Ellagic acid	554.71	120	13.95	1222.18
Coumaric acid	663.61	20	3633.67	44336.62
Vanillin	290.71	12.9	0.00	0.00
Ferulic acid	294.07	20	2994.48	82452.02
Naringenin	245.06	30	718.86	35628.49
Daidzein	567.68	35	2.01	50.09
Querectin	332.72	40	1.41	68.82
Cinnamic acid	531.82	10	1.44	10.99
Apigenin	669.77	50	0.00	0.00
Kaempferol	277.29	20	0.00	0.00
Hesperetin	374.08	20	0.00	0.00

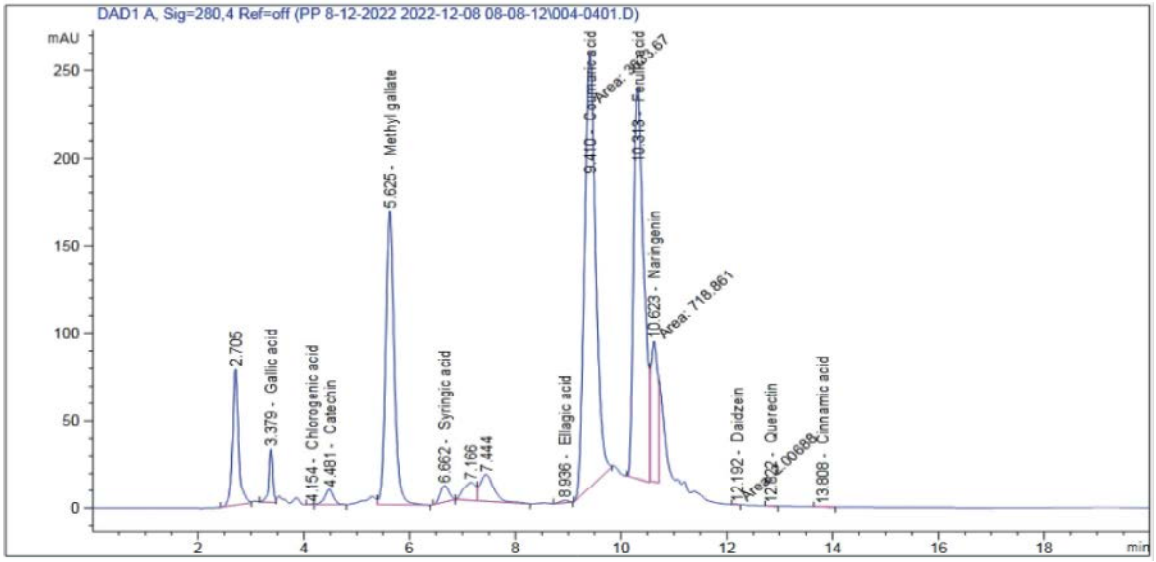


Fig. 3A: Phenolic compounds of extract mango seed kernel

Noteworthy this work could be considered the first report on the TPC and DPPH scavenging activity and assessment cytotoxity on cell human of 'keitt mango' Agro-industrial waste.

Fig. 3, A and B: Phenolic compounds quantitatively identified by HPLC in the extract obtained from 'Keitt mango' seed kernel extract compared with standard.

On the other hand, these results shown that FTIR spectroscopy is a high-throughput method that can be used to quantitatively predict mango kernels extract of in a large data set about of total polyphenols and its less costly and non-laborious compared with HPLC technique.

The spectrum of phenolic compounds are shown in Fig. 4, whereas the phenolic compounds O-H bond from 1440 - 1395 and 950 - 910 cm^{-1} note the broad peak due to O-H stretch superimposed on the sharp band due to C-H stretch.

