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ANTIMICROBIAL AND ANTIOXIDANT POTENTIAL OF BANANA (*Musa sapientum*) PEEL EXTRACTS OF VARIETY GRAND NAINE, MAJOR COMPOUND 5-HYDROXYMETHYLFURFURAL IN METHANOLIC PEEL EXTRACT AND ITS DERIVATIVES

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ABSTRACT

Banana peel extracts were prepared by using various solvents. Gas Chromatography-Mass Spectrometry analysis of methanolic extract showed 5-Hydroxymethylfurfural (24.78 %) as major compound which was isolated from extract and chemically transformed into two derivatives viz. [5-(3-Nitrophenyliminomethyl)furan-2-yl] methanol (2) and 5-Hydroxymethylfurfurali deneacetophenone (3). The peel extracts, 5-HMF and its derivatives were tested for their antibacterial potential against *Klebsiella pneumoniae and Pseudomonas aeruginosa,* antifungal potential against *Fusarium oxysporum* and antioxidant potential. Maximum antibacterial potential was exhibited by methanol extract with minimum inhibitory concentration (MIC) at 30 and 45 µg/ml against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, respectively. Ethyl acetate extract possessed maximum antifungal potential with MIC value at 300 µg/ml. 5-Hydroxymethylfurfurali deneacetophenone exhibited maximum antioxidant potential with median inhibitory concentration (IC_{50}) value of 25.50 and 30.00 µg/ml against DPPH and NO radical scavenging assays, respectively. Hence, banana peels can be used to form biologically active compounds as safe and biodegradable antimicrobial and antioxidants.

Keywords: Antimicrobial activity, Antioxidant activity, Banana, 5-Hydroxymethylfurfural, Methanol extract

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Over the last three decades, new synthetic antibiotics have been produced by various pharmaceutical industries to cure diseases caused by various micro-organisms. The use of such synthetic antibiotics always remains questionable due to their side effects (Sirajudin *et al.,* 2014). Moreover, the emergence of drug resistant microorganisms is one of the big challenges for the successful treatment of pathogenic diseases by using synthetic antibiotics. Therefore, researchers have mainly focused their attention on the development of better herbal drugs. Various phytochemicals present in fruits and vegetables have been screened for their numerous human health benefits (Gollo *et al*., 2020; Koss-Mikolajczyk *et al*., 2019; Mohammadi *et al*., 2019).

Among the human pathogenic gram negative bacteria, *Klebsiella pneumoniae* (*K*. *pneumoniae)* and *Pseudomonas aeruginosa* (*P*. *aeruginosa*) have leading role and pose serious threat to public health nowadays and they are mainly responsible for morbidity and mortality worldwide especially in developing countries (Sharmeen *et al*., 2012; Ulloa-Urizar *et al*., 2015). Gram negative bacteria have lipo-polysaccharide cell wall thus have ability to live in unfavourable environment. Furthermore, they develop antibiotic resistance through target site modifications, production of antibiotic inactivating enzymes, expression of efflux pumps and metabolic inactivation (Bassetti *et al*., 2018). Among the plant pathogenic fungi, *Fusarium oxysporum* (*F*. *oxysporum*) is of main concern as the fungus causes huge economic loss by inducing wilting and necrosis in major crop plants. It is difficult to eradicate as fungal spores survive in soil even in the absence of host plant. These microorganisms can survive the treatment with synthetic antimicrobial drugs making the standard treatment ineffective and infections persist. Hence, search for natural plant based antimicrobials to combat such micro-organisms are highly demanding.

Antioxidants are the substances that scavenge Reactive Oxygen Species (ROS) such as hydroxyl radical, superoxide anion, hydrogen peroxide which are generated both endogenously and exogenously causing oxidative stress and lead to a number of diseases (Lu *et al*., 2019; Ghazala *et al*., 2015; Arun *et al*., 2015). Various secondary metabolites of plant origin such as flavonoids, tocopherols, phenols, ascorbates and carotenoids are efficient free radical scavengers and have role in health care processes. Nowadays, natural antioxidants of plant origin have gained much importance as they have fewer side effects as compared to synthetic antioxidants. So, research for new plant based natural antioxidants is very active domain of research.

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Banana a tropical fruit belongs to *Musaceae* family. The plant is a perennial herb with height of 2-9 m. It has long hard pseudostem, crown of deep green large leaves and tuberous rhizome. The fruits develop in a large hanging cluster comprising of 3-20 tiers. Banana is considered as man's first food. About 16% of the total fruit production in the world is contributed by banana fruit. All parts of the plant are used for treating diseases in traditional medicinal system in India. Plant roots are used as anti-malarial, anti-leprotic and anti-stress agents. Flowers and juice of stem are used in the treatment of dysentery, diabetes, diarrhea, pain and snakebite. Fruits of the plant help in curing heartburns, anaemia and regulate blood pressure. Banana peel extracts possess various pharmacological and biological activities such as antioxidant (Mokbel and Hashinaga 2005), antitumour (Nagarajaiah and Parkash 2011), anti-inflammatory, antidiabetic, antihypersensitive (Chabuck *et al*., 2013), antifungal (Brooks 2008), antimicrobial (Shah *et al*., 2012) and antiviral (Singh and Immanuel, 2014). The peels being non-edible part of fruit constitute 30-40% (w/w) of the banana fruit and are considered as waste. Disposal of peels usually represents a problem that is further aggravated by legal restrictions. They are also prone to microbial spoilage. Therefore, new aspect regarding the use of peels as raw material for the production of value added products having pharmaceutical importance has gained lot of interest.

Various researchers have tested antimicrobial and antioxidant potential of banana peel extracts (Mordi *et al*., 2016; Siddique *et al*., 2018; Baskar *et al*., 2011). But, none of them have investigated antioxidant and antimicrobial activity of *M. sapientum* of variety Grand Naine. Moreover, antioxidant and antimicrobial activity of major compound (5-HMF) in methanolic peel extract and its derivatives have also been tested in this study and structure activity relationship was developed.

