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ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF MICROWAVE ASSISTED EXTRACTION OF APPLE PEEL

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ABSTRACT

The microwave assisted extraction (MAE) technique is emerged with great advantages over other conventional technique. Apple peel are the potential source of polyphenols, apple of Himachal variety was subjected to extract polyphenols by using microwave assisted technique and solvent extraction (SE) technique using methanol, ethanol, acetone and water as solvents. The extract obtained from the MAE showed the antimicrobial and antifungal activity against pathogenic bacteria strain named *Staphylococcus aureus*, *E. coli* and *Salmonella* spp. Moreover apple peel also showed the potentiality to check the growth of *Fusarium verticillioides, Aspergillus niger and Penecillium spp.*

KEYWORDS: ***Apple Peel, Microwave Assisted Extraction, Antibacterial activity, Antifungal Activity***

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INTRODUCTION

Polyphenols present in fruits and vegetables had a wide range of health beneficial property like antioxidant, anticarciogenic and antihypertensive property contributes for healthier life[1]. Apple (*Malusdo mestica*) the king of temperate is known to contain almost all nutrients required to human health. Apple is the richest source of polyphenols with a supplementary of dietary fibers, vitamin C.[2] The phenolic content in apple is mainly concentrated in peel[3,4,5,6]. Apple peels are good source of caffeic, P-coumanic and ferulic acids which are a type of phenolic acids[7]. Annually, about 67.9 Mt [8] of apple produced globally of which about 20-30 % get process which generates about 13% of apple peel as waste of the total processed apple. As apple peel is among the richest sources of polyphenols, using various bio refinery processes that can convert these waste products to value added products like phenolic, flavonoids and phenolic acids. The microwaves assisted extraction (MAE) is an efficient technique for the extraction of phenolic from fruits and vegetables peels[9]. The MAE technique is more advantageous over the conventional solvent extraction methods by less consumption of solvents, minimizing extraction time and maximum recovery of constituents[10,11,12]. In present study the antibacterial and antifungal properties were evaluated in microwave assisted extract of apple peel and compared with solvent extract.

MATERIALS AND METHODS

(i) Sample preparation

The apple (*Malusdo mestica)* of the Himachal variety was purchased from the local market in Varanasi, Uttar Pradesh, India. The apple surface was cleaned thoroughly with tap water to remove the dirt. Apple peels were removed manually and drowned into saline water and peels were further dried in vacuum oven (Sonar, Model No. VORP 5030, Associated Scientific Technologies, New Delhi, India) at 40 °C. The dried apple peels were grinded into coarse powder and mixed with four different solvents i.e. Methanol, Ethanol, Acetone and Distilled Water in the proportion of 1:50.

(ii)Microwave assisted extraction of polyphenols of from the apple peel powder

The peel powder mixed with the solvents was treated with microwaves in the microwave oven at 110 °C for 50 sec, which was further filtered with Whatman no.1 filter paper to separate the polyphenols. Thus obtained solvents with polyphenols were evaporated in vacuum oven at 40 °C and remained powder were used for determining the antioxidant activity and total phenolic content of the extract.

(iv)Extraction of polyphenols from apple peel powder by solvent extraction method

The mixture of peel powder and solvents were incubated for 10 hours in shaking incubator (Labtech, Model no. LSI-3016 R, Diahan labtech Germany) at 25 °C and at 200 rpm. The mixtures were filtered using Whatman No.1 filter paper. The filtered solvents were evaporated up to 80% and remained was used for analytical measurement.

(v) Total phenolic content (TPC) assay

TPC of the extract was determined by the method involving Folin-Ciocalteau reagent and Gallic acid standards[13]. Gallic acid was used for generating the standard curve having the concentration ranging from 20 to 100 mg/ml. 2.5 ml of 10 times diluted FC reagent was added to each tube and mixed well for 1 min and 2 ml of 7.5 % $Na_2CO_3$was added to it and allowed to incubate for 30 min at 37 °C and further the absorbance was measured at 760 nm and standard graph was plotted. The reaction mixture was incubated at 37 °C for 30 min and the absorbance was recorded at 760 nm. The total phenolic content equivalent to Gallic acid was determined from standard graph.