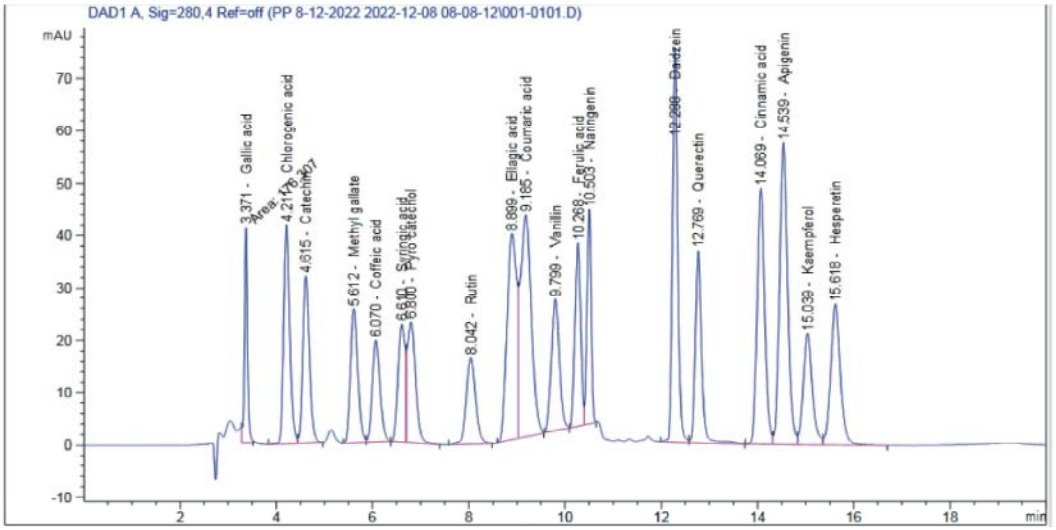


Fig. 3-B: Phenolic STD

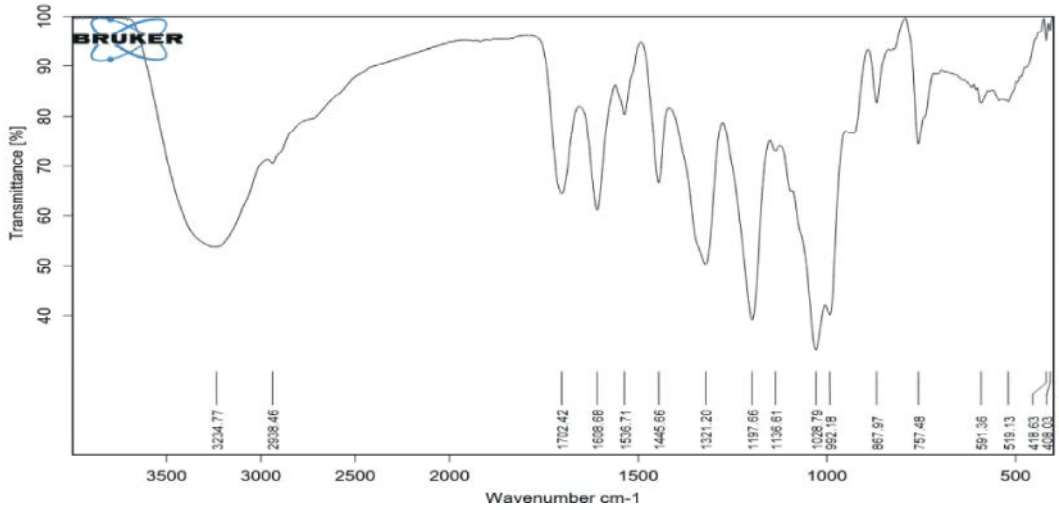
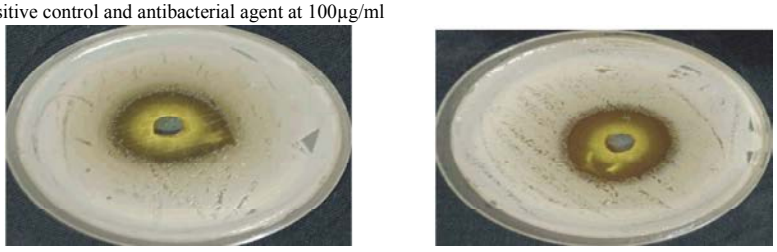


Fig. 4: FTRI of phenolic compounds at mango kernel extracts

Table 3: Antibacterial activity of *Mangifera indica* kernel extracts (MKE) and neomycin against *Staphylococcus aureus* and *Escherichia coli O.157*

	Diameter of inhibition zone or clear zone (mm)	
Sample name	*S.aureus*	*Escherichia coli O.157*
MKE (600 µL)	22	17
Neomycin (100µg/ml)*	26	23