MATERIALS AND METHODS

Plant material

The banana fruits of variety Grand Naine were taken from the Department of Fruit Science, PAU, Ludhiana and were identified by Dr. Karanbir Singh Gill (Assistant Professor of Horticulture), PAU, Ludhiana. The voucher specimen (No. PAU20185632) was deposited in the Department of Fruit Science, PAU, Ludhiana.

Banana peel extracts

The peels were separated manually, washed and dipped in citric acid (0.5%) for 2 hrs to prevent enzymatic blackening. They were dried under shade for 72 hrs. The peels were then powdered using a blender with having particle size < 2mm. Various extracts of the powdered material (10 g) were prepared in 100 ml of different solvents (ethanol, methanol, ethyl acetate, dichloromethane and petroleum ether) by Soxhlet extraction for 4 hrs. The filtered extracts were filtered, evaporated to dryness, weighed and stored at 4°C until further use.

GC-MS analysis of methanol extract of banana peel

GC-MS analysis of methanol extract was performed using SHIMADZU GC MS QP 2010 using CARBOWAX capillary column and Helium as a carrier gas. Methanol extract (0.2µl diluted 100:1 in acetone) of banana peels was injected into the column at a flow rate of 1µl/minute. The injector was operated at 260 ºC and the oven temperature was initially kept at 50ºC for 5 minutes, then gradually increased to 280ºC at a rate of 10 °C/minute and kept for 10 minutes. The interface temperature was kept at 260ºC and electron input (70eV) was used as ionization mode. Simultaneous injection of a mixture of straight-chain hydrocarbons (C8 to C20) under the same conditions was also carried out into the column to obtain linear retention. Matching of mass spectra with peel extracts constituents with Wiley and NIST libraries and retention time comparison with the authentic samples was carried out to identify compounds.

Isolation of 5-hydroxymethylfurfural (5-HMF) from methanolic extract of banana peel

5-HMF (1) was isolated from the dry methanolic extract by column chromatography. For this, silica gel (600 g, 60-120 mesh size) was activated at 110°C for 1 hr and was packed in a column. The dry methanolic extract (10 g) was chromatographed over silica gel and the column was eluted with solvents of increasing polarity (hexane and ethyl acetate). 5-HMF was collected in hexane: ethyl acetate (5%) fraction as a pure compound. The identification and purity of the compound were determined by comparison with the authentic sample, thin layer chromatography and by spectral techniques.

Chemical transformations of 5-HMF

Synthesis of [5-(3-Nitrophenyliminomethyl) furan-2-yl]methanol (2)

Schiff base (2) was prepared by condensation of 5-HMF (0.01 mole, 0.97 g) with *m*- nitroaniline (0.01 mole, 1.38 g) in 15 ml of ethanol. Acetic acid (5-10 drops) was added dropwise and mixture was stirred at room temperature (Muzammil *et al*., 2015). The product was identified on basis of spectral data (Figure 1a).

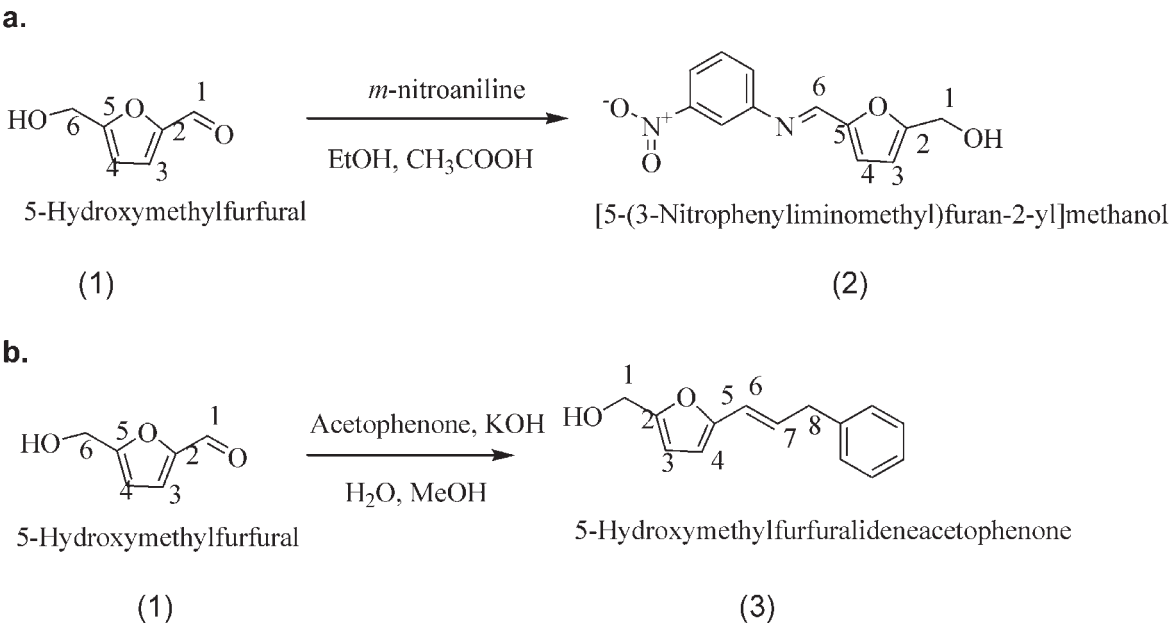


Fig. 1. a. Synthesis of [5-(3-Nitrophenyliminomethyl)furan-2-yl]methanol (2), b. Synthesis of 5-Hydroxymethylfurfuralideneacetophenone (3)

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Synthesis of 5-Hydroxymethylfurfuralidene-acetophenone (3)

5-HMF (0.02 mol, 2.52 g) and acetophenone (0.02 mol, 2.4 ml) were dissolved in 4 ml of methanol and 0.3 ml of potassium hydroxide (aq, 20%). Reaction mixture was stirred at room temperature for 7-8 hrs. Acidification of mixture was done with acetic acid (10%) to precipitate the solid (Skowronski *et al*., 1993). The resultant solid product formed was recrystallized from benzene and was confirmed by spectroscopic analysis (Figure 1b).

