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ORIGINAL PAPER

# Phytochemicals Identification Using GC-MS in Four Extracts of Fruit Peels and Enactment of Extracts Against *Pseudomonas Aeruginosa* MZ269380

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## Abstract

Organic wastes constitute a major share of Municipal Solid Wastes. The most beneficial approach to handling such wastes is to recover the bioactive constituents by biovalorization, making full use of them in the food, pharmaceutical as well as cosmetics industry. In this paper, fruit residual waste is collected from the waste of a residential and fruit-juice centre in Purba Medinipur district of West Bengal (India). Physico-chemical analysis of the powder generated from these wastes indicates a heterogeneous nature of powder particles having a diameter ranging from 10 to 55 µm. The moisture content of the powder was nearly 4%. Important minerals like C, K, Ca, N, O, Na, Mg, Zn, Si, P and K were detected in the powder. Four different extracts were prepared from the powder using solvents: ethanol, methanol, petroleum ether and butanol. Biochemical analysis of each extract showed high antioxidant properties. Each extraction was profiled using Gas Chromatography-Mass Spectroscopy analysis to screen the presence of functional bioactive molecules. A total of 33 molecules were identified, among which notable were N-Hexadecanoic acid; Vitamine-E; Squalene; Ergosta-7; Oleic acid; Hexadecane; 1,16-Dichloro etc. Methanol, Ethanol, Petroleum ether and Butanol extracts showed cytotoxic potential against Pseudomonas aeruginosa MZ269380 having MIC (mg/Ml) values of 19.5, 12.5, 24.5 and 8 respectively. Biocompatibility assay of each extract showed no significant damage to goat's erythrocytes at MIC concentration. Biovalorisation of fruit peels produces useful phytochemicals that can be used in the biomedical, food processing and cosmetics industries.

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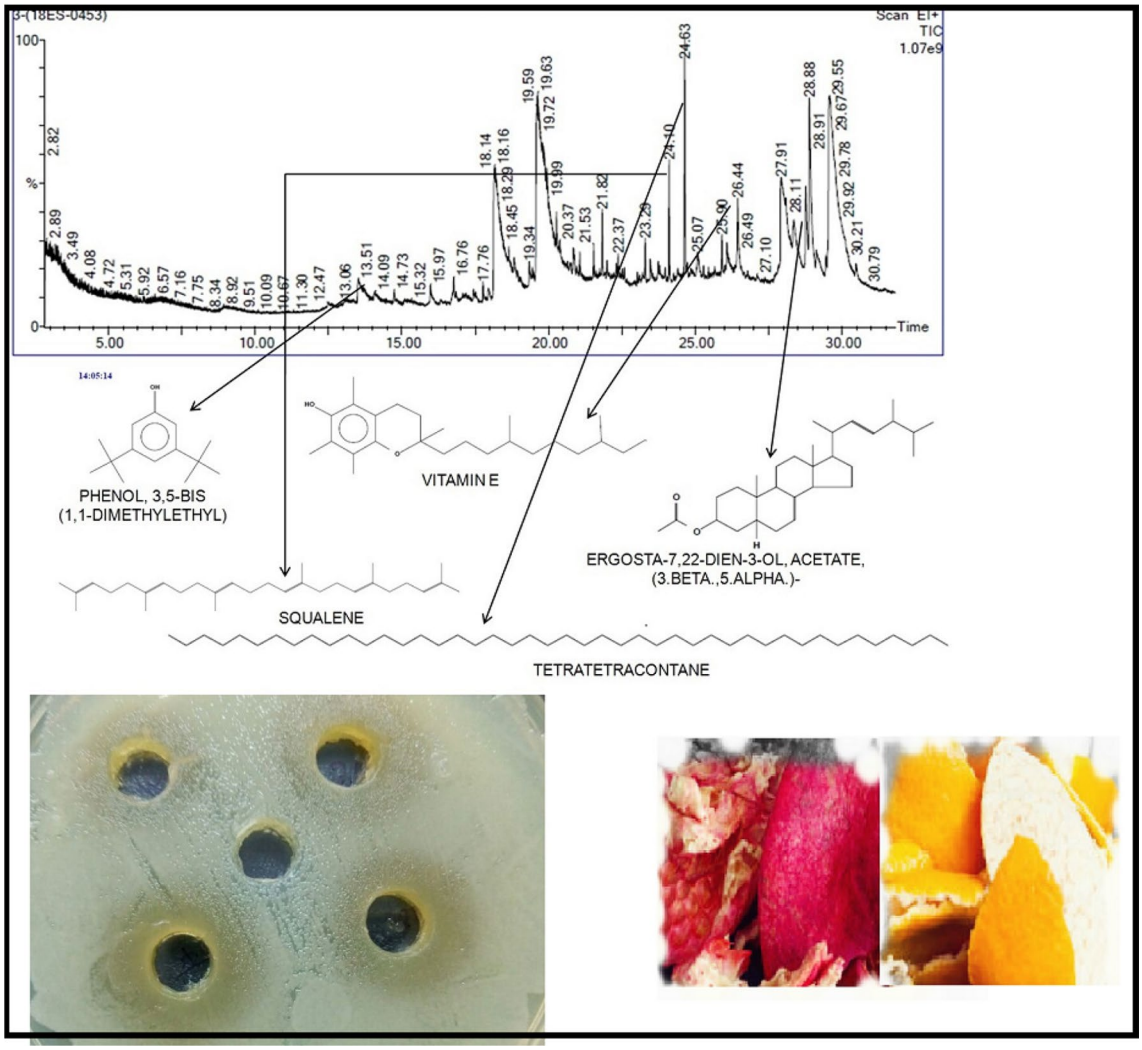
**Graphical Abstract**




**Keywords** Fruit peels · Extracts · Gas chromatography-mass spectroscopy · Phytochemicals · Antimicrobial · Haemolysis assay

## Statement of Novelty

This study investigated the potential use of fruit peels in bio-valorisation of important phytochemicals having wide application in industries. Fruit wastes collected from fruit juice centres and utilized for this study. So, isolation of important phytochemicals can be done and simultaneously Municipal solid wastes which contain the majority of organic wastes will be reduced and effectively handled. Identified bioactive molecules complete biological elucidation will help in development of various food supplements, food coatings, pharmaceutical and cosmetic products. can be reduced. This finding provides an option for converting peels of fruits into value-added products for multifunctional utilization.

## Introduction

Fruits and vegetables play a very crucial role in our diet and human life and thus the demand for such important food products has increased very constantly as a result of the expanding world population and the substituting dietary habits [1, 2]. The Food and Agriculture Organization (FAO) has quantified that up to 60% of losses and waste in fruits and vegetables are listed, which is in increased amount among all the other types of foods. The processing operations of fruits and vegetables produce notable wastes or by-products, which constitute about 25–30% of a whole product group. The organic waste generated from such food items has become

the major source of municipal solid waste (MSW), which has been coming out as a responsive environmental issue [3]. The fruit and vegetable wastes are mainly composed of seed, skin, rind, and pomace, comprised of good sources of mostly valuable bioactive compounds, such as carotenoids, polyphenols, dietary fibers, vitamins, enzymes, and oils, among others. These phytochemicals are utilized in different industries, including the food industry, where these are used for the development of functional or enriched foods, the health industry for medicines and pharmaceuticals, and the textile industry, among others. Towards sustainable development, the use of waste for the production of various significant bioactive components is an important step. The bioactive constituents found in the food wastes, their extraction techniques, and the possible application of the resulting bioactive compounds.

Bioactive compounds are those which have actions in the body that may promote good body health. Bioactive compounds are being studied for precaution and protection from cancer, heart disease, and other diseases. Dietary antioxidants are highly effective in eradication of intrinsic free radicals and therefore prevention of various diseases including amyloid beta (Aβ) aggregation [4]. These food bioactive compounds occur in small quantities in foods and are extra nutritional constituents. Many bioactive compounds have been discovered having various functional properties owing to their chemical nature. These bioactive compounds are grouped accordingly, and they vary widely in function and chemical structure. Accordingly, the methods which are used to examine and analyze these compounds are of engrossment. Many attempts have been made to provide selective and sensitive analytical processes for the easy determination and characterization of bioactive compounds. The solvent extraction method is one of the most important factors affecting the extraction efficiency of bioactive compounds from plant materials and their consequent health benefits. The solvent system selection mainly depends on the definite chemical nature of the bioactive compounds being besieged. The extraction of water soluble (hydrophilic) compounds from biological tissue required polar solvents like acetonitrile, methanol, ethanol or water, and few more whereas, for non-polar (lipophilic) compounds, n-hexane and chloroform diethyl ether or dichloromethane. Among the commonly used solvents, hexane and water reported to be having least and highest polarity respectively. Mixture of non-polar and polar solvents in different ratios helped in fractionation of different phytochemicals during the purification process. Apart from polarity of bioactive compounds, thermal stability is also an important parameter. Therefore, the extraction procedures should be wisely followed. Commonly practiced methods are heating under reflux, sonification, soxhlet extraction and others [5, 6]. Koeffi and co-workers reported that antioxidant rich compounds best extracted in methanol as solvent system [7]. In Ivorian plants extract prepared in ethanol exhibited higher concentrations of phenolics compared to acetone, methanol and water [8]. There are many bioactive compounds, among which flavonoids are the most abundantly found in fruits. It is suggested that to prevent and lower the risk of chronic diseases, dietary intake of flavonoids is advised [9, 10]. According to recent research, flavonoids exhibit a common molecular mechanism of action that widely hinders cell proliferation and angiogenesis, downregulates endogenous antioxidants, and modulates transcription factors and their associated kinases [11]. Fruit peels contain a large amount of antioxidant compounds compared to the other fruit fractions [12, 13] and antimicrobial molecules [14]. The peels have higher amounts of polyphenols and flavonoids compared to the fruit flesh. Anthocyanin, flavonol, kaempherol and xanthone glycoside were also isolated from peels of many fruits [15, 16].