*Neomycin was used as Positive control and antibacterial agent at 100µg/ml



S. aureus *E. coli*

Fig. 5: Inhibition zones of *Mangifera indic*a kernel extracts (Keitt) on *S.aureus* and *E.coli*. O.157. at 600 µL

Table 4: Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) of *Mangifera indica* kernel extracts (MKE) against different test microbes

	Staphylococcus aureus.		*Escherichia coli O.157*	
Sample	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
MKE Concentration	78.125	625	1250	2500

whereas, MIC = Minimum inhibition concentration, MBC = minimum bactericidal concentration

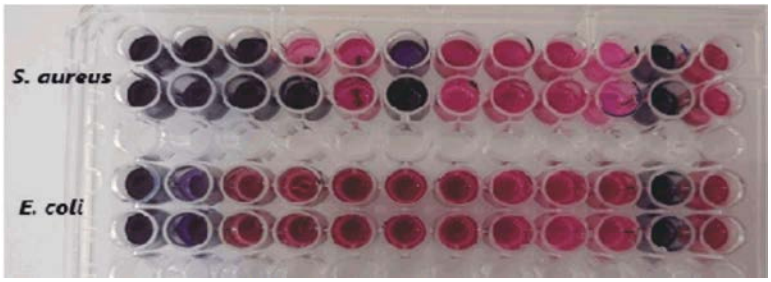


Fig. 6: MIC assay of *Mangifera indic*a kernel extracts (Keitt) on *S. aureus*, *E. coli*. O.157

Antibacterial Activity: The results of antimicrobial activity studies in Table 3 and Fig 5 showed extract of MSK exhibited higher antimicrobial activity against *Staphylococcus aureus* followed by *E. coli*. The antimicrobial activity of extracts of mango seed kernel was similar to the commercial antibiotic Neomycin which showed 22 cm and 17 cm zone of inhibition against *Staphylococcus aureus* and *E. coli*, respectively. It was found that the antimicrobial activity of mango seed kernel extract with concentration of 600 mg/ml showed maximum zone of inhibition against both gram negative bacteria *Staphylococcus aureus* and *E. coli*.

Mutua *et al*. [23] also reported the similar findings against *E. coli* bacteria. The strong antimicrobial activity exhibited by the mango kernel extracts could be due to their high amount of phytochemical composition such as flavonoids, terpenes, tannins and coumarins [33].

The antibacterial activities of *Mangifera indica* kernel extract of Kit mango were investigated by using agar well diffusion method. Antibacterial activities of extract were expressed in terms of the average diameter of the presence inhibition zone.

From the observation and measurement of diameter of inhibition zones formed, it has been demonstrated that the extracts from the *Mangifera indica* kernel (Keitt mango) had significant potential as an antibacterial compounds. The results clearly justified that the extract of have shown antibacterial activity. For the known antibiotic, which was the Neomycin, the diameter of the inhibition zones were between 23 to 26 mm.

The extracts from Kiett seed kernels gave great results, these results are agree with Mirghani *et al.* [34]; Ahmed *et al.* [35]; Kabuki [9] and Khammuang and Sarnthima [36].

On the other hand, the minimum inhibitory concentration (MIC) of the extracts was determined as shown in the in Table (4) and Fig. (6).

Besides that, the antibacterial activity against Gram-positive bacteria (*Staph.aureus*) have higher results compared to the Gram-negative bacteria (*E. coli*), whereas diameter of inhibition zone were 22 and 17 mm, respectively. One of the reasons that could possibly contribute to this phenomenon was the observation that Gram-negative bacteria are more resistant to antibiotics than are Gram-positive bacteria due to its complex outer membrane [37].

On the other hands, Intisar *et al*. [38] carried out, the methanol extract of Mangifera indica seeds, which contained coumarins, terpenes, tannins and flavonoids showed high antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, where as four phenolics and flavonoid compounds were isolated and identified by NMR and LC-MS. From the above mentioned data it be concluded that the mango Keitt kernels are rich in nutrients and could be utilized for cooking and some food products. These results reported that the mango kernels extracts had antimicrobial against gram-positive and gram-negative bacteria, moreover, the active antimicrobial component may be due to polyphenols.

