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Determination, identification of bioactive compounds extracts from yellow banana peels and used in vitro as antimicrobial

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Abstract

In this study, bioactive compounds of six solvent different extracts, from the dry yellow banana peels were present. Methanol was the best solvent extraction .The yield extract , total phenolic content and flavonoids concentration were 1.96 g, 81.89 mg G.A./g and 55.44mg RU/g from extract . The FT-IR spectra were recorded pronounced peaks belonging to the vibration of 3361 cm^{-1}, 1662 $cm^{-1}$and 1231 cm^{-1} in the spectra corresponds to the characterized peaks of flavonoids. GC-Ms chromatography clearly show the presence 17 component This analysis revealed that methanolic extract contain Pyrogallol (22.24%), Cis-9-Hexadecenal (21.20%) , Pentadecanoic acid (18.81%), Benzoic acid (16.04%) , Octadecanoic acid (6.18%) and Cis-9-Hexadecenoic acid (4.40%) . 300 mg / ml concentration from methanolic extract had broad inhibitory spectrum against gram positive and negative bacteria , yeast and molds .

Keywords: Banana peels, Total phenolic , Flavonoids, GC-MS Chromatography , antimicrobial.

Introduction

Phenolic compounds are basically involving plant metabolic system which is different widely in the structures, biological properties and mechanisms of action. It is known that different phytochemical components, especially polyphenols (such as phenolic acids, flavonoids, propanoids vinyl, tannins, etc.) to be responsible for scavenging free radicals and antioxidant activities of plants [1].

Flavonoids are some of the most common phenol are distributed widely in plant tissue. It are often responsible together with the carotenoids and chlorophyll whom blue, purple, yellow, orange and red colors. The flavonoid family includes flavones, flavonols, iso-flavonols, proanthocyanidins, anthocyanins, catechins , and anthocyanidins [2-3] . The treatment of food processing wastes is now becoming a very serious environmental issue. Peels are the major by-products obtained during the processing of various fruit and some studies show that these are good sources of polyphenols, carotenoids and other bioactive compounds which possess various beneficial effects on human health [4-6]. Potential applications for banana peel depend on its chemical composition. Banana peel is rich in dietary fiber, proteins, essential amino acids, polyunsaturated fatty acids and potassium [7] . Banana and tomato peels have been reported to be a good source of carotenoids [8-9] .

Various solvents were used for phenolic compounds extract from plants . Aqueous, methanol, ethyl acetate, acetone and petroleum ether were used flavonoids extract from *Achillea millefolium* L. and *Marrubium peregrinum* L. and It used as antioxidants [10-11].

In study found the isopropyl extracts of yellow banana peel was comparatively effective particularly on *Escherichia coli* , *Staphylococcus* sp. and *Klebsiella* sp. The inhibition zone was (1,0.6 and 0.7)cm respectively. Hence, the mid polar extracts of yellow banana peel can be vitally used as a natural food preservative, which will increase the shelf life of the food sample [12]. The objective of this work was to extraction Bioactive compounds from yellow banana peel by various solvents, Determination and identified of active compounds , and used as antimicrobial.

Materials and Methods

Chemicals

Acetone, methanol, ethanol, ethyl acetate, and benzene were obtained from Scharlab S. L., Spain. Standards of phenolic acids (gallic acid) and of flavonoids (rutin hydrate), Folin-Ciocalteu's reagent , fehling's solution aluminium chloride ($AlCl_3$) , KOH and ferric chloride (Fe Cl_3) were obtained from Sigma-Aldrich Chemicals Co., U.K. Sodium hydrogen carbonate ($NaHCO_3$) from Samarra Pharmaceutical Co. Iraq. The culture media (Mueller-Hinton agar , Nutrient broth and Potato Dextrose agar) were obtained from Hi-media Co. India.

Microbial isolates

Used bacteria isolates were *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Lactobacillus casei* obtained from Chr. Hansen Lab. *Bacillus* sp. and *Pseudomonas aeruginosa*. The last two isolates were obtained from Food Science Department/ Agriculture College /Basrah University / Iraq. Isolates of fungi were *Aspergillus niger* , *Penicillium* sp., *Fusarium solani*, *Rhizopus nigricans* and *Alternaria alternata* from Food Science Department/ Agriculture College /Basrah University / Iraq. And *Saccharomyces cerevisiae* from Saf-instant Co. France

Samples collection

Yellow banana (*Musa acuminate*) peel were collected and drying by oven at 65 ˚C for 24 hours. Dried plant peel were grind by electric mill and stored in a refrigerator at 4˚C.