Bioactive compounds include various steps such as extraction, isolation, purification, identification, and further characterization processes. The aim of this dissertation is to ascertain phytochemicals present in ethanol, methanol, petroleum ether and butanol extracts in fruit peels by GC–MS and thereafter, evaluation of antioxidant and antimicrobial properties. Biocompatibility of all extracts was evaluated by hemolysis assay using goat erythrocytes.

## Materials and Methods

### Sample Collection and Processing

The fresh peels of fruits collected from residential and fruit-juice centre in Purba Medinipur district of West Bengal (India) were from pomegranate and orange fruits. The collected samples were washed uniformly in running tap water, followed by rinsing in sterile double distilled water. The peels were made into small pieces and uniformly exposed to the sun for drying. The sun dried peels were powdered in a mechanical grinder into fine powder, passed through a sieve having fine pores to get homogenous particle size. The powder was kept in air tight containers for further experiments.

### Determination of Moisture Content

The moisture content of the peel powder was evaluated according to Dutta and Singh [17]. Briefly, powder sample fresh weight was measured before and after the water elimination by evaporation by moisture analyzer (Kern-MLS). Percentage moisture in the sample was determined using the formula,

$$Moisture(\%) = W1 - W2/W1 \times 100$$

where, $W_1$ = Initial Sample Weight and $W_2$ = Final Sample Weight.

## Physiochemical Characterization of Fruit Peels Powder

The fine powder of fruit peels was studied using Scanning Electron Microscope (SEM) by a ZEISS-Scanning Electron Microscope (SEM) to get the size, homogeneity and morphological features of particles in the sample. Images were captured by the proprietary JEOL software [18]. Energy Dispersive X-Ray Spectroscopy (EDS) was performed to study for the minerals present in the powder sample.

## Soxhlet Method of Extracts Preparation

The extraction procedure was carried out according to Redfern and co-workers with minor modifications [19] 0.100 mg of dried powder was filled on the thimble and positioned inside the Soxhlet apparatus with 500 ml of each solvent separately for 5 h. After the extraction process, it was filtered using filter paper (Whatman No 1) and thereafter, allowed to evaporate to dryness using a vacuum evaporator. The weight of dried extracts was recorded and the stock solution of 10 mg/mL was made using 1.0% DMSO.

## Phytochemicals Screening of Extracts

Dissection of plant extracts for profiling the various phytochemicals in plant tissues is important to check the nutritional and medicinal importance of plant specimens. Standard methodologies including GC-MS studies were followed to evaluate the important bioactive compounds present in each extract [17, 20]. The characterization of each identified component in the extracts was studied by the National Institute of Standards and Technology (NIST) library [21].

## Antimicrobial Property Evaluation of Extracts

In vitro antimicrobial activity of the extracts was screened against *Pseudomonas aeruginosa* MZ269380. The stock culture of test bacteria was maintained at −20 °C in nutrient medium with glycerol (50%). Active cultures were prepared by inoculating fresh nutrient broth (Luria-Bertani) medium with a loopful of cells from the stock cultures at 37 °C for overnight. To get desirable cell counts for bioassays, overnight grown bacterial cells were subculture in fresh nutrient broth at 37 °C. Agar well diffusion assay and Minimum Inhibitory Concentration (MIC) estimation was done to evaluate antimicrobial potential of the extracts on test bacteria.

The Agar-well diffusion method was studied following the procedure of Singh and co-workers [22]. Bacterial suspensions (100 µl) from overnight grown culture were uniformly spread with autoclaved glass spreader on nutrient agar plates of diameter 90 mm. For the petri-plate, five wells of diameter 6 mm were made and filled with varying concentrations (0, 25, 50, 75 and 100 mg/mL). The positive and negative controls used in the study were Ceftriaxone antibiotic and Phosphate buffer (pH 7.4). The plates were then incubated at 37 °C for 24 h. After completion of the incubation period, growth inhibitions around the wells for each extract and antibiotic were recorded in mm and tabulated.

Minimum Inhibitory Concentration (MIC) of four extracts against *P. aeruginosa* MZ269380 was determined to check the antimicrobial potential present in all extracts [23]. So, to determine the MIC of each the extract, standard procedure was followed. Here, varying concentrations of each extract and antibiotic (0–100 µg/ml) and fresh bacterial suspension in nutrient medium ($1 \times 10^5$ cells/ml) were added to each experimental set, and incubated at 37 °C overnight in an incubator with shaker (130 rpm). Absorbance at 600 nm indicates bacterial growth. The decrease in absorbance at 600 nm demonstrates antimicrobial activity of the extracts. After termination of the incubation period of 16 h, addition of 5 mg/ml concentration of 2,3,5-Triphenyl Tetrazolium chloride (TTC) was done to each set and further incubated for 1 h. TCC is a growth indicator. TTC is reduced to red formazan and thus the change from colorless to red color, which indicates the viability of the bacterial cells. The MIC of each extract and antibiotic against the test bacterial isolate was determined by observing the color change of TTC in each experimental set. The concentrations at which no changes of color occur indicate complete cell death.

## Hemolysis Assay

Haemolytic assay on goat erythrocytes was done to check the haemo-compatibility of the extract constituents according to our standardized protocol [23]. The haemolysis assay was performed for seven different experimental setups; (a) 0.1% Triton X100 (as a positive control), (b) Untreated Erythrocytes (negative control), (c) Ethanolic extract, (d) Methanolic extract, (e) Petroleum ether extract, (f) Butanolic extract and (g) Ceftriaxone. In brief, fresh goat blood sample was collected from local butcher shop (Haldia). Plasma and serum were removed from the sample by centrifuging at 5000 rpm at 4 °C for 5 min. Thereafter, each extract and antibioitic (MIC values) in PBS (pH 7.4) was mixed with the blood cells and incubated for 1 h at 37 °C. PBS and 0.1% Triton X-100 was used as negative and positive control, respectively. After completion of the incubation period, the cells were centrifuged and the absorbance of the supernatant containing ruptured erythrocytes is measured at 540 nm.

The percentage hemolysis was determined by the following equation:

$$Hemolysis(\%) = [ODt - ODc/OD100 - ODc] \times 100$$

where $OD_t$ is the absorbance of the supernatant from samples incubated with the particles; $OD_c$ is the absorbance of the supernatant from controls (normal saline); OD100% is the absorbance of the supernatant of positive controls incubated in the presence of 0.1% Triton X-100, which causes complete lysis of RBCs.

### Statistical Analysis

All experimental results were presented as Mean ± SE. Mean data of the results obtained was analyzed using ANOVA and subjected to least significant difference (LSD) wherever required. Experiments were conducted at least three times.

## Results and Discussions

### Fruit Waste Collection and Sample Preparation

Segregation of fruit residuals from organic waste was done by the fruit vendors of Haldia, West Bengal. Fruit residues collected had significant amounts of fruit peels and seeds of

**Table 1** Total yield of phytochemicals in extracts prepared from fruit peels powder

| Extract | Yeild (mg/g) |
|---|---|
| Methanol extract (ME) | 245 ± 6.4 |
| Ethanol extract (EE) | 26.3 ± 0.7 |
| Petroleum ether extract (PEE) | 3.2 ± 0.4 |
| Butanol extract (BE) | 21.3 ± 0.4 |

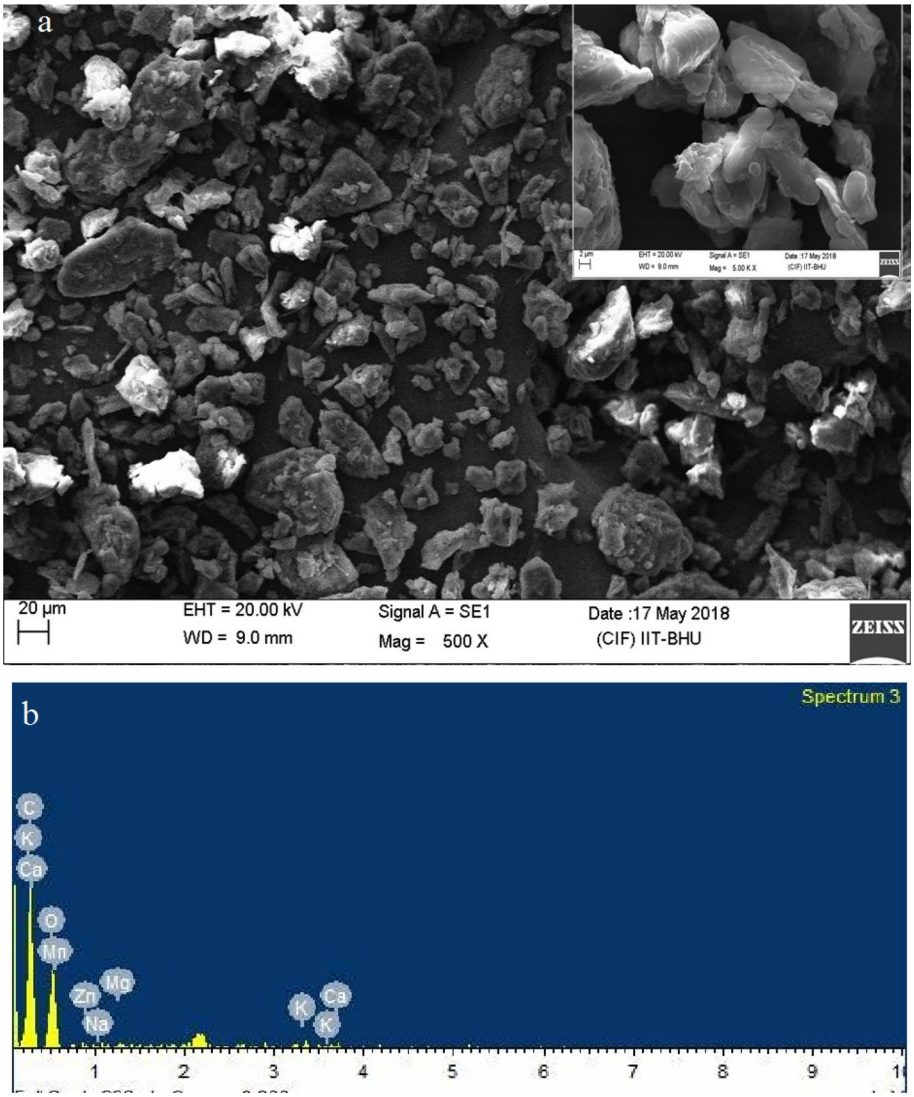



**Fig. 1** Physico-chemical study of fruit peels powder. **a** SEM images of the fruit peel fine powder of magnification (500 X). Inset SEM images of magnification 5.0 K X. **b** EDX of sample exhibiting presence of various minerals