Determination of antimicrobial activity

Procurement of microorganisms

Two gram-negative bacterial cultures *i.e K. pneumoniae* (Accession number-KF424316.1), *P. aeruginosa* (Accession number- KF853103.1) and one fungal culture *i.e. F. oxysporum* were procured from Laboratory of Pulses Microbiology, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Pure cultures of *P. aeruginosa* and *K. pneumoniae* were maintained on nutrient agar (NA) medium slants whereas pure culture of *F. oxysporum* was maintained on potato dextrose agar (PDA) medium slants.

Screening of antimicrobial activity

Antibacterial and antifungal activity of various banana peel extracts, 5-HMF and its derivatives against *K. pneumoniae*, *P. aeruginosa* and *F. oxysporum* were tested by disc plate method and agar well diffusion technique according to standard procedure reported by Davis and Stout (1971) and Balouiri *et al*., (2016), respectively.

Determination of antioxidant activity

Antioxidant potential of banana peel extract, 5-HMF and its derivatives were tested by using two *in vitro* methods involving 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and nitric oxide (NO) scavenging assay according to the standard procedure described by Blois (1958) and Green *et al*. (1982), respectively. The percentage inhibition (I%) was calculated using formula as below:

$$I\ (\%) = \frac{\text{Absorbance of control- Absorbance of sample}}{\text{Absorbance of control}}$$

Median inhibitory concentration (IC_{50}) *i.e.,* concentration at which 50% inhibition is scavenged, was determined from the graph.

Statistical analysis

The results were expressed in terms of Critical difference (CD) for the evaluation of antimicrobial potential and p = 0.05 was accepted as significant. The results of antioxidant activity were expressed as mean ± standard deviation. Tukeys B test using SPSS version 20 was used for the evaluation of antioxidant potential data and $p< 0.05$ was accepted as significant.

RESULTS AND DISCUSSION

Yield of extracts

Yield of banana peel extracts increased with an increase in polarity of solvents. The yield of methanol extract (11.08%) was found to be highest followed by ethanol (9.5%), ethyl acetate (7.43%), dichloromethane (5.86 %) and petroleum ether (2.2%) extract (Table 1.).

Table 1. Yields of banana peels extracts prepared by using different solvents

Solvent extracts	Yield (%)
Methanol	11.08
Ethanol	9.5
Ethyl acetate	7.43
Dichloromethane	5.86
Petroleum ether	2.2

Chemical composition of methanolic extract of banana peel

GC-MS analysis of a methanolic extract of banana peel showed the presence of fifty-one compounds representing 99.61 % of the total (Table 2). 5-Hydroxymethylfurfural commonly called 5-HMF (24.78%) was major compound (Fig. 2). This was followed by cycloeucalenol acetate (21.98%), 9,12,15-octadecatrienoic acid (8.15%), 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (6.23%), hexadecanoic acid (5.79%) and 8-dodecen-1-ol acetate (5.02%). But earlier reports in literature showed the presence of pyrogallol (22.24%) and Vitamin-E (31.35%) as major compounds in the methanolic and ethanolic extract of banana peel, respectively (Niamah 2014; Waghmare and Kurhade 2014). 5-HMF have been reported to exhibit various biological activities such as antioxidant, antityrosine enzyme effects, antiischemic, morphological hepatocyte protective effects, improving blood rheology, antiapoptosis mechanism and affecting glycyrrhizin metabolism (Li *et al*., 2011; Yamada *et al*., 2011).

Characterisation of compounds

Methanolic extract of banana peel was subjected to column chromatography using hexane as solvent and polarity was increased by using ethyl acetate to isolate 5-HMF (1) as a major compound. The dark brown coloured liquid formed has boiling point ranging from 114 -116ºC. The purity of compound was checked by TLC and the structure was confirmed by spectroscopic analysis. IR spectrum of the pure compound showed broad band at 3371 cm^{-1} due to O–H stretching, 2841 and 2717 cm^{-1} due to C–H stretching of –CHO group and 1781 cm^{-1} due to C=O stretching of –CHO group. Further, this compound showed bands at 2930 cm^{-1} due to C–H stretching of methylene group attached to furan ring, 1672 cm^{-1} due to C=C stretching in furan ring, 1192 cm^{-1} due to C–O stretching in C–OH bond, 1022 cm^{-1} due to C–O–C stretching in furan ring and 966 cm^{-1} due to =C–H bending in furan ring. ^1H NMR spectrum ($CDCl_3$, 400 MHz) 'δ' in ppm showed singlet at δ 9.59 due to one aldehydic proton, singlet at δ 7.27 due to -OH group, doublet at δ 7.21 due to one proton