(vi)Determination of sensitivity of apple peel extract against pathogenic bacteria

The sensitivity of peel extract against three food borne pathogens named *Staphylococcus aureus, Salmonella typhii and E. coli* was performed according to protocol of Uhlmann et al. [14]. For this Muller Hinton Agar media were prepared and were swabbed with three different bacterial species. The apple peel extract which was vacuum dried was mixed with Dimethyl Sulphoxide (DMSO) in the ratio of 2:1. Then 15µl of extract solution was poured into the petriplates that containing bacteria. The plates were then incubated for 24 hours at 37℃. The clear zone around the drop was noticed.

(vii)Determination of minimum inhibitory concentration of apple peel extract against pathogenic bacteria

The LB broth media was prepared and 100 ml of LB broth was poured into the micro titter plate and 100 ml of the extract solution (peel extract and DMSO mixture) was also poured into the same plate. Then serial dilutions were prepared and the 10ml of bacterial species suspension were mixed. This plate was then incubated at 37℃ for 24 hours. After that, streaking was done by taking the sample from each well into different petriplates containing MHA media. Then these streaked plates were incubated at 30℃ for 24 hours and the growth was checked [14].

(viii)Determination of sensitivity of apple peel extract against pathogenic fungi

The sensitivity of apple peel against pathogenic fungi were determined by following the protocol of Droby et al.[15].*Penicillium chrysogenum, Aspergillus niger and Fusarium verticillioides* were cultured and grown on potato dextrose agar plates. 15µ of apple peel extract that dissolved in the DMSO was dropped into the plates

where the fungus has been swabbed. After 48 hours of incubation at 30 °C the sensitivity was checked and the diameter of inhibition zone around the drop was measured.

(ix)Determination of minimum inhibitory concentration of apple peel extract against fungi

100 µL of the nutrient broth prepared for the fungi was poured into the well microtitre plate. 100 µL of the extract solution of the concentration 5000 µg/ml was poured into the first well of the plate and was serially diluted. 10 µL of the fungal suspension made in saline was dropped into the all well of the plate. After 48 hours of the incubation at 30 °C streaking of the fungi was carried into the PDA plate. The growth of the fungi was checked for MIC [15].

(x) Statistical analysis

All the data were expressed as mean ± standard error of mean and was calculated from three independent experiments. One-way analysis of variance (ANOVA) was applied by using the SYSTAT software to measure the test for significance as described by Snedecor and Cochran[16].

RESULTS AND DISCUSSION

1. Total Phenol

Anthocyanin plays great role for the development of colour in fruits and vegetables. The amount of total polyphenols in the dark red varieties of apple is higher compare to that of less dark varieties of apple due to the presence of the anthocyanin[17]. Upon microwave extraction it was found that among the four solvents used methanol extract was found to have the highest total phenol contents with 106.64 mg/100g Gallic acid equivalents.

Table 1
Phenol content in various solvents extract

Solvents used for extraction	Total phenol Content
Methanol	106.64 ± 0.51[a]
Ethanol	102.47 ± 0.49[a]
Acetone	98.09 ± 0.38[a]
Water	83.84 ± 0.74[b]

Values with different superscripts in column is significantly different (p<0.05)
Each value is mean ± SEM of triplicate sample

It can be clearly concluded that from Table 1 that methanolic peel extract exhibited highest phenolic content. The highest value of total phenolic compound was detected in the methanol extract whereas the lowest content was obtained in the water extract. The phenolic content of water and methanol extract was significantly different (P < 0.5). The ability of solvents to extract polyphenol was as follows: Methanol extract > ethanol extract > acetone extract > water extract. In this study methanol proved to be the best solvent for the extraction polyphenol from apple peel. It has been indicated that methanol is the best solvent for catechin extraction[18].