pomegranates and oranges. The fine powder prepared from the dried peels of both pomegranate and oranges was utilized for preparation of solvent extracts (Sect. 2.3). Powder generated from these samples was used to make four different extracts using Methanol, Ethanol, Petroleum Ether and Butanol using Soxhlet apparatus. The residue generated from fruit vendors, if not properly disposed, can be the source of many harmful organisms, including pathogenic microbes.

## Physiochemical and Morphological Characterization of Fruit peel Powder

The moisture content of powder derived from fruit waste was 4.25%. The sundried fruit samples exhibited low moisture content, which indicates that the sample can be stored for a long duration because high moisture content in the sample promotes microbial growth [26]. Fruit is reported to be the storehouse of important minerals having usefulness for human health. pH of the powder when dissolved in deionised water was 3.5. SEM images of the sample at 500 X magnification showed powder having heterogeneous sized particles (5–40 µm ) with sharp edges and porosity. The inset image (Magnification 5.0 KX) showed an undulating surface with diverse shaped pores of varying Fig. 1). EDS micro analysis of powder revealed the presence of different minerals as shown in the figure. Visual analysis from the image clearly reveals that there is high Carbon, Potassium and Calcium in the powder. Minerals like Zince, Magnesium and Sodium are also present in the sample.

**Table 2** Phytochemicals screening of solvent extracts prepared with peel powder

| Phytochemicals | Extracts | | | |
|---|---|---|---|---|
| | Ethanolic | Methanolic | Petroleum ether | Butanolic |
| Amino acids | ++ | + | + | ++ |
| Anthraquinones | +++ | + | +++ | − |
| Phytosterol | ++ | ++ | + | + |
| Tannin | ++ | + | + | ++ |
| Cardiac glycosides | ++ | ++ | + | + |
| Reducing sugar | ++ | ++ | + | ++ |
| Phenols | + | + | + | ++ |
| Flavanoids | ++ | ++ | ++ | + |
| Alkaloids | ++ | + | + | ++ |
| Tri-terpenoids | + | + | ++ | + |
| Resins | + | + | − | + |

+ present; − Absent

## Qualitative Phytochemical Analysis

The final yield of four extracts is presented in Table 1 and stock solution each extracts of 10 mg/mL was prepared using 1% DMSO for further experiments. The sample of each extract was used for screening commonly reported phytochemicals. The results are given in Table 2 which showed the presence of amino acids, anthraquinones, phytosterols, tannins, cardiac glycosides, reducing sugar, phenols, flavanoids, alkaloids, tri-terpenoids and resins. The presence of such important bioactive components in significant amounts isolated from fruit waste demonstrates that MSW containing fruit wastes can be reduced by utilizing these wastes to isolate important phytochemicals [23]. These phytochemicals are reported to have important applications in food pharmaceuticals, pharmaceuticals and cosmetics [23, 24].

## Quantitative Phytochemical Analysis

The results of quantitative analysis of various phytoconstituents of ethanol, methanol, petroleum ether and butanol extracts of fruit peels powder are shown in Table 3. The total protein content was estimated for each of the extracts using the standard plot of BSA solution ($y = 0.0034x + 0.0044$; $R2 = 0.9989$). The total carbohydrate content is calculated from the standard curve of glucose ($y = 0.0012 + 0.867$, $R2 = 0.9945$). The total phenolic content was estimated from the standard curve of gallic acid ($y = 0.014x\text{-}0.036$, $R2 = 0.9031$). The total flavonoid content was evaluated on the standard curve of quercetin ($y = 0.0001x + 0.011$; $R2 = 0.988$). The total Vitamin C of each extract is estimated by an oxidation-reduction method using ascorbic acid as standard. The result showed that the fruit peels are rich in important phytochemicals which are related to antioxidant

**Table 3** Total protein content (TPC), total carbohydrate content (TCC), total phenol content (TPC), total antioxidant capacity (TAC ), total flavanoid content (TFC in), Vitamin C in and LD50 in µg for radical scavenging of each extract

| Extract | TPC µg/mg | TCC µg/mg | TPC µg/mg | TAC µg/mg | TFC µg/mg | Vitamin C µg/mg | $LD_{50}$ of DPPH |
|---|---|---|---|---|---|---|---|
| Ethanol | 10.67 ± 0.51 | 11.72 ± 0.84 | 38.22 ± 0.81 | 160.22 ± 1.33 | 111.21 ± 1.01 | 1.01 ± 0.43 | 70 |
| Methanol | 9.12 ± 0.76 | 10.41 ± 0.82 | 33.12 ± 1.21 | 180.22 ± 1.09 | 118.33 ± 0.98 | 1.11 ± 0.88 | 56 |
| Petroleum Ether | 6.32 ± 0.19 | 6.45 ± 0.54 | 28.87 ± 0.76 | 175.34 ± 0.88 | 120.22 ± 1.11 | 1.38 ± 0.67 | 62 |
| Butanol | 10.13 ± 0.23 | 11.28 ± 0.34 | 36.34 ± 1.33 | 156.23 ± 0.97 | 109.11 ± 0.76 | 1.21 ± 0.48 | 43 |

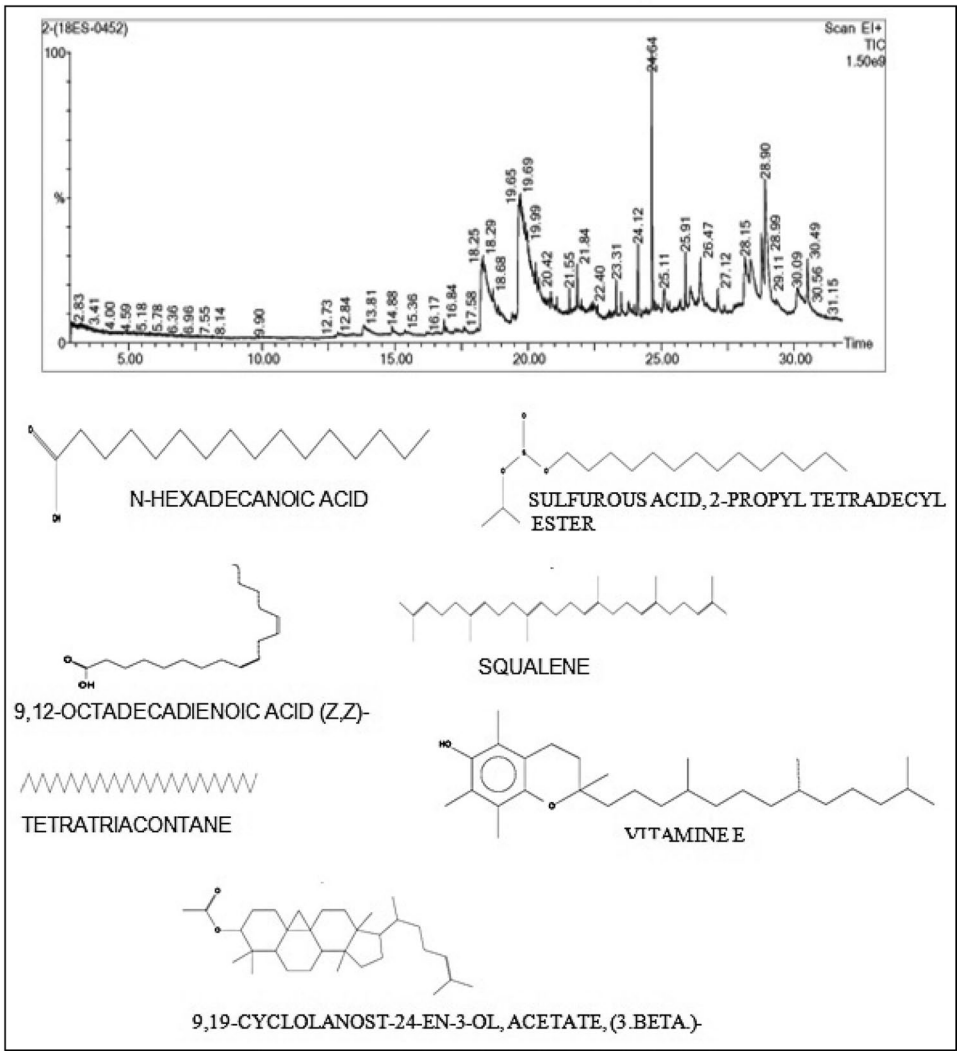



**Fig. 2** GC-MS chromatogram of fruit peels ethanolic extract. Chemical structure of selected bioactive components of the extract

and antimicrobial properties. The total antioxidant capacity is significantly high in the all extract, which demonstrates good free radical scavenging activity. In this study, DPPH· is used as a source of stable free radical. DPPH· is widely used to test the potential of any chemical compound or herbal extracts in eradicating free radicals [25]. The $LD_{50}$ value, a measure of the extract concentration that was required for 50% inhibition of the free radical DPPH·, was determined and was recorded for each of the extracts. Hence, dietary compounds rich in antioxidants are considered as food supplements to prevent free radical damage to cellular biomolecules like DNA, proteins and lipids. Equally, the phytomolecules be exploited for food preservation and storage.