Preparation of plant extracts

10 gm. from dried banana peel transferred to flask and added to 100 ml of solvents with different polarities water, ethanol, methanol, ethyl-acetate, acetone and respectively and stored at 25±2 C˚ . After 24 h, infusions were filtered through Whatman. 6 filter paper and residue was re-extracted with equal volume of solvents. After 48 h, the process was repeated. Combined supernatants were evaporated to dryness under vacuum at 40 ˚C using Rotary evaporator. The obtained extracts were kept in sterile sample tubes and stored in a refrigerator at 4˚C [10].

Identification

Preliminary qualitative test: the chemical family of the isolated compound was implemented using several tests such as:

-Test of Phenolic Compounds

0.5 ml ferric chloride 1% add to 1ml from phenolic extracts .The appearance green color was indicate for presence of total phenolic compounds [13].

-Test for Flavonoids

A 1ml from alcoholic KOH (5N) adds to 1ml from extracts .The presence of yellow precipitate was indicate for the presence of flavonoids [14].

-Test for tannins

1ml of freshly prepared 10%KOH was added to 1ml from the extract, a dirty white precipitate indicates the presence of tannins [15].

-Test for glycosides

To 1ml of the extract in the test tube, 10ml of 50% H_2SO_4 was added. The mixture was heated in boiling water for 15min. 10ml of fehling's solution was added, a brick red precipitate indicates the presence of glycosides [15].

-Test for saponins:

Emulsion test: 5 drops of olive oil was added to 3ml of the extract in a test tube, the mixture was vigorously shaken. A stable emulsion indicates the presence of saponins [15].

Determination of total phenolic contents

The concentration of phenolic compounds in plant extracts was determined using spectrophotometric method [16]. 1 mg/ml from plant extract solution was used in the analysis. 0.5 ml of solution of banana peels extract was mixing with 2.5 ml of Folin-Ciocalteu's reagent (10%) and dissolved in water and 2.5 ml from $NaHCO_3$ (7.5%). Blank was concomitantly prepared, by add all the components of the reaction except the banana peels extract. The samples were then incubated at 45˚C for 45 min. The absorbance was determined using 765 nm. The results were compared with standard curve from gallic acid (GA) then the content of phenolic compounds in banana peels extracts was expressed in terms of gallic acid amounting to (mg of GA/g of extract) by using the following equation from standard curve

$y = 0.0003x$; $R^2 = 0.9328$.

Determination of flavonoid concentrations

The flavonoids content in the assayed banana peels extracts was determined using by[17]. 1ml from plant extract solution (concentration of 1 mg/ml) and 1 ml of 2% $AlCl_3$ solution dissolved in methanol. Then incubated at room temperature for one hour. The absorbance was determined using 415 nm. The results were compared with standard curve from solution of rutin (RU) then the content of flavonoid in extracts was expressed in terms of solution of rutin equivalent (mg of RU/g of extract) by applying the following equation of standard curve.

$y = 0.0032$; $R^2 = 0.9785$

Identification of phenol compounds for the plant extract

Infrared spectra

The Methanolic banana peels extract spectra was recorded in a JASCO FTIR4200 (Japan) using 200 mg KBr pellet disc. The FTIR spectrum of the protective film was recorded by carefully removing the film, mixing it with KBr and making the pellet [18].

Gas Chromatography- Mass Spectrum Analysis (GC-MS)

GC-MS technique was used in this study to make a distinction of the phytochemical components present in the methanolic extract of banana peel. GC-MS technique was conducted in the GC-mass Lab. Agriculture College, Basrah University, Basrah City ,Iraq. GC-MS analysis of this extract was performed using GC SHIMADZU QP2010 Ultra and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with DbB5ms capillary column. Detection of GC-MS, was used electron ionization power system by ionization energy of 0.70 KV. Helium gas (99.999%) was used as the carrier gas at a purge flow rate of 3ml/min and an injection volume of 2µl was employed (split ratio: 20). The pressure 106.1kPa, total flow 40.7 ml/min, Injector temperature 250 C; Ion-source temperature 200 C. The oven temperature was programmed from 50 C (isothermal for 2 min.), with an increase of 280 C for 10 min. The time of running was 28 min. The percentage of area the peak of each compound account by comparing its average peak area to the whole areas. Software adopted to handle mass spectra and chromatograms was a GC MS solution ver.2.53[19].