2. Antimicrobial activity of apple peel extract

The study of the antimicrobial property of the polyphenol against *Staphylococcus aureus* a gram positive bacterium, *E. coli and Salmonella typhii* which are gram negative bacterial species represented in the Table 2. Different solvent extract showed the different response for the growth of bacteria of *S. aureus, E. coli* and *Salmonella typhii.* From the above study it was observed that methanol extract showed the higher response to inhibit the growth of *S. aureus, E. coli* and *Salmonella* with the minimum inhibitory concentration of 250 µg/ml, 2000 µg/ml and 1000 µg/ml, respectively (Table 3). These findings were complementary to those reported by Baydar et al.[19] and Ozakan et al.[20] in which it was demonstrated that polyphenol extracts from various fruit peel inhibited the growth of *S. aureus, E. coli, P. aeuroginosa, E. aerogenes, E. faecalis, M. smegmatis, P. vulgaris, P. fluorescens, S. enteritidis and Y. enterocolidica.* The apple peel extract showed the more effectiveness towards the gram positive bacteria than the gram negative bacteria. From the observations it is evaluated that the minimum inhibitory concentration (MIC) polyphenol is different for different bacterial species and is also varied with the solvent used for the extraction. According to the Table 3 it becomes clear that different bacteria have the different clear zone around them for antimicrobial sensitivity for polyphenols and also have the different MIC. *S. aureus* had the lowest MIC of around 500 µg/ml while *E. coli* have the highest MIC of 3000 µg/ml and the *Salmonella typhii* shows the MIC of around 1500 to 2000 µg/ml depends upon the type of the solvents.

Table 2
MIC for different bacteria with polyphenol extract of different solvents

Bacteria	MIC for Methanol extracted polyphenols (µg/ml)	MIC for Ethanol extracted polyphenols (µg/ml)	MIC for Acetone extracted polyphenols (µg/ml)	MIC for Water extracted polyphenols (µg/ml)
S. aureus	250 ± 50 [a]	500 ± 45 [b]	500 ± 43 [b]	1000 ± 50 [c]
E. coli	2000 ± 50 [a]	2000 ± 50 [a]	2000 ± 55 [a]	3000 ± 65 [b]
Salmonella typhii	1000 ± 70 [a]	1500 ± 60 [b]	1000 ± 65 [a]	2500 ± 62 [c]

Values with different superscripts in rows are significantly different (p<0.05)
Each value is mean ± SEM of triplicate sample

Table 3
Bacteria with the diameter of zone of inhibition for polyphenols of different solvents

Bacteria	Diameter of zone of inhibition for Methanol extracted polyphenols (mm)	Diameter of zone of inhibition for Ethanol extracted polyphenols (mm)	Diameter of zone of inhibition for Acetone extracted polyphenols (mm)	Diameter of zone of inhibition for Water extracted polyphenols (mm)
S. aureus	13±0.3[a]	12±0.3[a]	10±0.1[a]	8±0.3[b]
E. coli	8±0.4[a]	8±0.4[a]	6±0.3[a]	4±0.6[b]
Salmonella typhii	10±0.2[a]	9±0.2[a]	10±0.4[a]	6±0.3[b]

Values with different superscripts in rows are significantly different (p<0.05)
Each value is mean ± SEM of triplicate sample

The apple peel contains the higher amount of dimer and trimers of (-)-epicatechin [21,7] which possess higher anti-microbial activity than the monomer ones. The maximum inhibition zone was observed in methanolic extracts than the ethanolic extracts than the water extracts. This results of this study showed that antimicrobial compounds from apple peel are more soluble in methanol than acetone than ethanol than water. The bacterial inhibition growth caused by apple peel extract can be described by the several mechanism of action. Polyphenols can penetrate the semi permeable membrane of bacteria where they react with the cytoplasm or cellular protein. *Salmonella* and *E. coli* are the gram negative bacteria and they showed the some resistance for growth inhibition of apple peel extract. It may be possible that lipid wall of gram negative bacteria represented a great barrier for extracted polyphenols to get into cytoplasm; hence somewhat little inhibition was occurred

Figure 1
Antibacterial action of apple peel extracts on the growth of pathogenic bacteria