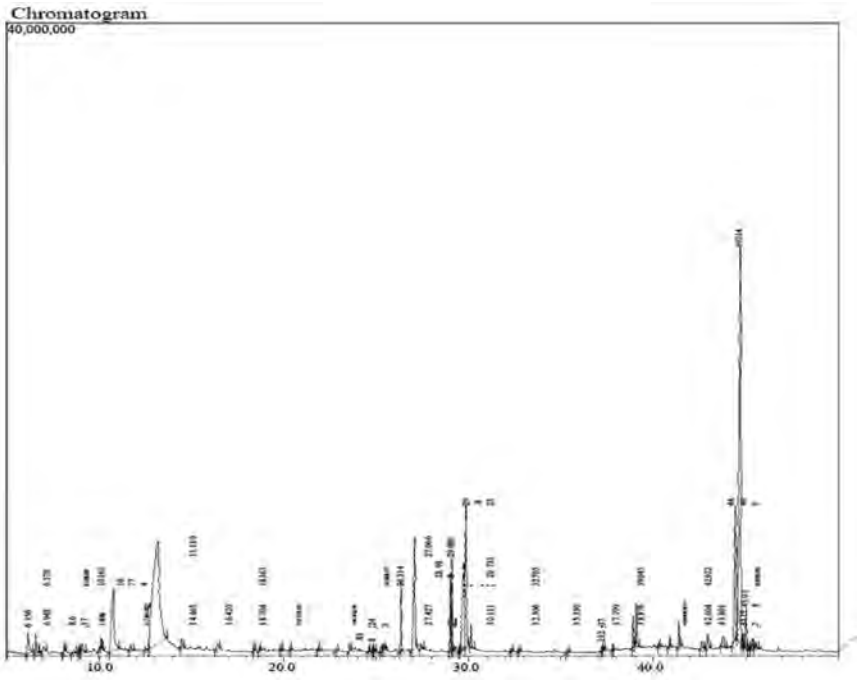


Fig. 2. GC-MS chromatogram of methanolic extract of banana peel

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Table 2. GC-MS of methanolic extract of banana peel

Retention Time (min)	Retention index literature	Retention index experimental	Area (%)	Name of compound
6.16	985	993	1.23	5-Methyl -2-furancarboxaldehyde
6.58	1156	1173	0.61	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
6.95	1023	1003	0.44	2H-Pyran-2,6(5H)-dione
8.13	1145	1106	0.36	Butyl cyclobutanecarboxylate
8.62	820	824	0.13	2,5-Dimethylfuran-3,4(2H,5H)-dione
8.90	1101	1109	0.21	2,5-Furandicarboxaldehyde
9.08	1209	1222	0.55	Neopentyl-2-furoate
10.01	1106	1115	1.03	Dimethyl malate
10.16	1058	1042	0.20	Monomethyl succinate
10.77	1251	1269	6.23	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one
11.71	1187	1193	0.35	2-Methyl-2H-pyran-3,4,5(6H)-trione
12.48	1072	1088	0.13	5-Formyl-2-furfuralmethanoate
13.19	1147	1163	24.78	5-Hydroxymethylfurfural
14.46	1287	1293	0.41	2-Methoxy-4-vinylphenol
16.42	1085	1091	1.37	Methyl-5-oxo-2-pyrrolidinecarboxylate
18.36	2204	2220	1.20	Methyl-2-heptenyl-2,6-difluorobenzoate
18.70	1537	1555	0.11	2,4-Ditert-butylphenol
19.85	1709	1720	0.21	Dodecanoic acid
20.35	1810	1818	0.09	9-Octadecene
21.86	1654	1648	1.06	9-Pentadecanone
22.82	1689	1680	0.06	Methyl tetradecanoate
23.55	1874	1869	1.26	Tetradecanoic acid
24.60	1609	1615	0.15	Methyl pentadecanoate
24.82	2145	2168	0.12	Neophytadiene
24.90	1765	1754	0.08	6,10,14-trimethyl- 2-pentadecanone
25.28	1649	1670	0.28	Pentadecanoic acid
25.44	2017	2020	0.28	1-Hydroxy-1-(m-nitrophenyl)-2-propyl 3-butanone
26.31	1863	1878	1.48	Methyl hexadecanoate
27.07	1875	1869	5.79	Hexadecanoic acid
27.43	1109	1124	0.29	9-Oxabicyclo[6.1.0]non-6-en-2-one
28.99	2079	2093	1.66	Methyl linolelaidate
29.08	2121	2101	1.99	9,12,15-Octadecatrienoic acid, methyl ester
29.17	2073	2085	1.07	Methyl 10-octadecenoate
29.49	2034	2077	0.11	Methyl stearate
29.73	1573	1588	5.02	8-Dodecen-1-ol acetate
29.82	2183	2191	8.15	9,12,15-Octadecatrienoic acid
30.11	2158	2176	0.63	Octadecanoic acid
32.31	2076	2088	0.09	2-Ethylhexyl 3-(4-methoxyphenyl)-2-propenoate
32.70	2246	2258	0.09	4,8,12,16-Tetramethylheptadecan-4-olide
35.33	2877	2853	0.14	Di-n-octyl phthalate
37.21	2049	2069	0.14	9,12-Octadecadien-1-ol
37.30	2054	2077	0.10	9,12,15-Octadecatrien-1-ol
37.78	1117	1103	0.13	3,3,5-Trimethylcyclohexanol
38.88	2634	2653	2.99	α-Tocospiro A
39.04	3517	3508	2.48	α-Tocospiro B
40.30	28159	2861	0.09	Stigmasta-4,6,22-trien-3-yl acetate
40.87	2547	2579	0.21	4,6-Cholestadien-3-β-ol benzoate
41.37	3121	3149	0.80	Vitamin E
42.60	2622	2632	0.41	Ergost-5-en-3-β-ol
42.92	2751	2778	0.84	Stigmasta-5,22-dien-3-ol
44.69	2913	2900	21.98	Cycloeucalenol acetate
Total			99.61	

Retention indices data from the literature (Andiamaharavo, 2014)

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attached to C_3 with coupling constant 3.60 Hz, doublet at δ 6.52 due to one proton attached to C_4 with coupling constant 3.56 Hz, singlet at δ 4.72 due to two protons attached to C_6 (Table 3).