## GC-MS Analysis of Extracts

Eextraction and characterization of phytochemicals having antimicrobial and antioxidant properties from plants have resulted in the relief of many medical complications caused by pathogens and pollutants. GC–MS have been widely used for identification and characterization of various bioactive compounds present in plants [26–29]. In this study, fruit peels powder extracts (ethanolic, methanolic, petroleum ether and butanolic) were analyzed by GC-MS for identification of various bioactive compounds present in four extracts. Bioactive compounds in each extract are identified and were shown in Figs. 2, 3, 4 and 5. Apart from a few common compounds each extract has a different compound identified in them. The National Institute of Standards and Technology (NIST) library (NIST Chemistry Web Book, 2008) identified the vital bioactive molecules in the extract [21]. The GC-MS data of the four extracts were analyzed in detail and were individually presented below.

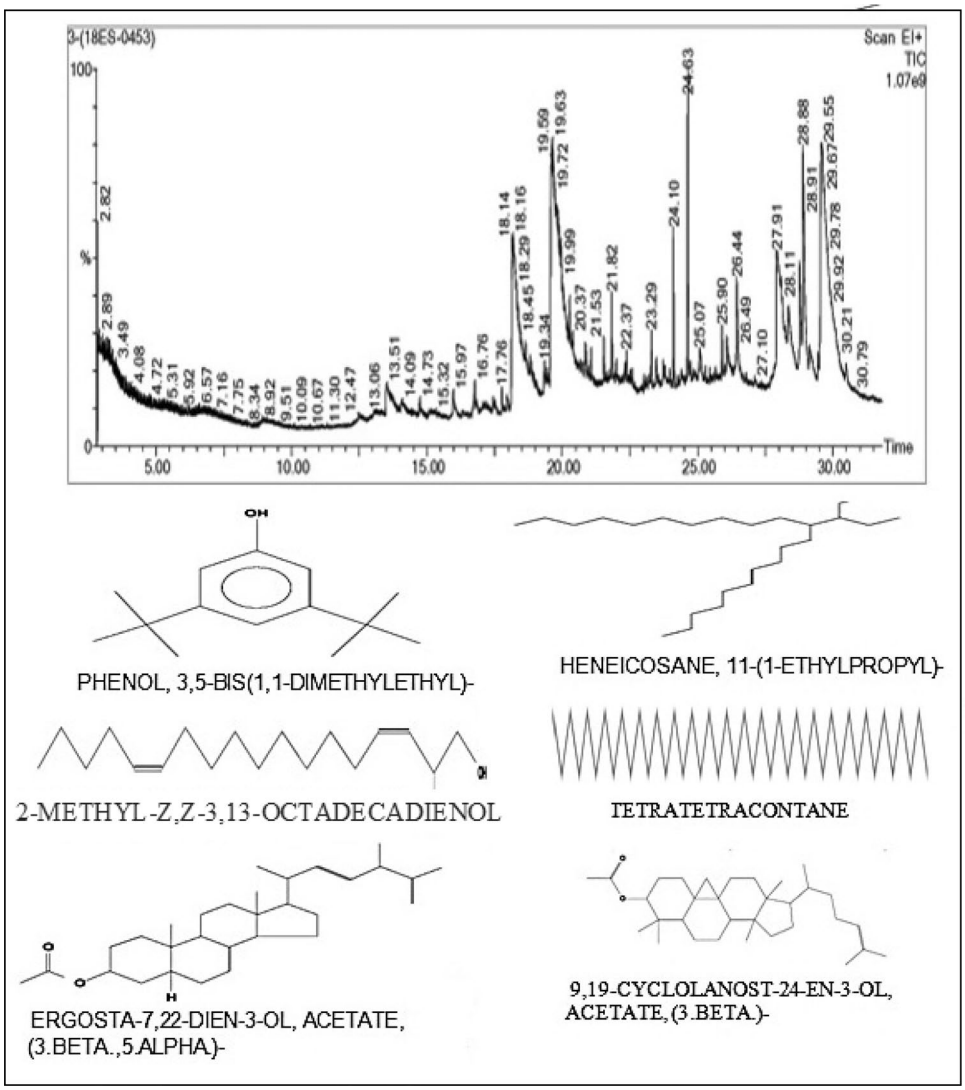



**Fig. 3** GC-MS chromatogram of fruit peels methanolic extract. The selected bioactive components identified in the extract chemical structure in the lower panel

## Ethanol Extract

GC-MS chromatogram analysis of the ethanolic extract of fruit peels (Fig. 2) showed 12 peaks. The peaks at different RT indicate the presence of 12 phytochemicals having specific functional groups i.e., chemical constituents. The twelve bioactive molecules were characterized and identified by the NIST library. The compounds identified are N-Hexadecanoic acid; Sulfurous acid, 2-Propyl tetradecyl ester; 9,12-Octadecadienoic acid(z,z); Vitamine-E; 9,19-Cyclolanost-24-en-3-ol, Acetate (3.Beta.); 7-Hexadecenal, (Z); Squalene; Tetratriacontane; Pregnan-16-one, 20-[(3-hydroxy-6-Methyl) oxacyclohex-3-yl] methyl; Tetradecane, 1-chloro-3,3-Bis-tert-butylsulfanyl-2-fluoroacrylonitrile and Oxirane, tetradecyl. MS analyzed spectrum and structure of each molecule in theethanol extracts of fruit wastes are shown in Suppl Fig. 1. Compounds identified inethanolic extract reported to have antioxidant and antimicrobial properties (N-Hexadecanoic acid). Few also have anti-inflammatory action in the biological system (squalene). The squalene acts as a defense molecule against certain pathogens causing human and animal diseases [30]. The traditional Siddha drug (Vajra kandi maathirai) cures various types of fevers and inflammatory diseases including COVID [31]. GC-MS analysis of the drug revealed its formulation. The bioactive components are 1 H-Imidazole, 4,5-dihydro-2-(phenylmethyl), and 9,12-Octadecadienoic acid (Z,Z)- a 9-Octadecenoic acid-(E) along with mercury[32]. 9,12-Octadecadienoic acid and Tetradecaneare used as flavoring agents in food [32, 33]. But Tetradecane was reported a scarcinogen and tumor inducer in mice by enhancing the mitosis cell division of murine spleen lymphocytes [33]. Oxirane is used as a chemical intermediate in fabric, foods and cosmetic industries. It is also widely used for synthetic waxes [34]. Vitamin E is used as a nutrient supplement due to its

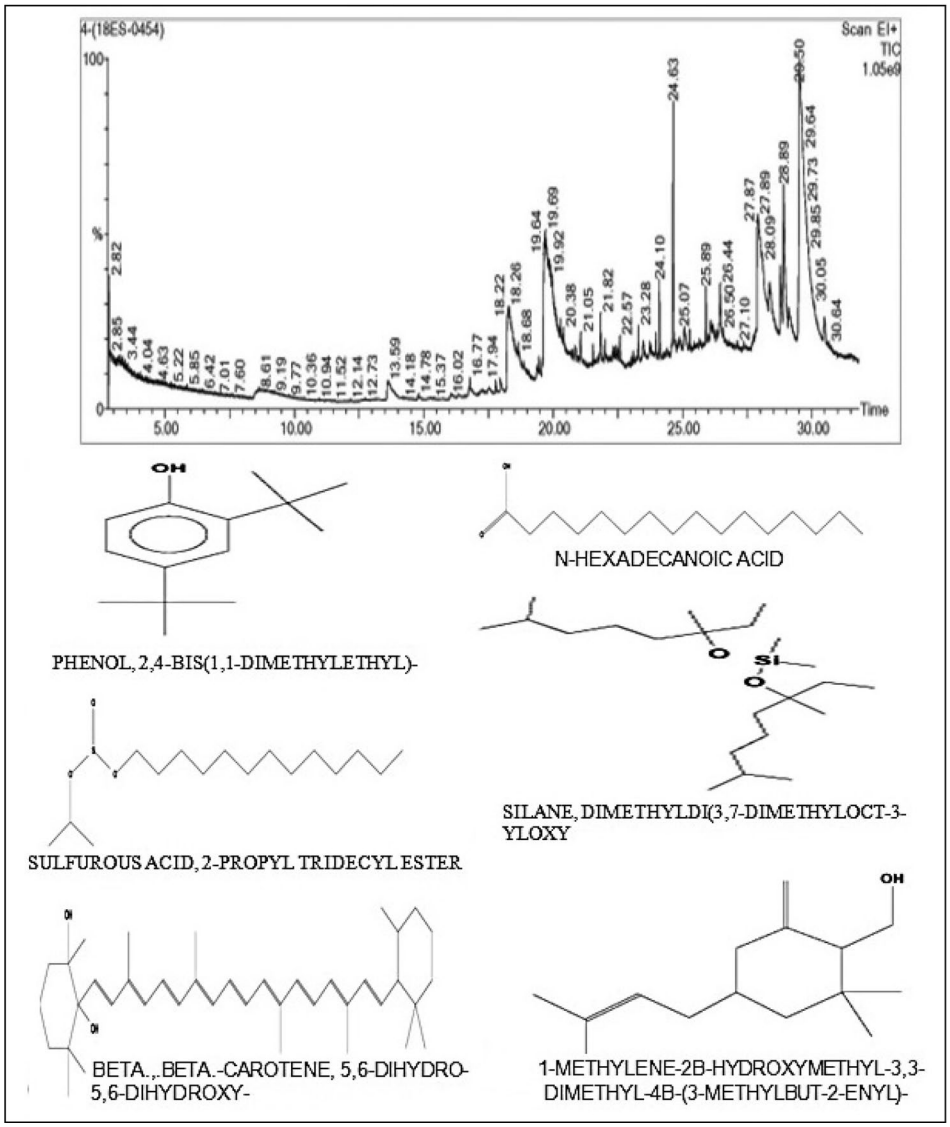



**Fig. 4** GC-MS chromatogram of fruit peels petroleum ether extract. The selected bioactive components identified in the extract chemical structure in the lower panel

diverse functions in the biological system. It helps in scavenging or retardation of oxidation reactions in cells [35].