Detection of inhibitory activity

Agar well diffusion assay by [20] was used without any modifications in assessing the inhibitory for bacteria and yeast test, 0.1 ml from old bacteria and yeast (10^5-10^6 cfu/ml) were trans to pleats contend Mueller-Hinton agar and Potato Dextrose agar . 3 wells (6 mm) worked in agar and add 0.2 ml from Methanolic banana peels extract were concentration (100 ,200, 300) mg / ml. after 24-48 hours for incubation measuring inhibition zones.

-Anti-molds activity: Inhibition of mycelial growth was assayed using the food poison method described by [21]. The autoclaved medium was maintained in a water batch at 45 C. 1 ml from methanolic banana peels extract were concentration (100 ,200, 300) mg / ml added to molten Potato Dextrose agar. 12ml from mixture was poured in Petri dishes. A 6 mm disc from mycelium of old culture of molds and transferred to the center of petri plate. 3 replicate petri plates were used per treatment. The control samples were used plates containing mycelium disc without methanolic. All plates were incubated at 25 C for 3-5 days. After molds growth was calculated as colony diameter against negative controls was measured in terms of percent mycelia inhibition by the formula:

Growth Inhibition (%) =[Dc-Dt/Dc] 100

The total phenolic contents in the examined extracts ranged from 45.67 to 81.89 mg GA/g. The highest concentration of phenols was measured in methanol, ethanol , water and acetone extracts. Ethyl acetate and benzene extracts contains considerably smaller concentration of phenols. While The concentration of flavonoids in plant extracts from banana peel ranged from 30.12 to 55.44 mg RU/g. Methanol, ethanol , acetone and ethyl acetate extracts contains the highest flavonoid concentration The lowest flavonoid concentration was measured in benzene and water extract.

FT-IR Spectral Analysis

Where, Dc: Diameter of colony in the control (mm) , Dt: Diameter of colony in the treatment (mm).

Results

The presence of total phenol and flavonoid were discovered in Acetone, methanol, ethanol, ethyl acetate, benzene and aqueous extracts while tannins, saponins and glycosides were absence in the extracts accept methanol on found tannins only table1.

Table 2 shows the yield of extract , total phenol compounds and flavonoids obtained from 10 g of dry banana peels was measured for each extracts . The highest yield of solid residue was obtained using methanol or ethanol as extraction solvents were (1.96 , 1.64) g respectively

The FT-IR analysis of the methanolic banana peels extract was done and the functional groups associated were determined (Figure. 1) . A broad band due to the O-H stretch is observed near 3361 cm $^{-1}$ .A band due to the alkane medium C-H sharp quadrant stretch is observed at 2926 cm $^{-1}$ and one due to the ester and carbonyl C=O ,=C-H generally strong stretch at 1725 and 1662 cm $^{-1}$. The aromatic alcohol OH was observed at 1316 cm $^{-1}$ a band, and at 1231 cm $^{-1}$. A band approving to the -C-O extension of an aromatic alcohol is observed in1044 cm $^{-1}$. The bands due to the aromatic OH wags are observed between 900–750 cm $^{-1}$ the frequency of the bands due to the OH wags depend on the substitution of the aromatic rings.

Table 1. Phytochemical screening of the crude extract from banana peels.

Extracts	Total phenol	flavonoid	tannins	saponins	glycosides
Acetone	+	+	-	-	-
methanol	+	+	-	+	+
ethanol	+	+	-	+	-
ethyl acetate	+	+	-	-	-
benzene	+	+	-	-	-
water	+	+	-	-	-