The IR spectrum of compound (2) showed absence of bands at 2841, 2717 and 1781 cm^{-1} due to -CHO group which indicated that aldehyde group has reacted. It showed bands at 1624 cm^{-1} due to C=N stretching, 1498, 1515, 1563 and 1585 cm^{-1} due to C=C stretching of aromatic ring, 3086 cm^{-1} due to =C-H stretching of aromatic ring, 1166 cm^{-1} due to C-N stretching and 1312 cm^{-1} due to N=O stretching of nitro group. It also showed bands at 3398 cm^{-1} due to OH group, 1658 cm^{-1} due to C=C stretching in furan ring and 1054 cm^{-1} due to C–O–C stretching in furan ring. ^{1}H NMR (DMSO, 400 MHz) 'δ' in ppm of compound (2) showed no singlet at δ 9.59 showed absence of –CHO group. It showed singlet at δ 8.04 due to one proton attach to C_6 and multiplet at δ 7.7-8.5 due to four protons of aromatic ring. It also showed singlet at δ 4.52 due to two protons attached to C_1, doublet at δ 6.57 due to one proton attached to C_3 with coupling constant 3.36 Hz, doublet at δ 7.20 due to one proton attached to C_4 with coupling constant 3.40 Hz and singlet at δ 5.54 due to –OH group (Table 3).

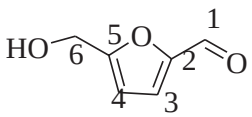
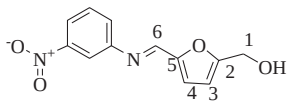
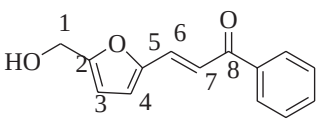
IR spectrum of compound (3) showed absence of bands at 2841, 2717 and 1781 cm^{-1} due to -CHO group which indicated that aldehyde group has reacted. It showed bands at 1606 cm^{-1} due to C=C stretching of chalcone moiety, 966 cm^{-1} due to C-H deformation of trans C=C of chalcone moiety and 1671 cm^{-1} due to C=O stretching. It showed bands at 3101 cm^{-1} due to =C-H stretching and 1426, 1448, 1471 and 1575 cm^{-1} due to C=C stretching of aromatic ring. It also showed bands at 3282 cm^{-1} due to hydroxyl group, 1636 cm^{-1} due to C=C stretching and 1079 cm^{-1} due to C-O-C stretching in furan ring. ^{1}H NMR (DMSO, 400 MHz) 'δ' in ppm of compound (3) did not show singlet at δ 9.59 indicated the absence of –CHO group. It showed doublet at δ 7.54 due to one proton attached to C_6 with coupling constant 6.64 Hz, doublet at δ 7.57 due to one proton attached to C_7 with coupling constant 6.68 Hz and multiplet at δ 7.4-8.0 indicated the five protons of aromatic ring. It also showed singlet at δ 5.76 due to two protons attached to C_1, doublet at δ 6.51 due to one proton attached to C_3 with coupling constant 3.36 Hz, doublet at δ 7.06 due to one proton attached to C_4 with coupling constant 3.28 Hz and singlet at δ 5.63 was due to –OH group (Table 3).

Antimicrobial activity

Antibacterial activity

Methanol extract of banana peel showed highest antibacterial activity against *K. pneumoniae* and *P. aeruginosa* with MIC at 30 and 45 µg/ml, respectively (Fig. 3). Petroleum ether extract showed the least antibacterial activity against both tested bacterias. Among the derivatives of 5-HMF (1), compound (3) having chalcone moiety showed highest antibacterial activity with MIC values at 45 and 60 µg/ml against *K. pneumoniae* and *P. aeruginosa,* respectively (Fig. 3). Compound (2) was found to be least effective against both the tested bacteria. All the treatments were found to be less effective than standard (tetracycline) which showed MIC value at 1 and 2 µg/ml against *K. pneumoniae* and *P. aeruginosa,* respectively.

Table 3. Spectroscopic data of 5-HMF and its derivatives

Compound	IR (cm^{-1})	^{1}H-NMR 400 MHz (δ)
 (1)	(KBr):3371 (O-H stretching), 2841 and 2717 (C-H stretching of -CHO group), 1781 (C=O stretching of -CHO group), 2930 (C-H stretching of methylene group) 1672 (C=C stretching), 1022 (C-O-C stretching) and 966 (=C-H bending)	9.59 (s, 1H, C_1),7.21 (d, 1H, ^3J= 3.6Hz, C_3), 6.52 (d, 1H, ^3J= 3.56 Hz, C_4) and 4.72 (s, 2H, C_6)
 (2)	(KBr):1624 (C=N stretching), 1498, 1515, 1563 and 1585 (C=C stretching of aromatic ring), 3086 (=C-H stretching of aromatic ring), 1166 (C-N stretching), 1312 (N=O stretching)	8.04 (s, 1H, C6),7.7-8.5 (m, 4H, ArH)
 (3)	(KBr):1671 (C=O stretching), 1606 (C=C stretching of chalcone moiety) and 966 (C-H deformation of trans C=C of chalcone moiety), 3101 (=C-H stretching), 1426, 1448, 1471 and 1575 (C=C stretching of aromatic ring)	7.54 (d, 1H, ^3J=6.64 Hz, C_6),7.58 (d, 1H, ^3J=6.68 Hz, C_7),7.4-8.0 (m, 5H, ArH)

s stands for singlet, d stands for doublet, m stands for multiplet, *J* is coupling constant