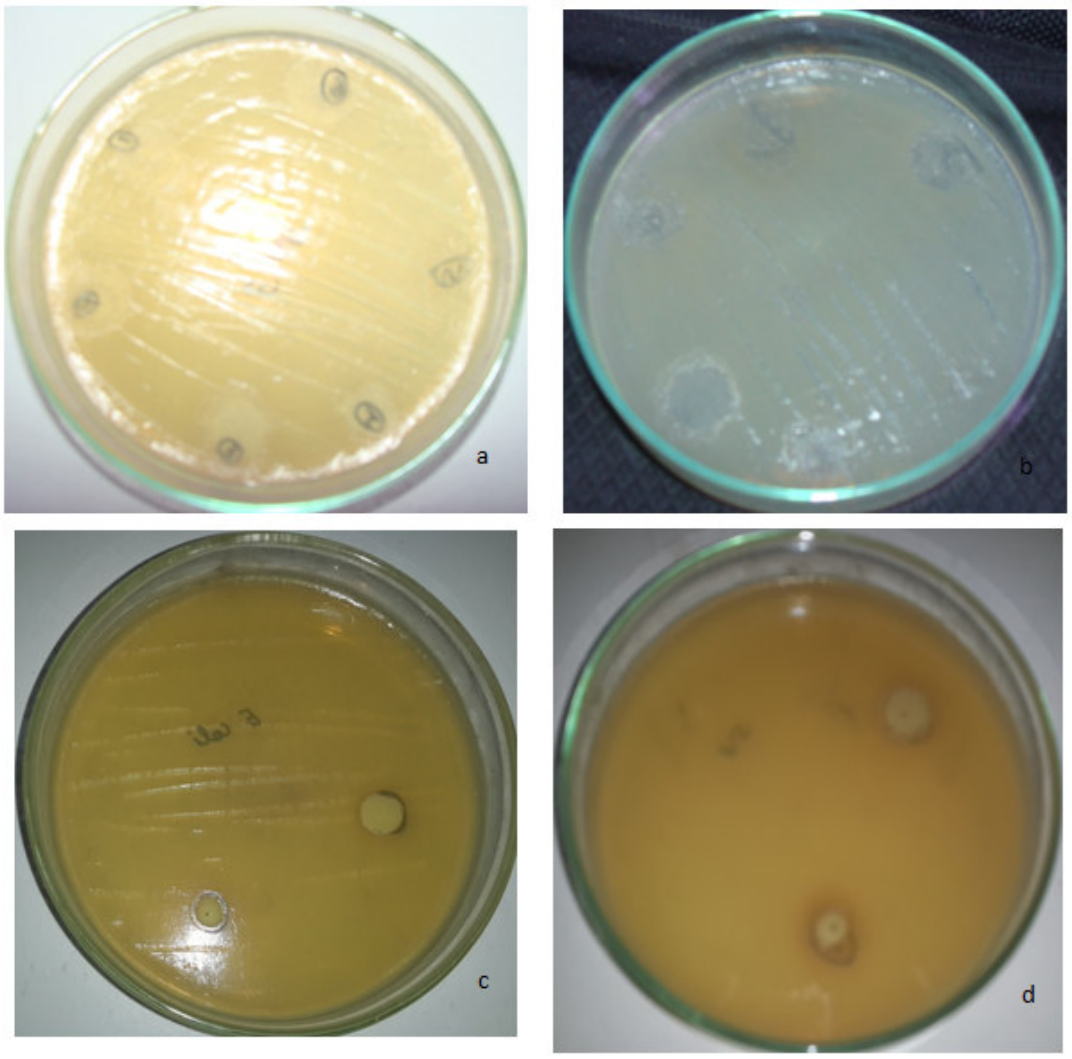

Plate a- Salmonella typhi, Plate b- S. aureus, Plate c and d - E. coli

3. Antifungal activity of apple peel extract

Various studied had been performed on the antifungal activity of polyphenols and researchers had decided that polyphenols present the tissue of plant had the capability to check the growth of fungus that causes the diseases [22]. Mycotoxins produced by different fungi had the adverse effect on the human health. Apple contains the significant amount of the sugar, nutrients and water which makes apple to susceptible for the fungal infection like *Aspergillus niger, penicillium, Fusarium verticillioides, Clostridium spp. and Alternaria spp.* [23]. Present study revealed that apple peel extract showed the notable fungi static effect. Upon solvent used for the extraction our study showed that peel ethanol showed the maximum inhibition of the growth in *Fusarium verticillioides*, *A. niger* and *Penecillium* among the other solvents. Peel methanol and peel ethanol extract were effective as compare to the acetone and water and were more effective even in low concentration for checking the growth of fungi. All extract showed the maximum inhibition against the *Fusarium verticillioides* whereas *Aspergillus niger* showed some resistant against the apple peel extract and had least diameter of the inhibition high degree of MIC. Table 4 clearly showed that *Fusarium verticillioides* had the lowest MIC of 3000 µg/ml having the 10 mm of inhibitions diameter for the ethanol peel extract whereas peel water showed the 4000 µg/ml against the *Fusarium verticillioides* with 7mm diameter of zone of inhibition. The *Aspergillus niger* had the highest degree of MIC of 5000µg/ml with peel ethanol and *Penecillium* had the 4000 µg/ml of MIC and 7mm diameter of inhibition zone. Certain fungi static effect has been attributed to isolated polyphenols like coumaric acid, caffeic acid, ferulic acid and sinapic acid as well as to isolated flavonoids such as (+)–catechin, kaempferol and quercetin[24]. Hence, the presence and solubility of some these compounds in extracts are likely to be responsible for the inhibitory effect of different extracts on the growth of mold.

Figure 2
***Antifungal action of apple peel extracts on a) Aspergillus niger , b) Fusarium verticillioides* and *c)* Penecilium spp.**