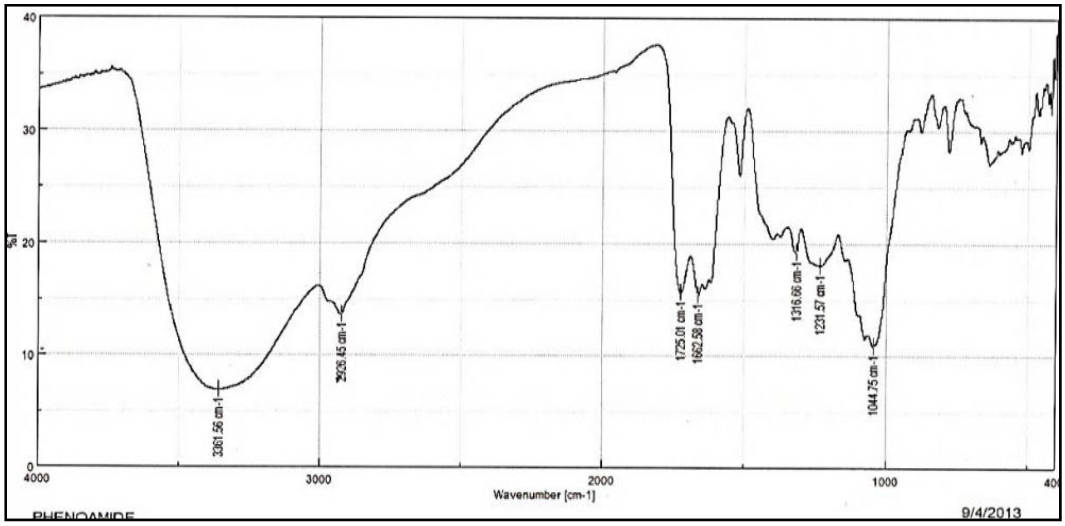


Figure 1. FT-IR spectrum of methanol extract from banana peel

Table2. Yields, phenol compounds and flavonoids for banana peels extracts

Extracts	Yields (g)	Total phenolic (mg G.A./g of extract)	Flavonoids(mg Ru/g of extract)
Acetone	0.88	67.14	49.32
methanol	1.96	81.89	55.44
ethanol	1.64	74.22	51.52
ethyl acetate	1.04	55.19	42.11
benzene	0.65	45.67	30.12
water	1.33	70.32	35.77

The GC-MS chromatogram of the seventeen peaks of the compounds detected was shown in (figure.2) . The GC-MS analysis clearly showed the presence of seventeen compounds (Table-3). The active principles with their Peak Numbers, retention time (RT), peak area, concentration (Peak area%) , names of compounds and Molecular structure of compound were presented in Table.3. The prevailing compounds were Pyrogallol (22.24%), Cis-9-Hexadecenal (21.20%) , Pentadecanoic acid (18.81%), Benzoic acid (16.04%) , Octadecanoic acid (6.18%) and Cis-9-Hexadecenoic acid (4.40%) . The most identified compound to have antimicrobial because of bioactive groups.