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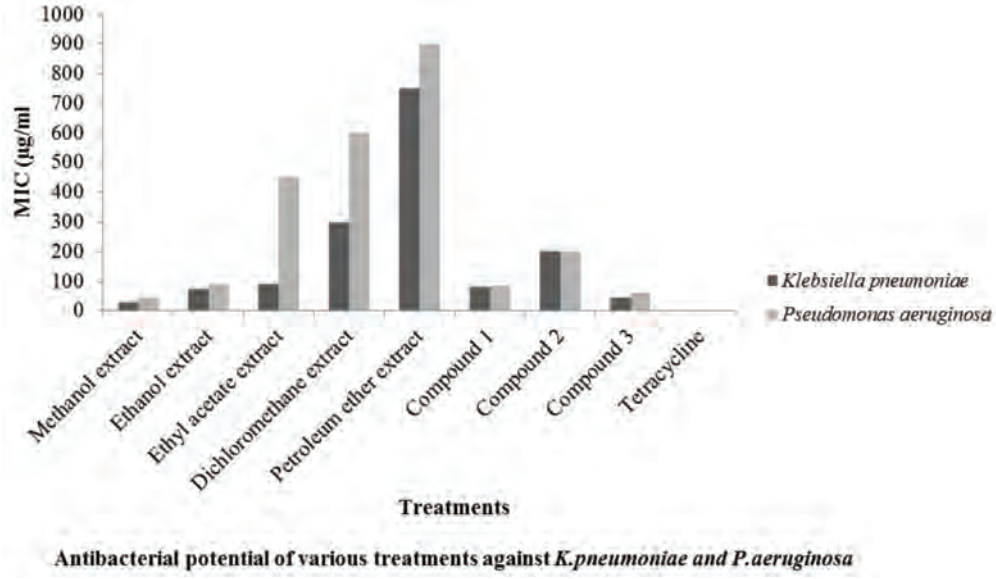


Fig. 3. Antibacterial potential of various treatments against *K. pneumoniae* and *P. aeruginosa* in terms of MIC values

On the basis of MIC values, antibacterial activity of all the prepared extracts, 5-HMF (1) and its derivatives against *K. pneumoniae* followed the order (Fig. 3):

Tetracycline > Methanol extract > 5-Hydroxymethylfurfuralideneacetophenone (3) > Ethanol extract > 5-HMF (1) > Ethyl acetate extract > [5-(3-Nitrophenyliminomethyl)furan-2-yl]methanol (2) > Dichloromethane extract > Petroleum ether extract

On the basis of MIC values, antibacterial activity of all the prepared extracts, 5-HMF and its derivatives against *P. aeruginosa* followed the following order (Figure 3):

Tetracycline > Methanol extract > 5-Hydroxymethylfurfuralideneacetophenone(3)>5-HMF (1) > Ethanol extract > [5-(3-Nitrophenyliminomethyl) furan-2-yl]methanol (2) > Ethyl acetate extract > Dichloromethane extract > Petroleum ether extract

MIC values of various extracts of banana peel, 5-HMF and its derivatives have been found to be the least in case of *K. pneumoniae* as compared to *P. aeruginosa*.

Table 4. DPPH free radical scavenging activity (%) of various banana peel extracts

Concentration (µg/ml)	Radical scavenging activity (%)						Mean values
	Methanol extract	Ethanol extract	Ethyl acetate extract	Dichloromethane extract	Petroleum ether extract	Ascorbic acid	
10	34.93±0.16	34.07±0.16	32.79±0.16	15.54±0.16	17.86±0.31	37.62±0.25	28.80^{k}
20	43.88±0.16	43.53±0.01	38.39±0.28	18.42±0.06	19.49±0.24	48.12±0.18	35.31^{j}
30	54.86±0.19	48.44±0.18	41.67±0.06	24.05±0.18	22.67±0.11	58.92±0.21	41.77^{i}
40	64.95±0.27	51.9±0.05	42.27±0.16	34.32±0.10	27.44±0.22	71.15±0.16	48.67^{h}
50	89.51±0.18	64.06±0.21	54.75±0.18	38.56±0.16	33.89±0.21	90.65±0.16	61.91^{g}
80	91.01±0.32	69.62±0.11	65.73±0.16	49.61±0.18	36.78±0.18	91.44±0.11	67.37^{f}
100	92.58±0.12	77.74±0.18	70.93±0.11	55.82±0.01	38.42±0.16	93.9±0.11	71.57^{e}
200	93.57±0.28	84.98±0.16	75.57±0.16	60.42±0.01	40.42±0.21	94.32±0.10	74.89^{d}
300	93.72±0.12	90.23±0.06	78.92±0.18	63.84±0.10	49.72±0.10	95.29±0.18	78.62^{c}
400	94.89±0.26	94.00±0.08	80.52±0.18	65.38±0.24	56.21±0.13	97.35±0.17	83.62^{b}
500	95.43±0.16	94.71±0.05	89.10±0.01	69.55±0.16	60.67±0.24	98.39±0.11	84.64^{a}
Mean values	77.14^{b}	68.56^{c}	60.97^{d}	45.05^{e}	37.9^{f}	79.74^{a}	

The data has been expressed as mean ± standard deviation. Mean values followed with different superscripts in the same row of above table are significantly different ($p<0.05$) using Tukeys-B test

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Table 5. DPPH free radical scavenging activity (%) of 5-HMF and its derivatives

Compound	Concentration (µg/ml)						Mean values
	10	20	30	40	50	100	
1	25.59±0.23	35.36±0.12	45.98±0.18	55.29±0.11	63.16±0.43	71.94±0.22	49.55^{c}
2	19.35±0.21	25.98±0.10	38.35±0.16	43.81±0.16	53.36±0.21	61.92±0.11	40.46^{d}
3	35.54±0.16	45.30±0.05	51.08±0.22	65.38±0.22	72.43±0.16	79.99±0.18	58.29^{b}
Ascorbic acid	37.62±0.25	48.12±0.18	58.92±0.21	71.15±0.16	90.65±0.16	93.40±0.11	66.73^{a}
Mean values	29.52^{f}	38.69^{e}	48.59^{d}	58.91^{c}	69.90^{b}	76.94^{a}	

Data has been expressed as mean ± standard deviation. Mean values followed by different superscripts in the same row of the above table are significantly different (p<0.05) using Tukeys-B test

Table 6. Nitric oxide free radical scavenging activity (%) of various banana peel extracts