### Methanol Extract

GC-MS chromatogram analysis of the methanolic extract of fruit peels (Fig. 3) showed thirteen peaks among which few are more prominent than others. The peaks at different RT indicate the presence of thirteen bioactive molecules of specific chemical constituents. The thirteen bioactive molecules were characterized and identified by the NIST library. The compounds identified are N-Hexadecanoic acid; Sulfurous acid, 2-Propyl tetradecyl ester; 9,12-Octadecadienoic acid (z,z;) Vitamine-E; 9,19-Cyclolanost-24-en-3-ol, Acetate (3.Beta.); 7-Hexadecenal, (Z); Squalene; Tetratriacontane; Phenol,3,5-bis(1,1-dimethylethyl)-; Heneicosane, 11-(1-ethylpropyl)-; Ergosta-7,22-dien-3-ol,acetate, (3.beta.,5.alpha.)-; 2-methyl-Z,Z-3,13-Octadecadienol and Octadecanoic acid, 2-oxo, methylester. MS analyzed spectrum and structure of each molecule in the methanol extracts of fruit wastes are shown in Suppl Fig. 1.

Plant extract having 9,19-Cyclolanost-24-en-3-ol, Acetate (3.Beta) reported to have in-vitro antibacterial activities [36]. Phenol,3,5-bis(1,1-dimethylethyl) used as a food additive because of its antioxidant property [37]. Octadecanoic acid or stearic acid used as food flavoring agent [38].

### Petroleum Ether Extract

GC-MS chromatogram analysis of the methanolic extract of *fruit peels* (Fig. 4) showed 10 peaks among which few are more prominent. The peaks at different RT indicate the presence of 12 phytochemicals of specific chemical constitutents. The thirteen bioactive molecules were characterized

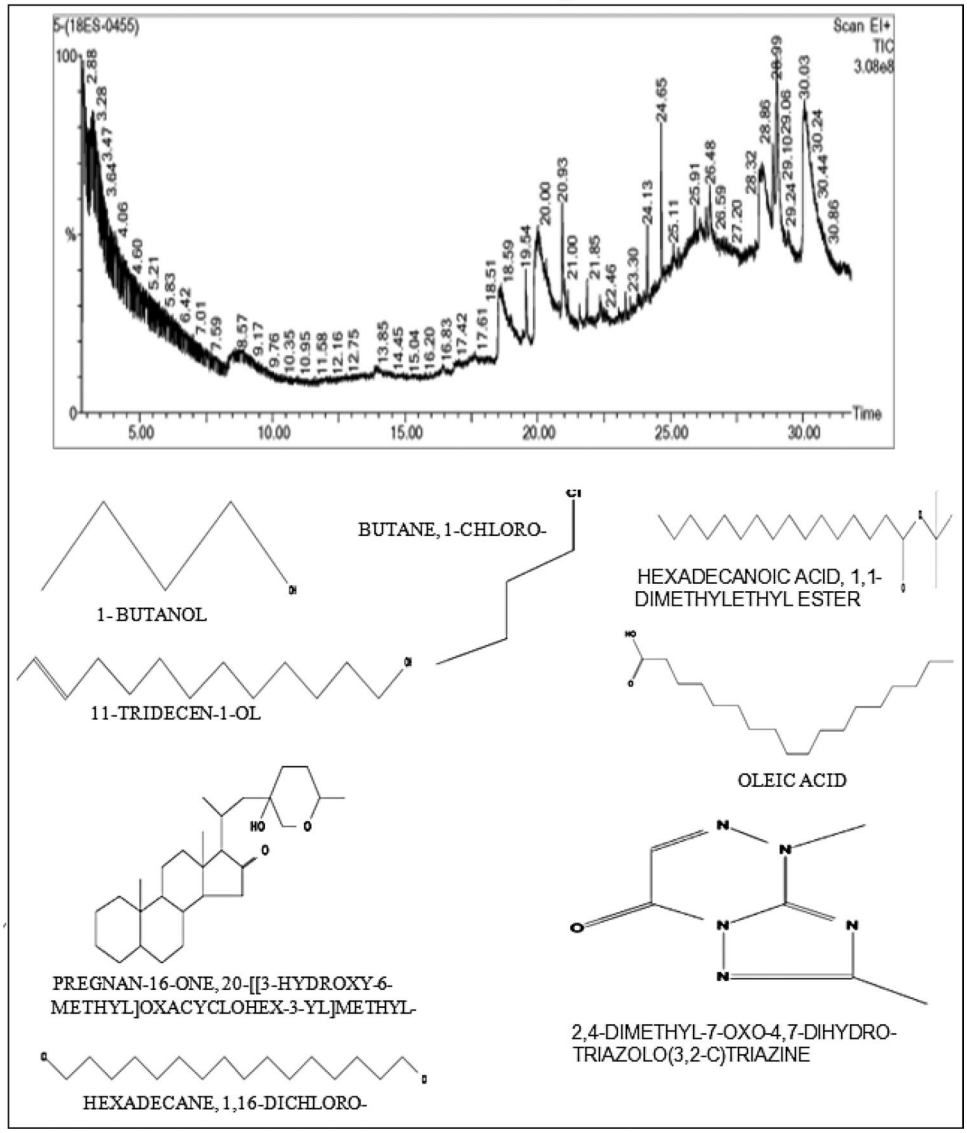



**Fig. 5** GC-MS chromatogram of fruit peels butanolic extract. The selected bioactive components identified in the extract chemical structure in the lower panel

and identified by the NIST library (Table…). The compounds identified are N-Hexadecanoic acid; Sulfurous acid, 2-Propyl tetradecyl ester; 9,12-Octadecadienoic acid (z,z); Vitamine-E; 9,19-Cyclolanost-24-en-3-ol, Acetate (3.Beta.); Phenol, 2,4-bis(1,1-Dimethylethyl)-; Tetrateracontane; Silane, Dimethyldi(3,7-dimethyloct-3-yloxy); .Beta.,.Beta.-Carotene, 5,6-Dihydro-5,6-dihydroxy- and 1-Methylene-2B-Hydroxymethyl-3,3-Dimethyl −4B-(3-Methylbut-2-enyl)-. MS analyzed spectrum and structure of each molecule in the methanol extracts of fruit wastes are shown in Suppl Fig. 1.

### Butanol Extract

GC-MS chromatogram analysis of the butanolic extract of *fruit peels* (Fig. 5) showed 14 peaks among which few are more prominent. The peaks at different RT indicate the presence of 12 phytochemicals of specific chemical constituents. The thirteen bioactive molecules were characterized and identified by the NIST library (Table 4). The compounds identified are N-Hexadecanoic acid; Sulfurous acid, 2-Propyl; 9,19-Cyclolanost-24-en-3-ol, Acetate (3.Beta.); Pregnan-16-one, 20-[[3-hydroxy-6-Methyl]oxacyclohex-3-yl]methyl; 1-Butanol; Butane, 1-Chloro-; Hexadecanoiv acid, 1,1-Dimethylethyl ester; 11-Tridecen-1-ol; Z-8-Methyl-9-tetradecenoiv acid; Oleic acid; 1-Octadecyne; Heptacosane, 1-chloro; 2,4-Dimethyl-7-oxo-4,7-Dihydro-trizolo (3,2-C)triazine; 4,22-Stigmastadiene-3-one and Hexadecane, 1,16-Dichloro.