GC-MS analysis

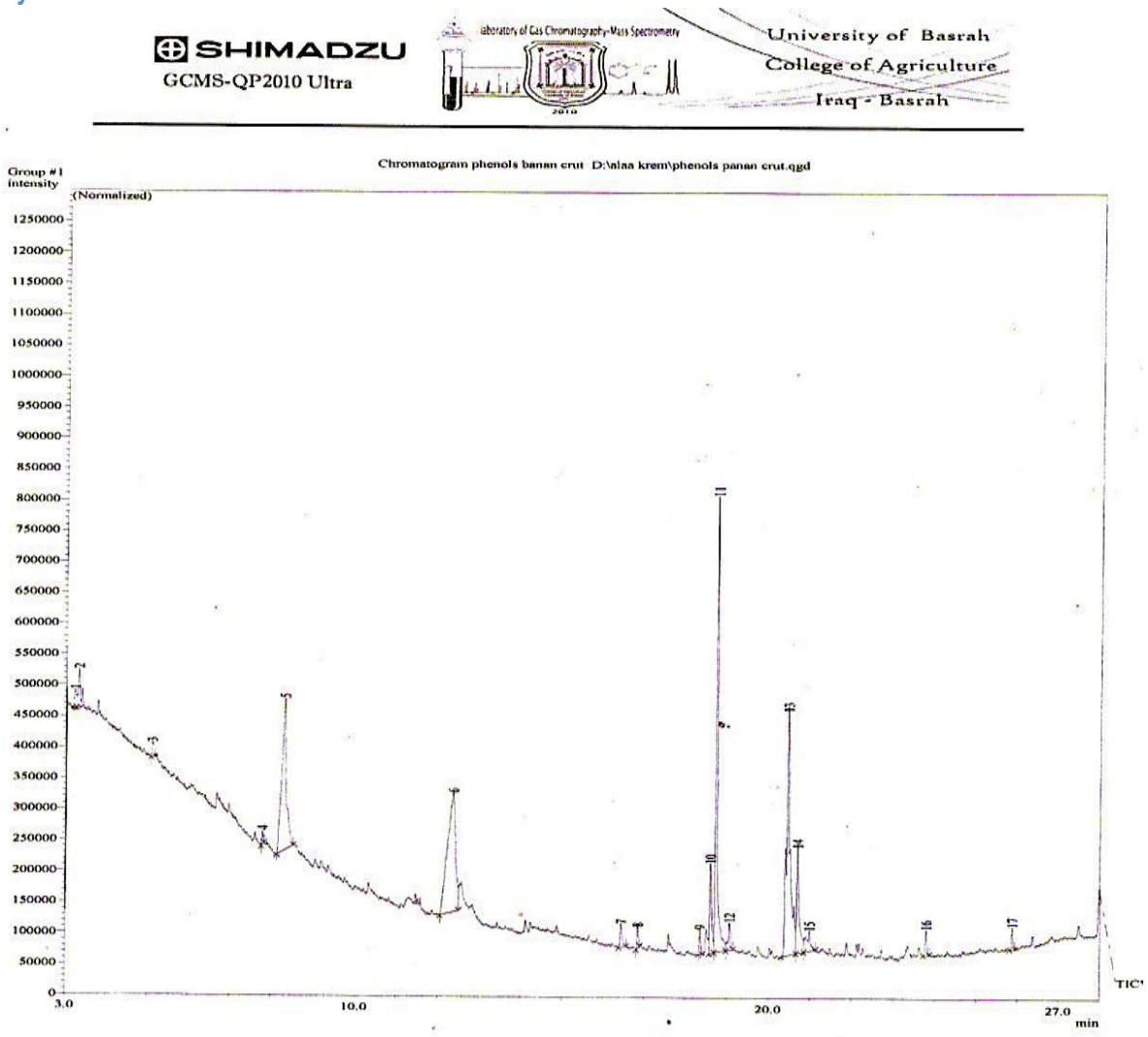
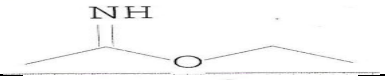
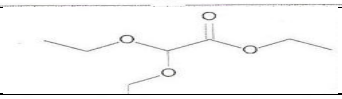
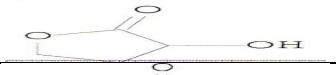
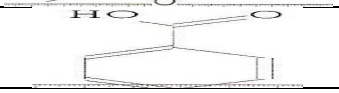
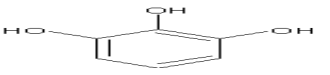
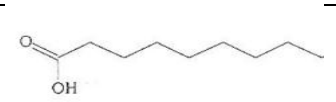
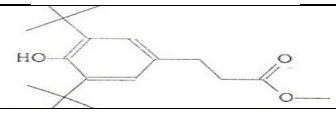
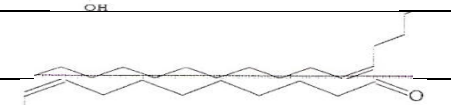
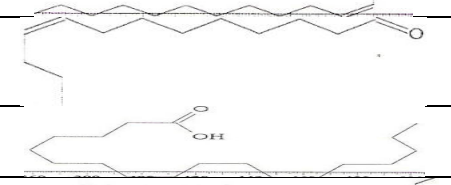
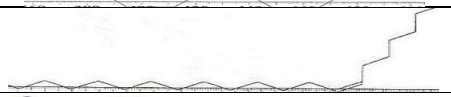
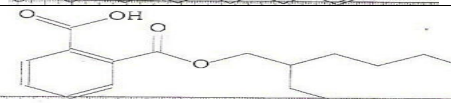