Concentration (µg/ml)	Radical scavenging activity (%)						Mean values
	Methanol extract	Ethanol extract	Ethyl acetate extract	Dichloromethane extract	Petroleum ether extract	Ascorbic acid	
10	31.7±0.37	29.43±0.24	21.00±0.92	15.08±0.24	14.91±0.37	39.16±0.24	25.22^{k}
20	40.87±0.64	42.9±0.85	35.59±0.29	18.49±0.73	17.43±0.37	49.54±0.37	34.14^{j}
30	61.55±0.24	45.51±0.61	40.87±0.24	20.25±0.50	20.59±0.37	62.44±0.14	41.87^{i}
40	65.85±0.56	59.93±0.38	44.60±0.37	28.22±0.24	28.7±0.24	66.01±0.37	48.89^{h}
50	67.87±0.64	65.44±0.24	61.63±0.37	32.97±0.33	35.6±0.61	69.6±0.40	55.52^{g}
80	72.74±0.42	70.71±0.56	65.93±0.48	47.52±0.56	37.62±0.14	73.71±0.48	61.37^{f}
100	76.63±0.42	75.17±0.64	68.52±0.14	54.01±0.24	40.14±0.64	78.74±0.14	65.54^{e}
200	79.88±0.51	78.42±0.56	71.12±0.56	56.68±0.42	45.25±0.49	81.34±0.51	68.78^{d}
300	84.42±0.87	81.42±0.50	79.72±0.85	60.74±0.70	46.71±0.42	87.58±0.24	73.43^{c}
400	89.42±0.31	84.1±0.78	82.88±0.29	62.28±0.24	52.55±0.24	90.34±0.37	76.93^{b}
500	90.83±0.37	89.29±0.24	86.37±0.24	65.93±0.42	55.57±0.18	92.86±0.51	80.14^{a}
Mean values	69.25^{b}	65.66^{c}	59.84^{d}	42.02^{e}	35.91^{f}	71.94^{a}	

The data has been expressed as mean ± standard deviation. Mean values followed with different superscripts are significantly different (p<0.05) using Tukeys-B test

Table 7. NO free radical scavenging activity (%) of 5-HMF and its derivatives

Compounds	Concentration (µg/ml)						Mean values
	10	20	30	40	50	100	
1	24.96±0.49	34.86±0.37	43.14±0.28	51.17±0.50	63.41±0.37	71.28±0.42	48.19^{c}
2	18.32±0.60	26.35±0.50	39.89±0.42	45.73±0.49	51.82±0.49	63.09±0.14	40.89^{d}
3	33.18±0.14	44.19±0.14	49.87±0.24	59.28±0.14	66.09±0.37	73.63±0.29	54.38^{b}
Ascorbic acid	39.16±0.24	49.54±0.37	62.44±0.14	66.01±0.37	69.60±0.40	78.74±0.14	60.92^{a}
Mean values	29.01^{f}	38.74^{e}	48.84^{d}	55.55^{c}	62.73^{b}	71.71^{a}	

The values indicated mean ± standard deviation of triplicate values. Mean values followed with different superscripts in the same row of the above table are significantly different (p<0.05) using Tukeys-B test

Antifungal activity

Ethyl acetate extract showed maximum antifungal activity with MIC value at 300 µg/ml against *F. oxysporum* (Figure 4). Dichloromethane and petroleum ether extracts showed least antifungal activities. Among the derivatives of 5-HMF (1), Compound (2) having azomethine linkage was most effective against *F. oxysporum* with MIC value at 400 µg/ml (Figure 4). 5-HMF (1) was found to be least effective. All the treatments were less effective as compared to standard (Clotrimazole) which showed MIC value at 10 µg/ml.

On the basis of MIC values, antifungal activity of

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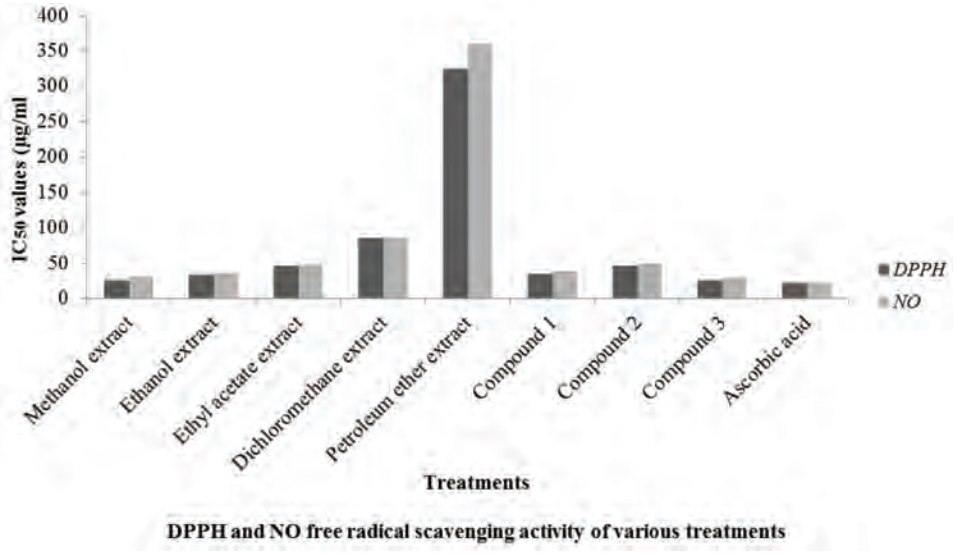


Fig. 5. DPPH and NO free radical scavenging activity of various treatments expressed in terms of IC_{50} values