Oleic acid is having Flavoring Agents in food additives [39], inhibitors of fatty acid and cholesterol biosynthesis [40]. It is used for formulations of many therapeutic or cosmetic products. It has special properties by which percutaneous absorption of cosmetics and drugs get enhanced

**Table 4** Phytomolecules identified by GC-MS study of four extracts prepared from fruit peels powder

| S.No. | Compound | Ethanol | | Methanol | | Pet-Ether | | Butanol | |
|---|---|---|---|---|---|---|---|---|---|
| | | RT-Value | %Area | RT-Value | %Area | RT-Value | %Area | RT-Value | %Area |
| 1 | N-Hexadecanoic acid | 18.295 | 15.776 | 18.159 | 15.153 | 18.275 | 9.741 | 18.595 | 8.039 |
| 2 | Sulfurous acid, 2-Propyl tetradecyl ester | 18.67 | 2.105 | 18.64 | 0.987 | 18.65 | 1.566 | X | X |
| 3 | 9,12-Octadecadienoic acid (z,z) | 19.705 | 38.910 | 19.63 | 23.422 | 19.69 | 19.501 | X | X |
| 4 | Vitamine-E | 28.148 | 5.664 | 27.913 | 10.377 | 26.443 | 1.469 | X | X |
| 5 | 9,19-Cyclolanost-24-en-3-ol, Acetate (3.Beta.) | 28.769 | 2.628 | 29.579 | 23.804 | 29.514 | 36.961 | 28.994 | 9.206 |
| 6 | 7-Hexadecenal, (Z) | 20.39 | 2.708 | 20.37 | 1.088 | X | X | X | X |
| 7 | Squalene | 24.122 | 1.936 | 24.102 | 1.255 | X | X | X | X |
| 8 | Tetratriacontane | 30.094 | 4.683 | 24.627 | 2.861 | X | X | X | X |
| 9 | Pregnan-16-one, 20-[[3-hydroxy-6-Methyl]oxacyclohex-3-yl]methyl | 28.349 | 5.362 | X | X | X | X | 28.453 | 13.464 |
| 10 | Tetradecane, 1-chloro- | 24.642 | 5.367 | X | X | X | X | X | X |
| 11 | 3,3-Bis-tert-butylsulfanyl-2-fluoro-acrylonitrile | 30.489 | 2.984 | X | X | X | X | X | X |
| 12 | Oxirane, tetradecyl- | 20.28 | 2.639 | X | X | X | X | X | X |
| 13 | Phenol,3,5-bis(1,1-dimethylethyl)- | X | X | 13.648 | -- | X | X | X | X |
| 14 | Heneicosane, 11-(1-ethylpropyl)- | X | X | 16.759 | -- | X | X | X | X |
| 15 | Ergosta-7,22-dien-3-ol,acetate, (3.beta.,5.alpha.)- | X | X | 28.353 | 3.538 | X | X | X | X |
| 16 | 2-methyl-Z,Z-3,13-Octadecadienol | X | X | 19.945 | 7.617 | X | X | X | X |
| 17 | Octadecanoic acid, 2-oxo ,methylester | X | X | 20.265 | 1.758 | X | X | X | X |
| 18 | Phenol, 2,4-bis(1,1-Dimethylethyl)- | X | X | X | X | 13.613 | 2.102 | X | X |
| 19 | Tetrateracontane | X | X | X | X | 24.627 | 2.821 | X | X |
| 20 | Silane, Dimethyldi(3,7-dimethyloct-3-yloxy) | X | X | X | X | 27.903 | 15.960 | X | X |
| 21 | .Beta.,.Beta.-Carotene, 5,6-Dihydro-5,6-dihydroxy- | X | X | X | X | 28.359 | 3.961 | X | X |
| 22 | 1-Methylene-2B-Hydroxymethyl-3,3-Dimethyl-4B-(3-Methylbut-2-enyl)- | X | X | X | X | 28.774 | 1.569 | X | X |
| 23 | 1-Butanol | X | X | X | X | X | X | 3.344 | 2.026 |
| 24 | Butane, 1-Chloro- | X | X | X | X | X | X | 3.424 | 2.072 |
| 25 | Hexadecanoiv acid, 1,1-Dimethylethyl ester | X | X | X | X | X | X | 19.545 | 1.752 |
| 26 | 11-Tridecen-1-ol | X | X | X | X | X | X | 19.995 | 18.434 |
| 27 | Z-8-Methyl-9-tetradecenoiv acid | X | X | X | X | X | X | 20.31 | 3.240 |
| 28 | Oleic acid | X | X | X | X | X | X | 20.415 | 1.251 |
| 29 | 1-Octadecyne | X | X | X | X | | | 20.926 | 2.514 |
| 30 | Heptacosane, 1-chloro | X | X | X | X | X | X | 24.647 | 2.386 |
| 31 | 2,4-Dimethyl-7-oxo-4,7-Dihydro-trizolo(3,2-C)triazine | X | X | X | X | X | X | 26.483 | 2.096 |
| 32 | 4,22-Stigmastadiene-3-one | X | X | X | X | X | X | 28.859 | 3.133 |
| 33 | Hexadecane, 1,16-Dichloro | X | X | X | X | X | X | 30.034 | 28.727 |

X: Compound not detected in the extract.

[41]. Therefore, oleic acid is used for the preparation of effective drug delivery systems. 4,22-Stigmastadiene-3-one possess antioxidant activity, metal chelating and ferric reducing power assays. Its antiproliferation activity is not yet proved [42].

Total of thirty three bioactives compounds, most of which are secondary metabolites of plants, are identified by GC-MS (Table 4). Comparative analysis of the four extracts showed two common bioactive compounds present in all such as N-Hexadecanoic acid and 9,19-Cyclolanost-24-en-3-ol, Acetate (3.Beta.). Chemical structure of identified bioactive compounds in the individual extract from fruit peel powder is illustrated along with the GC chromatogram in the Supplementary file.

## Antimicrobial study of extracts on *P. aeruginosa* MZ269380

*P. aeruginosa* MZ269380 was tested against each extract. Table 5 shows the MIC values of the extracts tested in which the growth of bacterial cells was totally inhibited. Figure 6 shows the diameter (in mm) of growth inhibition

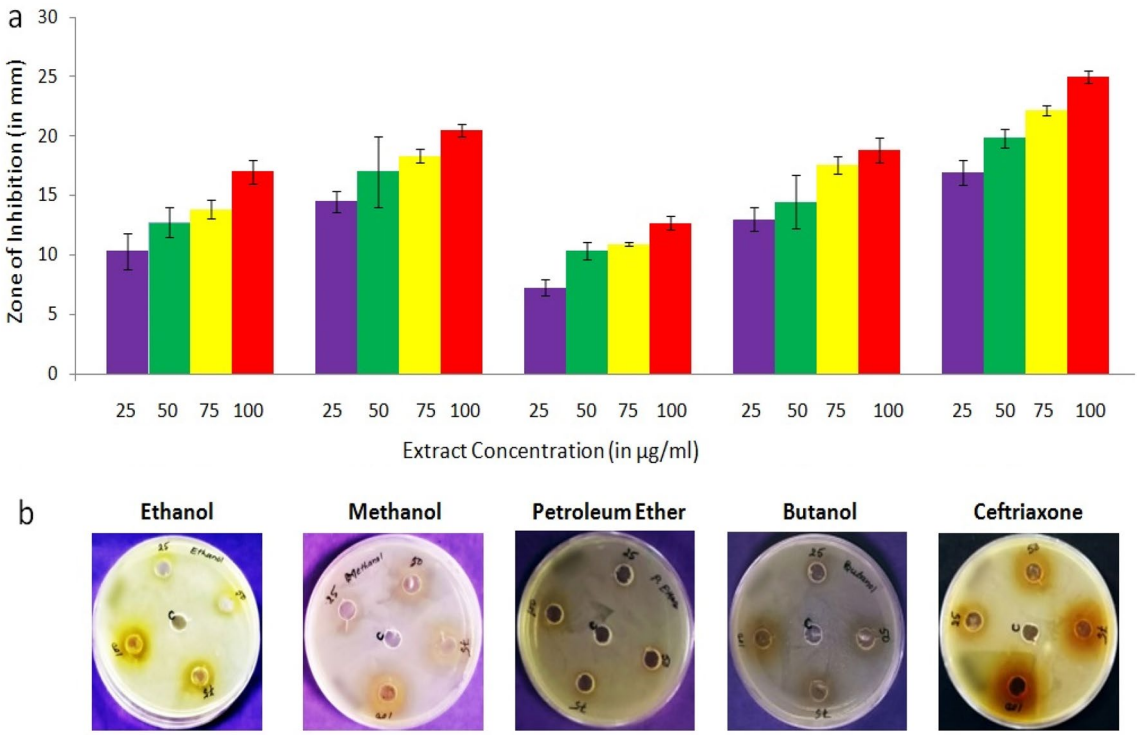



**Fig. 6** Antimicrobial study of four extracts by agar diffusion assay on *Pseudomonas aeruginosa* MZ269380. Histogram depicting zone of inhibition (**a**) and representative plates showing zone of inhibition around each well filled with different concentration of extracts. Central well of each plate is filled with negative control, Phosphate buffer. Ceftriaxone antibiotic used as negative control in this study

**Table 5** MIC values (mg/mL)

| Extract | MIC |
|---|---|
| Ethanol extract (EE) | 12.5 |
| Methanol extract (ME) | 19.5 |
| Petroleum ether extract (PEE) | 24.5 |
| Butanol extract (BE) | 0.45 |
| Ceftriaxone | |

around each well loaded with different concentrations of extracts. The clear zone around each well demonstrates dose-dependent extraction activity. Plant extracts and purified phytochemicals have been reported to have both antioxidant and antimicrobial properties. There have been a number of reports published over decades on the role of bioactive components in fruits having such properties.