Figure 2. GC-mass chromatogram of methanol extract from banana peel

Table 3. the main compounds identified by GC-MS in the extracts of banana peel

Peak	RT	Area	Area%	Name of compounds	Molecular structure of compounds
1	3.124	93352	0.8	Ethanimidic acid	
2	3.310	187166	1.61	Acetic acid	
3	5.107	83748	0.72	2-hydroxy-gamm-butyrolactone	
4	7.789	48149	0.41	4H-pyran-4-one	
5	8.139	1868284	16.04	Benzoic acid	
6	12.371	2590730	22.24	Pyrogallol	
7	16.444	127862	1.10	Tetradecenoic acid	
8	16.848	79915	0.69	1-Nonadecene	
9	18.356	91178	0.78	Benzenepropanoic acid	
10	18.606	512474	4.40	Cis-9-Hexadecenoic acid	
11	18.735	2191361	18.81	Pentadecanoic acid	
12	19.065	147700	1.27	9-Tricosene	
13	20.464	2469457	21.20	Cis-9-Hexadecenal	
14	20.703	719478	6.18	Octadecanoic acid	
15	20.995	244835	2.10	9-Tricosene	
16	23.797	103790	0.89	1,2-Benzenedicarboxylic acid	
17	25.881	88730	0.76	2,6,10,14,18,22-Tetracosahexaene	

Antimicrobial activity

Table 4 shows the inhibition zones diameters for three concentrations from methanolic banana peels extract. Increase concentrations gave increase in bacteria inhibition. The gram positive bacteria were most affected compared with gram negative and yeast. The range of inhibition zones for bacteria were 13 to 24 mm at concentration 300 mg extract /ml (Figure.3). Inhibition zone of *Saccharomyces cerevisiae* was 15mm at 300 mg extract /ml and no zones at 100 and 200 mg extract /ml.

Anti-molds activity The results of anti-molds of methanolic banana peels extract were presented in table 4 In this study, all concentrations showed inhibition activity against the molds except

Aspergillus niger no effect when to add 100mg from methanolic banana peels extract (Figure.3) (table5).

Table 4. Zones of inhibition for concentrations methanolic banana peels extract

Bacteria and yeast	Zones of inhibition for concentration (mm)		
	100mg	200mg	300mg
E. coli ATCC 25922	-	-	14
Staphylococcus aureus ATCC 25923	12	17	24
Lactobacillus casei	12	15	21
Bacillus sp.	10	14	22
Pseudomonas aeruginosa	-	-	13
Saccharomyces cerevisiae	-	-	15