Subsequently, mango seed kernel extract can use as a natural food additive for extending the shelf-life of a variety of food products, such as beef burger products, Yasser mohamoud, [39], as preservative stability of buffalo ghee (butter-fat) Puravankara *et al*. [27], for improving stability of some oils and biscuit production by Mohamed and Girgis [40], for pan bread supplementation El-Soukkary *et al*. [41], as sources of phytochemicals in

biscuit [24]. On the other hand, a research under published of using mango kernels extract as preservative in soft drinks instead of sodium benzoate.

The agro-industrial waste, especially a mango kernel, may be considered a good source of bioactive phenolic compounds, with promising uses in food and pharmaceutical products. This thereby represents an alternative use for mango waste, giving it added value (valorization) and helping to reduce its environmental effects. However, additional studies are necessary in order to establish the most suitable recovery method as well as the bioavailability and safety of bioactive compounds from mango kernel. Finally, the present work could be considered as the first report on bioactive phenolics from the Egyptian of ' mango Keitt ' cultivar.

As food spoilage is usually associated with the growth of different pathogenic bacterial strains and oxidation of food components, further study is required to investigate the application of these extracts in food preservation

Cytotoxic Effect Kiett Mango Kernels on Human Normal Fibroblast Cell Line (BJ1):

Methodology of MTT Assay: The Sample Was Tested Against the Normal Human Epithelial Cell Line:

1- BJ1 (normal Skin fibroblast).

Cytotoxic activity test (In vitro bioassay on human tumor cell lines) was conducted according to Mosmann, [19] and determined by the Bioassay-Cell Culture Laboratory, National Research Centre, Egypt. Sample concentration range between (100 to 0.78 µg/ml) using MTT assay.

The present study has assessed for the first time the cytotoxic activity of mango kernel extract against normal human epithelial cell line.The results did not show negative effects on normal human epithelial cell in terms of LC_{50} of (50 µg/mL)(Lethal concentration of the sample which causes the death of 50% of cells in 48 hrs.) or LC_{90} (90 µg/mL) (Lethal concentration of the sample which causes the death of 90% of cells in 48 hrs) for methanolic extracts, this meaning to until 100 µg / ml from MKE is very safe if added in food industries as preservative material. However, we need further confirmation in animal models and responsible phytochemical isolations in pure form.

Normal cell lethality, bioassay for cytotoxicity assessment showed at LC_{50} and LC90 for the kiett kernels extract, which was not found of toxic effect among all concentrations studied extracts. Salawu *et al*. [42] have been reported that LC_{50} values in the range of 100-500 µg/mL are considered moderately toxic.

The extract of mango kernels showed the non-toxic at level LC_{50} until 453.21± 18.9 µg/mL.

The initial results indicated that the extracts could be used for food preservative applications based on the antimicrobial, antioxidant and cytotoxicity properties of the tested extract, at least with mango Keitt kernels extract, especially with has unique characteristics such as stable against heat (121°C, 15 minutes), freezing (-20°C, 16 hours) and pH treatment (pH 3 to 9), these conditions which normally used in food processing. However, efficacy, stability and safety issues need to be addressed with both in vitro and in vivo studies, these results agreement with which reported [43].

Gold-Smith *et al*. [44] presented a complete review on the potential role and action mechanisms of mangiferin against cancer. On the other hand, They described that mangiferin has potent antioxidant and anti-inflammatory effects, causing cell cycle arrest, reducing proliferation and metastasis, promoting apoptosis in malignant cells and protecting the cells and biomolecules (e.g., DNA) against oxidative stress and damage.

In addition, mangiferin exhibits low toxicity (it has a broad intake safety margin) and has not shown cytotoxic effect on normal cells; thus, mangiferin will be a candidate for cancer therapies.

Gallic acid derivates such as galloyl glucosides have been associated with the antioxidant and antiproliferative activities of mango peels and kernel extracts; Torres-León *et al*. [45] reported pentagalloyl glucoside as the major phenolic compound identified in mango seed kernel ('Ataulfo' cultivar from Mexico) and suggests that it is related with the DPPHscavenging activity of kernel extracts. Additionally, Jiang *et al*. [46] reported that the pentagalloyl glucoside from mango peels possessed potent scavenging effects on hydroxyl radicals, superoxide anions and singlet oxygen.