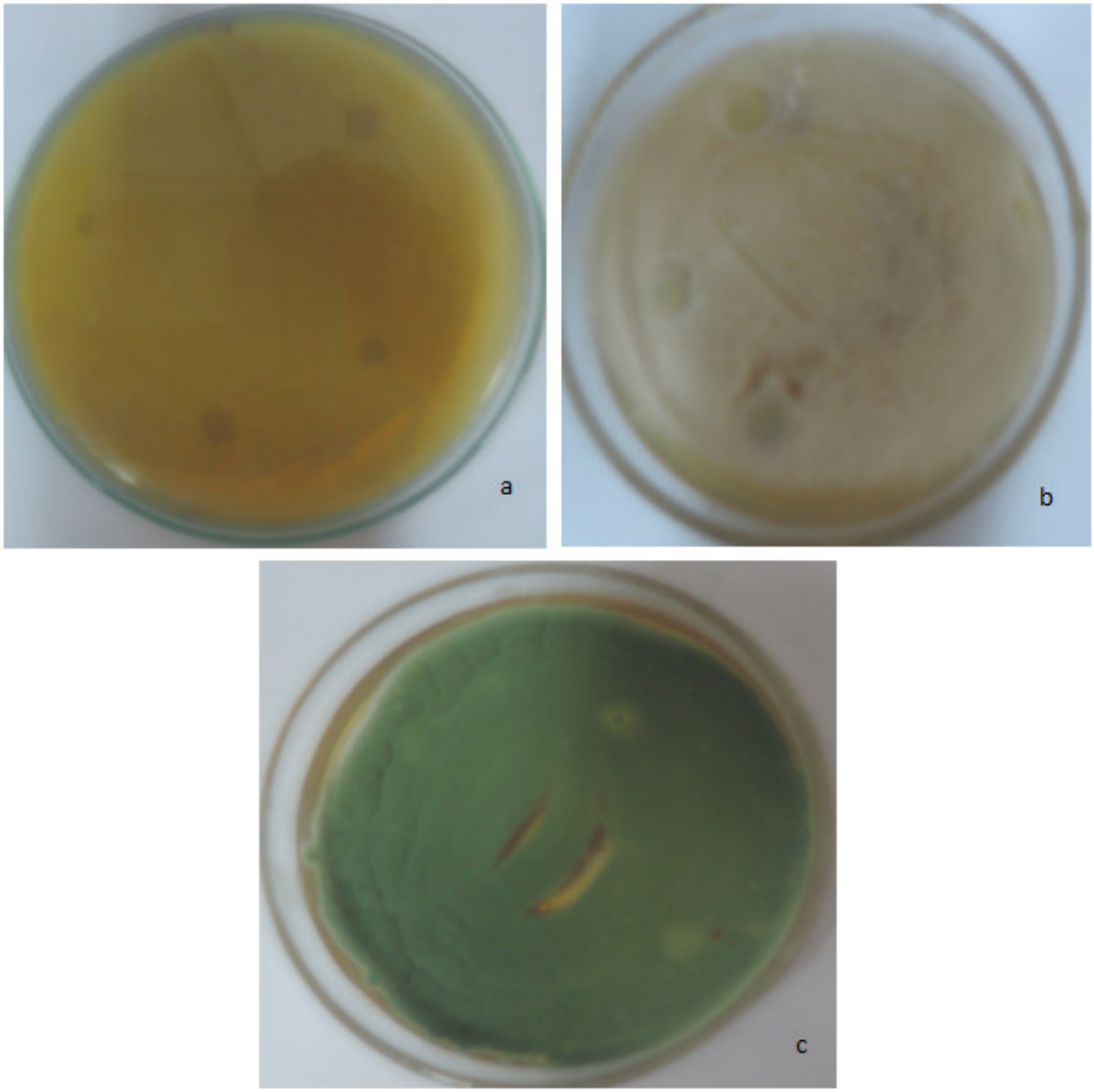

Table 4
MIC of polyphenols for different fungi

Fungai	MIC for Methanol extracted polyphenols (µg/ml)	MIC for Ethanol extracted polyphenols (µg/ml)	MIC for Acetone extracted polyphenols (µg/ml)	MIC for Water extracted polyphenols (µg/ml)
Aspergillus niger	4000 ± 55 [a]	4000 ± 45 [a]	4500 ± 43 [b]	5000 ± 50 [c]
Penecillium spp.	3500 ± 150 [a]	3500 ± 100 [a]	4000 ± 335 [b]	4500 ± 365 [c]
Fusarium verticillioides	3000 ± 190 [a]	3000 ± 110 [a]	3500 ±135 [b]	4000 ± 162 [c]

Values with different superscripts in rows are significantly different (p<0.05)
Each value is mean ± SEM of triplicate sample

Table 5
Fungi with the diameter of zone of inhibition for polyphenols of different solvents

Fungi	Diameter of zone of inhibition for Methanol extraceted polyphenols (mm)	Diameter of zone of inhibition for Ethanol extraceted polyphenols (mm)	Diameter of zone of inhibition for Acetone extraceted polyphenols (mm)	Diameter of zone of inhibition for Water extraceted polyphenols (mm)
Aspergillus niger	5 ± 1.21 [a]	5 ± 1.2 [a]	4 ± 0.81 [a]	3 ± 1.2 [b]
Penecillium spp.	7 ± 0.81 [a]	8 ± 0.81 [a]	6 ± 0.47 [a]	4 ± 0.401 [b]
Fusarium verticillioides	9 ± 0.23 [a]	10 ± 0.094 [a]	8 ± 0.81 [a]	6 ± 0.23 [b]

Values with different superscripts in rows are significantly different (p<0.05)
Each value is mean ± SEM of triplicate sample

CONCLUSION

From the present study the microwave assisted extraction had proved to be the better technique for the extraction of polyphenols from apple peel with advantages of shorter duration of extraction time, minimum use of solvent and had the maximum recovery of phenolic components. Along with the microwave assisted extraction the use of methanol enhances the total polyphenol extraction. The extract had the remarkable sensitivity against the gram positive bacteria than the gram negative bacteria. Peel extract also showed the notable sensitivity against the fungal growth.

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