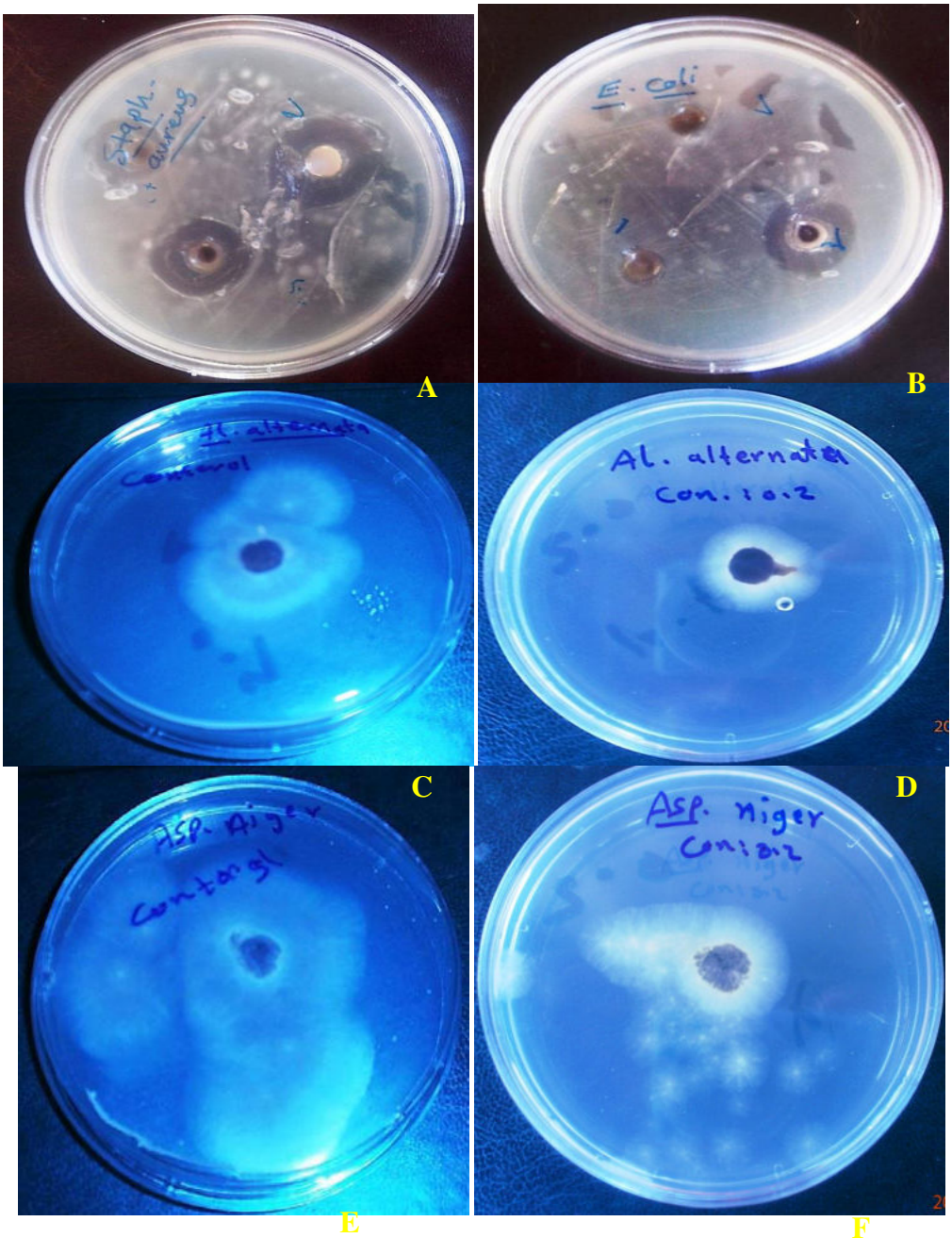


Figure 3. Pictures of microbes inhibition by methanolic banana peels extract

Table 5. The percentage of inhibition for concentrations methanolic banana peels extract

molds	The percentage of inhibition		
	100mg	200mg	300mg
Aspergillus niger	0	22	40
Fusarium solani	26	39.5	50
Rhizopus nigricans	22	40	83
Alternaria alternata	28.89	50	66.66
Penicillium sp.	24	44.45	60

Discussion

The total phenolic and flavonoids contents in banana peels extract depends on extract method and solvent type. High solubility of phenol compounds in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction [22-23].

FT-IR associated KBr pellet method was used, for the determination of flavonoids from the ethyl acetate, methanol and water extract of *Uncaria gambir*[24].

Pyrogallo and Benzoic acid from phenol compound were used as antibacterial or antifungal [25] . Other compounds belonging to the group of volatile fatty acids were active in the inhibition of microbial. These oils were secondary metabolites that are highly enriched in compounds like cinnamaldehyde, cinnamic acid, benzaldehyde, eugenol, benzoic acid, monoterpenes, triterpenes, and sesquiterpenes [26] . The mechanisms involved are cytoplasm granulation, cytoplasmic membrane rupture and inactivation and/or inhibition of intracellular enzymes. It has been reported that lytic enzymes act on the fungal cell wall, causing breakge of β-1,3glycan, β-1,6 glycan and chitin polymers [27]. In study found ,The isopropyl extract of banana peels was comparatively effective particularly on *Escherichia coli* and *Staphylococcus* sp. [12] . Past studies indicated that, The methanol was the best solvent for the steady extraction of antimicrobial compounds from medicinal plants compared with other solvents such as water, hexane and ethanol [28] . Thence, in this study The methanol was used for extract the active antimicrobial components from leaves of red cabbage. The results of the current study showed certain novel and significant antimicrobial activities against the tested bacteria and *Saccharomyces cerevisiae* yeast. The methanolic extracts of leaves and aerial parts from some plants were antifungal and antiaflatoxigenic activities against the aflatoxigenic fungus *A. flavus* [29] . This gives evidence that banana peels extract has various antibacterial components practicing and various mechanisms of action. A broad spectrum of anti-bacterial impacts of the banana peels extract In the present study can be attributed to the methanol procedure of extraction that is suitable for extracting all the fractures efficiently.

Methanol extract contained the highest the total phenolic and flavonoids concentration was identification and It was as antimicrobial.

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