Out of four extracts of fruit peels, screened for potential antibacterial activity against test bacteria, butanol and ethanol provided more prominent antimicrobial activity as compared to the other two extracts (methanol and petroleum ether). The reason for having potent antibacterial activity in ethanol and butanol extracts could be a high concentration of phytochemicals having antibacterial activity. Antimicrobial properties have been reported for N-Hexadecanoic acid [44, 45], Pregnan-16-one, 20-[[3-hydroxy-6-Methyl]oxocyclohex-3-yl]methyl; 9,12-Octadecadienoic acid (z, z), Oleic acid, Vitamin E, 11-Tridecen-1-ol, and a few others [46]. Unsaturated fatty acids have remarkable cytotoxic effects on pathogenic bacteria, including P. aeruginosa, Neisseria gonorrhoeae Porphyromonas gingivalis, Fusobacterium nucleatum and even Helicobacter pylori [46, 47]. Oleic acid, being an unsaturated fatty acid, acts as an inhibitor of FabI (bacterial enoyl-acyl carrier protein reductase). FabI is a potential target for many antibacterial drugs [48]. Vitamin E is considered the main antioxidant in biological membranes. Vitamin E helps in decreasing the MIC value of antibiotics when applied together and thus, shows a potential adjunct antibiotic treatment choice against communicable diseases caused by dreadful drug-resistant bacteria (P. aeruginosa, Burkholderia cenocepacia and methicillin-resistant Staphylococcus aureus Hartmann. According to Naguib and co-workers, vitamin E can be used to increase killing efficacy by acting as a lipocalin antibiotic binding inhibitor [49] Therefore, this vitamin enhanced the effective concentration of antibiotics around cells of bacteria, signifying that vitamin E could be used as an antibiotic adjuvant for the treatment of infectious pathogens, including multidrug-resistant bacteria. In this study remaining two extracts (petroleum ether and methanol) had MIC values of 24.5 and 19.5 respectively significantly

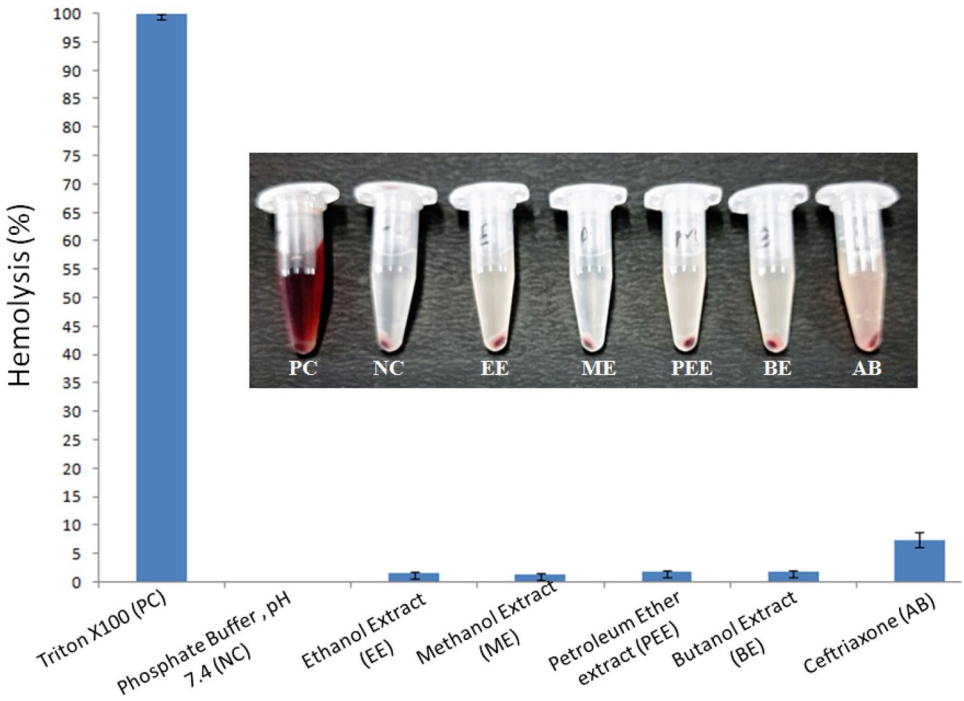



**Fig. 7** Hemolysis assay on goat's RBCs treated with four extracts along with standard antibiotic (Ceftrixone) at the respective MIC values. Phosphate buffer, pH 7.4 and Triton x 100 used as negative and positive controls respectively. Inset shows image of goat's RBCs hemolysis

higher than the former two (ethanol and butanol) against any of the assayed bacteria. These extracts may contain a low concentration of antibacterial phytochemicals or may not have antimicrobial compound (s).

### Haemo-Compatibility of Fruit Peels Extract

The hemolysis assay is a simple and important blood compatibility test since it is reported to be an indicator of cytotoxicity of the desired material. the rupturing phenomenon is also reported during contact with foreign substances due to excessive osmotic stress exerted from the incompatible material surface [51].

The hemolytic percentage of extracts was found to be promising because ruptured RBCs were insignificant for the concentration of extract tested at MIC values of each extract (Fig. 7). The percentage hemolysis for ethanol, methanol, petroleum ether and butanol were 1.59, 1.44, 1.82, and 1.91 respectively. Antibiotic at its MIC value shows hemolysis of 7.2%. Ceftriaxone antibiotic reported to induced hemolytic in child suffering from sickle cell anemia [52]. This show fragile RBCs are susceptible to this antibiotic. Our result corroborate with reported result of ceftriaxone antibiotic. ASTMF756-00 (2000) standard validated those materials with a hemolysis percentage greater than 5% are not safe for biological systems and regarded as hemolytic. Materials having hemolysis percentage of less than 2% is considered to be a nonhemolytic material [53, 54]. Our data obtained from the assay demonstrate the nonhemolytic nature of the extracts at MIC value. Thus, demonstrating biocompatible nature of extracts prepared from fruit peels.

## Conclusion

This study reveals that the biovalorisation of fruits wastes may yield industrial useful natural compounds for health benefits. The extracts prepared from fruit peels contained antioxidant and antimicrobial rich compounds along with many others having different properties like moisturizer, flavoring agents, etc. Total of thirty three phytochemicals identified by GC-MS analysis. N-Hexadecanoic acid and 9,19-Cyclolanost-24-en-3-ol, Acetate (3.Beta.) identified as common bioactive compounds in all extracts. MIC values of four extracts against P. aeruginosa MZ269380 were in the order Butanolic < Ethanolic < Methanolic < Petroleum Ether. Extracts showed biocompatibility. Hemolysis assay showed least RBC damage when treated with each extract. Hemolysis percentage was below 5%. Although, tested antibiotic ceftrixone showed high antimicrobial activity compared to four extracts, its hemolytic nature was not promising. The results show potential perspectives in exploitation of the fruits wastes using advanced techniques for isolation and purification of important phytochemicals for food, cosmetics and pharmaceutical industries.

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## Declarations

**Conflict of interest** Authors do not have any conflicts of interest.

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**Consent to Participate** NA.

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## References

1. Schieber, A., Stintzing, F.C., Carle, R.: By-products of plant food processing as a source of functional compounds-recent developments. Trends Food Sci. Technol. **12**, 401–413 (2001)
2. Vilariño, M.A., Carol, F., Caitlin, Q.: Food loss and waste reduction as an integral part of a circular economy. Front. Environ. Sci. **5**, 21 (2017)
3. Salehiyoun, A.R., Sharifi, M., Di Maria, F., et al.: Effect of substituting organic fraction of municipal solid waste with fruit and vegetable wastes on anaerobic digestion. J. Mater. Cycles Waste Manag. **21**, 1321–1331 (2019)
4. Koulakiotis, N.S., Purhonen, P., Gikas, E., Hebert, H., Tsarbopoulos, A.: Crocus-derived compounds alter the aggregation pathway of Alzheimer's Disease: associated beta amyloid protein. Sci. Rep. **10**(1), 18150 (2020)
5. Zhang, Q.W., Lin, L.G., Ye, W.C.: Techniques for extraction and isolation of natural products: a comprehensive review. Chin. Med. **13**, 20–45 (2018)
6. Abubakar, A.R., Haque, M.: Preparation of Medicinal plants: basic extraction and fractionation procedures for experimental Purposes. J. Pharm. Bioallied Sci. **12**(1), 1–10 (2020)
7. Koffi, E., Sea, T., Dodehe, Y., Soro, S.: Effect of solvent type on extraction of polyphenols from twenty three ivorian plants. J. Anim. Plant. Sci. **5**, 550–558 (2010)
8. Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D.G., Lightfoot, D.A.: Phytochemicals: extraction, isolation, and identification of Bioactive Compounds from Plant extracts. Plants (Basel). **6**(4), 42 (2017). doi: https://doi.org/10.3390/plants6040042
9. Paul, K., Kumpulainen, J., Järvinen, R., Rissanen, H., Heliövaara, M., Reunanen, A., Hakulinen, T.A., Aromaa.: Flavonoid intake and risk of chronic diseases. Am. J. Clin. Nutr. **76**(3), 560–568 (2002)
10. David, V., Rodriguez-Mateos, A., Corona, G., Oruna-Concha, M.J., Spencer, J.P.: E,: polyphenols and human health: prevention of disease and mechanisms of action. Nutrients. **2**(11), 1106–1131 (2010)
11. Panche, A.N., Diwan, A.D., Chandra, S.R.: Flavonoids: an overview. J. Nutr. Sci. **5**, e47 (2016)
12. Lim, Y.Y., Lim, T.T., Tee, J.J.: Antioxidant properties of guava fruit: comparison with some local fruits. Sunway Acad. J. **3**, 9–20 (2006)
13. Ghasemi, K., Ghasemi, Y., Ebrahimzadeh, M.A.: Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. Pak J. Pharm. Sci. **22**, 277–281 (2009)
14. Bor, T., Sulaiman, O., Aljaloud, R., Gyawali, S.A.: Antimicrobials from Herbs, Spices, and Plants. Fruits, Vegetables, and Herbs **3**, 551–578 (2016)
15. Chinnici, F., Bendini, A., Gaiani, A., Riponi, C.: Radical scavenging activities of peels and pulp from cv. Golden Delicious apples as related to their phenolic composition. J. Agric. Food Chem. **52**, 4684–4689 (2004)
16. Berardini, N., Frezer, R., Conrad, J., Beifuss, U., Carle, R., Schieber, A.: Screening of mango (Mangiferaindica) cultivars for their contents of flavanol O- and xanthone C-glycoside, anthocyanidins and pectin. J. Agric. Food Chem. **53**, 1563–1570 (2005)
17. Dutta, A., Singh, M.: Comparative analysis of aqueous extracts of Amaranth and Coriander in scavenging free radical activity and protection of DNA against oxidative damage. Chiang Mai J. Sci. **38**(4), 560–571 (2011)
18. Sathya, B.V., Velpandian, Kumar, M.P.: Physicochemical characterization andinstrumental analysis of the polyherbal siddha contraceptive formulation maavilingathymathirai. World J. Pharm. Sci. **3**(9), 789–799 (2014)
19. Redfern, J., Kinninmonth, M., Burdass, D., Verran, J.: Using soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. J. Micro Bio Ed. **15**(1), 45–46 (2014)
20. Devmurari, V., Ghodasara, T.J., Jivani, N.P.: Antibacterial activity and Phytochemical Study of Extract of Triumfetta homboidea Jacq. Int. J. Pharm. Tech. Res. **2**(2), 1182–1186 (2010)
21. Mallard, W.G., Linstrom, P.J.: (eds.) (eds.): NIST Standard Reference Database, National Institute of Standards and Technology. NIST Chemistry Web Book. (2008). https://webbook.nist.gov)
22. Das, M., Roy, S., Guha, C., Saha, A.K., Singh, M.: In vitro evaluation of antioxidant and antibacterial properties of supercritical $CO_2$ extracted essential oil from clove bud (*Syzygium aromaticum*). J. Plant. Biochem. Biotechnol. **30**, 387–391 (2021)
23. Goswami, S.R., Sahareen, T., Singh, M., Kumar, S.: Role of biogenic silver nanoparticles in disruption of cell–cell adhesion in Staphylococcus aureus and Escherichia coli biofilm. J. Ind. Eng. Chem. **26**, 73–80 (2015)
24. Chockchaisawasdee, S., Stathopoulos, C.E.: Extraction, isolation and utilisation of bioactive compounds from fruit juice industry waste. In: Utilisation of Bioactive Compounds from Agricultural and Food Waste. CRC Press, Boca Raton (2017)
25. Morsy, K.M., Enas, M., Rasha, E.: Impact of pomegranate peel nanoparticles on quality attributes of meatballs during refrigerated storage. LWT **89**, 489 (2018)
26. Dash, S., Nath, L.K., Bhise, S., Bhuyan, N.: Antioxidant and antimicrobial activities of Heracleum nepalense D Don root. Trop. J. Pharm. Res. **4**, 341–347 (2005)
27. Ralte, L., Khiangte, L., Thangjam, N.M.: GC–MS and molecular docking analyses of phytochemicals from the underutilized plant, Parkia timoriana revealed candidate anti-cancerous and anti-inflammatory agents. Sci. Rep. **12**, 3395 (2022). https://doi.org/10.1038/s41598-022-07320-2
28. Singh, A.S., Vellapandian, C.: Phytochemical Studies, antioxidant potential, and identification of bioactive compounds using GC-MS of the ethanolic extract of luffa cylindrica (L.) Fruit. Appl. Biochem. Biotechnol. **194**(9), 4018–4032 (2022)