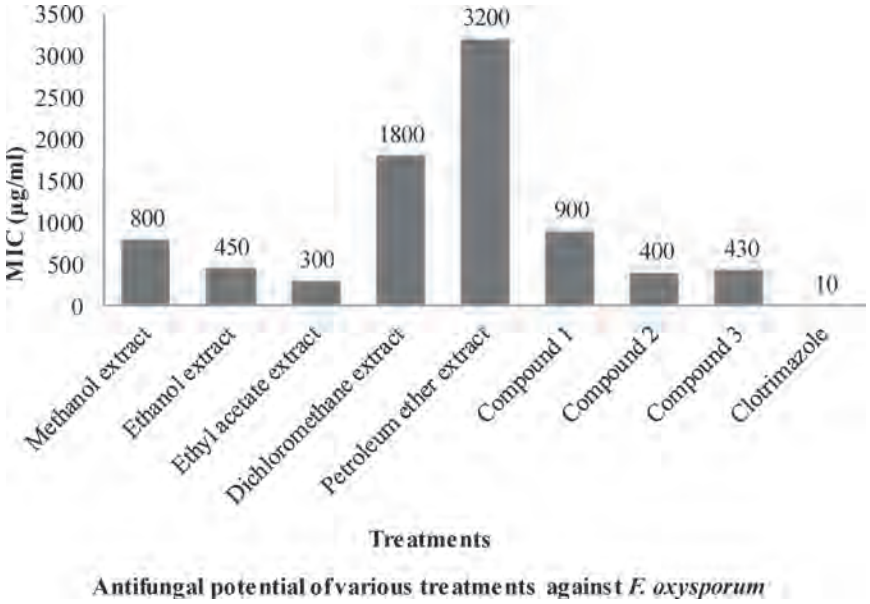


Fig. 4. Antifungal potential of various treatments against *F. oxysporum* in terms of MIC values

all the prepared extracts, 5-HMF (1) and its derivatives against *F. oxysporum* followed the following order (Fig. 4):

Clotrimazole > Ethyl acetate extract > [5-(3-Nitrophenyliminomethyl)furan-2-yl]methanol (2) > 5-Hydroxymethylfurfuralideneacetophenone (3) > Ethanol extract > Methanol extract > 5-HMF (1) > Dichloromethane extract > Petroleum ether extract

Various mechanisms have been proposed for the antimicrobial potential of plant extracts which stated that plant extracts have membrane disrupting compounds capable of causing leakage of cellular contents, interference with metabolic pathways and active transport system (Khameneh *et al*., 2019). According to another statement, organic acids present in plant extracts are responsible for changing cytoplasmic membrane permeability by altering its structure via interaction with proteins, changes in membrane potential and intracellular pH (Burt *et al*., 2007). Also, the active components of various plant extracts have inhibitory effects on cell wall construction, microbial DNA replication, energy synthesis and biofilm production (Mickymaray, 2019). Among 5-HMF and its derivatives, compound (3) having a chalcone moiety and hydroxyl group exhibited maximum antibacterial

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potential as compared to 5-HMF (1) having aldehyde and hydroxyl group. This was further supported by the fact that various chalcone derivatives have been tested for their antimicrobial, cytotoxicity and genotoxicity and hence proved as starting materials for preparation of drugs (Ozdemir *et al*., 2017). Compound (2) having azomethine linkage commonly called schiff bases and hydroxyl group exhibited maximum antifungal potential. Various Schiff bases have been reported to show antifungal activity due to the presence of azomethine linkage (Da Silva *et al*., 2011).

Antioxidant activity

Antioxidant activity of banana peel extracts, 5-HMF and its derivatives against DPPH and NO radical was concentration dependent and increased with an increase in concentration as well as with an increase in polarity of solvents used to prepare extracts (Tables 4-7). Petroleum ether being the least polar median concentration (IC_{50}) value of 360 and 325 µg/ml in DPPH and NO free radical scavenging assay, respectively. Methanol extract showed maximum free radical scavenging activity with IC_{50} value of 26.5 and 30.50 µg/ml in DPPH and NO free radical scavenging assays, respectively. Among 5-HMF (1) and its derivatives, compound (3) having chalcone moiety showed maximum radical scavenging activity with IC_{50} value of 25.50 and 30.00 µg/ml against DPPH and NO radical scavenging assays respectively (Figure 5). Compound (2) showed least activity with IC_{50} value of 47.00 and 49.00 against DPPH and NO radical scavenging assays respectively. All the treatments exhibited lower radical scavenging activity than standard ascorbic acid with IC_{50} value of 22.50 and 22.0 µg/ml in DPPH and NO radical scavenging assays, respectively.

On the basis of IC_{50} values, antioxidant potential of all prepared extracts, 5-HMF and its derivatives in both the tests followed the following order (Fig. 5):

Ascorbic acid > 5-Hydroxymethylfurfuralideneacetophenone (3) > Methanol extract > Ethanol extract > 5-HMF (1) > Ethyl acetate extract > [5-(3-Nitrophenyliminomethyl)furan-2-yl]methanol (2) > Dichloromethane extract > Petroleum ether extract

5-HMF exhibits potential antioxidant behavior on the cellular, molecular and genetic levels. It not only effectively scavenges free radicals and cellular reactive oxygen species but also protects the cellular membrane from oxidative stress and has a paramount role in the defense system (Zhao *et al*., 2013).

From the above study, it was concluded that banana peels which are considered as major problem by food processing industries and pollution controlling agencies, may be used for the production of value added products. As the peels have higher content of phytochemicals as secondary metabolites as compared to other parts of fruit, it protects inner parts of fruit from microorganisms and insects. India is the second largest producer of banana fruit with annual production of 46.26 lakh tonnes. The peels of fruit constitute about 30-40% of total fruit weight. Thus, complete utilization of banana peel waste to form value added products will provide extra income to food and pharmaceutical industries and may also solve the problem of environmental pollution.

Authors' contribution

Conceptualization and designing of research work (SK); Execution of lab experiments and data collection (R); Analysis of data and interpretation (SK, PS); Preparation of manuscript (SK,R)

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