Namngam *et al*. [47] isolated different phenolic fractions from kernels of 'Kaew' and 'Choke-Anan' cultivars from Thailand. They observed that the rich fraction of galloyl glucosides (particularly tetragalloyl glucoside and hexagalloyl glucoside) exhibited the highest free radical scavenging activities as well as inhibitory effects against oxidation process mediated by enzymes.

The galloyl glucosides also exhibited antiproliferative effects against breast cancer (MDA-MB-231), liver cancer (HepG2) and leukemia (HL-60). These properties have been associated with their antioxidant activity, which acts on the inflammatory mechanisms that are associated with cancer development [45, 48].

Finally, the interaction effects (additivity, synergism and antagonism) between phenolic compounds have been studied. Rocha *et al*. [49] reported that the mixtures of hydroxycinnamic acids, hydroxybenzoic acids and their derivatives, exhibit important effects on the induction of differentiation and cellular apoptosis in colon, liver, prostate, breast and lung tumor cell lines.

Conclusion, mango seed kernel has attracted considerable interest from scientists due to its unique physicochemical characteristics. The recovery and utilization of valuable compounds from mango by-products is an important challenge for scientists, where contain high levels of various health-enhancing substances (phenolic compounds).

DPPH scavenging and antibacterial activities of the mango kernel extracts may be partially caused by galloyl groups since penta-O-galloyl-glucoside and gallic acid revealed high antioxidant and antibacterial activities. Therefore, the Ferulic acid -rich extracts of mango kernel may serve as natural sources and may have important pharmaceutical potential. In addition, the Egyptian mango seed kernel (Keitt cultivar) as waste seems promising as a food additive for extending the shelf-life of a variety of food products and create new sources of bioactive compounds. These could reduce environmental problems and provide a greater economic returns to agro-industries. Also, it will help to play a role in minimizing waste generation worldwide.

As the results showed constructive values, where the mango seed kernel (Keitt cultivar) fruit is rich in polyphenolic compounds were isolated, identified and quantified by HPLC and FTIR, for the first time with high resolution HPLC.

This method allowed separating and identifying 19 phenolic compounds. The highest amount of phenolic compounds were ferulic acid, Coumaric acid, Methyl gallate, naringenin, catechin, gallic acid, syringic acid, ellagic acid, hlorogenic acid, querectin, daidzein and cinnamic acid (1.06814e4 µg/ml on a dry wt basis), as far as it is known, have not been reported before in mango seeds kernel.

Based on the above results, it could be concluded that mango kernels extract could be used as a potential source for as a preservative potent, instead of chemicals preservative in addition and avoid negative effects for it and meanwhile as a potential source for enrich biological value of functional foods by phenolics compounds which have scavenging activity of free radicals as antioxidants, also addition, utilization of mango seed waste generated worldwide.

The study serves as scientific evidence for the use of mango kernel extract as a natural preservative. Further studies are needed to study the effect on other pathogenic bacteria and isolate antimicrobial compounds at this point.

These findings showed that the Mango kernel extract had strong antimicrobial activity *Escherichia coli* O157:H7 and *Staph aureus*.

However, further research is needed, especially sensory analysis and nutritional aspects to determine if the addition of Mango kernel extract would change the taste of the original food. Since the food industry is looking toward naturally occurring antimicrobials agents to control the food borne pathogens. As a result, mango kernel extract has the potential to satisfy consumer demand for a natural antimicrobial substance in food as an acceptable alternative to artificial preservatives.

Besides that, the future study must involve various extraction reagents at different concentration so that better comparisons can be achieved to make sure that the compounds are safe and has no bad effects on the health. Furthermore, study needed to identify the exact active compound underlying this high antioxidant activity and antibacterial effect.

Nomenclature: FDA: Food and Drug Administration, AAE: Ascorbic Acid Equivalent, GAE: Gallic Acid Equivalent, DMSO: Dimethyl Sulfoxide, LC: Lethal Concentration, DPPH: 2, 2-diphenyl-1-picrylhydrazyl, ZOI: Zone of Inhibition and KMKE: keitt mango kernel extract.

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