29. Ferdous, N., Rahman, M., Alamgir, A.N.: Investigation on phytochemical, cytotoxic and antimicrobial properties of ethanolic extracts of *Centella asiatica* (L.) Urban. J. Med. Plants. **5**, 187–188 (2017)
30. Ezhilan, B.P., Neelamegam, R.: GC-MS analysis of phytocomponents in the ethanol extract of Polygonum chinense L. Pharmacognosy Res. **4**(1), 11–14 (2012). https://doi.org/10.4103/0974-8490.91028
31. Ezhilan, B.P., Neelamegam, R.: GC-MS analysis of phytocomponents in the ethanol extract of Polygonum chinense L. Pharmacognosy Res. **4**(1), 11–14 (2012)
32. Shiva, S., Mari, S., Amuthan, A., Shanmugam, R.: Repurposing Siddha mercurial drug for mild to moderate COVID-19 - case series and exploration of its chemical profile. J. Ayurveda Integr. Med. **13**(2), 100469 (2022)
33. National Center for Biotechnology Information: : "PubChem Compound Summary for CID 3931, 9,12-Octadecadienoicacid. (2022). https://pubchem.ncbi.nlm.nih.gov/compound/9_12 Octadecadienoic-acid
34. National Center for Biotechnology Information: : PubChem Compound Summary for CID 12389, Tetradecane. (2022). https://pubchem.ncbi.nlm.nih.gov/compound/Tetradecane
35. National Center for Biotechnology: : Information PubChem Compound Summary for CID 23741, 1,2-Epoxyhexadecane. (2022). https://pubchem.ncbi.nlm.nih.gov/compound/1_2-Epoxyhexadecane
36. National Center for Biotechnology: : Information PubChem Compound Summary for CID 425400, Tocopherol succinate. (2022). https://pubchem.ncbi.nlm.nih.gov/compound/Tocopherol-succinate
37. Madureira, A.M., Ascenso, J.R., Valdeira, L., Duarte, A., Frade, J.P., Freitas, G., Ferreira, M.J.: Evaluation of the antiviral and antimicrobial activities of triterpenes isolated from Euphorbia segetalis. Nat. Prod. Res. **5**, 375–80 (2003)
38. National Center for Biotechnology Information: : "PubChem Compound Summary for CID31404, Butylated hydroxytoluene (2022). https://pubchem.ncbi.nlm.nih.gov/compound/Butylated-hydroxytoluene
39. National Center for Biotechnology Information: : "PubChem Compound Summary for CID 5281, Stearic acid. (2022). https://pubchem.ncbi.nlm.nih.gov/compound/Stearic-acid,
40. National Center for Biotechnology Information: : "PubChem Compound Summary for CID 445639, Oleic acid, (2022). https://pubchem.ncbi.nlm.nih.gov/compound/Oleic-acid.
41. Gabriele, V., Gnoni, N., Francesco, J.H., Math, Geelen, S.: Luisa.: Oleic Acid as an Inhibitor of Fatty Acid and Cholesterol Synthesis, Olives and Olive Oil in Health and Disease Prevention. Academic Press. Chapter 152 - Pages 1365–1373 (2010)
42. Ruiz, M.A., José, L., Arias, Visitación, G.: Skin creams made with olive oil olives and olive oil in health and disease prevention. Academic Press, Cambridge (2010)
43. Liu, C.M., Kao, C.L., Wu, H.M., Li, W.J., Huang, C.T., Li, H.T., Chen, C.Y.: Antioxidant and anticancer aporphine alkaloids from the leaves of Nelumbo nucifera Gaertn. cv. Rosa-plena. Molecules **19**(11), 17829–17838 (2014)
44. Agoramoorthy, M., Chandrasekaran, V., Venkatesalu, M.J.H.: Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India. Braz J. Microbiol. **38**, 739–742 (2007)
45. Aparna, V., Dileep, K.V., Mandal, P.K., et al.: Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. Chem. Biol. Drug Des. **80**(3), 434–439 (2012)
46. Zahara, K., Bibi, Y., Arshad, M., Kaukab, G., Al Ayoubi, S., Qayyum, A.: *In-vitro* examination and isolation of antidiarrheal compounds using five bacterial strains from invasive species Bidens bipinnata L. Saudi J. Biol. Sci. **29**(1), 472–479 (2022)
47. Casillas-Vargas, G., Ocasio-Malavé, C., Medinam, S., Morales-Guzmán, C., Valle, D., Carballeira, R.G., Sanabria-Ríos, N.M.: Antibacterial fatty acids: an update of possible mechanisms of action and implications in the development of the next-generation of antibacterial agents. Prog. Lipid Res. **82**, 101093 (2021)
48. Wright, H.T., Reynolds, K.A.: Antibacterial targets in fatty acid biosynthesis. Curr. Opin. Microbiol. **10**(5), 447–453 (2007)
49. Heath, R.J., Rock, C.O.: Fatty acid biosynthesis as a target for novel antibacterials. Curr. Opin. Investig. Drugs **5**(2), 146–153 (2004)
50. Naguib, M.M., Valvano, M.A.: Vitamin E increases antimicrobial sensitivity by inhibiting bacterial lipocalin antibiotic binding. mSphere **3**(6), e00564-18 (2018). https://doi.org/10.1128/mSphere.00564-18
51. Yuan, W., Feng, Y., Wang, H., et al.: Hemocompatible surface of electrospun nanofibrous scaffolds by ATRP modification. Mater. Sci. Eng. C. **33**, 3644–3651 (2013)
52. Bernini, J.C., Mustafa, M.M., Sutor, L.J., Buchanan, G.R.: Fatal hemolysis induced by ceftriaxone in a child with sickle cell anemia. J. Pediatr. **126**, 813–825 (1995). https://doi.org/10.1016/s0022-3476(95)70417-5
53. Fazley, M., Elahi, G.G., Lu, W.: Hemocompatibility of surface modified silk fibroin materials; a review. Rev. Adv. Mater. Sci. **38**, 148–159 (2014)
54. Stefania, L., Anna, M.A., Claudia, B., Marco, C., Alessandro, D., Gemma, L., Claudio, R., Gabriella, T., Luana, G., Ersilia, F., Paolo, F., Ilaria, C., Agnese, M.: Evaluation of in vitro cell and blood compatibility and in vivo analgesic activity of plant-derived dietary supplements. J. Integr. Med. **17**, 213–220 (2019)