Evaluation of antimicrobial and antioxidant properties of *Mangifera indica* bark extracts to prolong the shelf life of fruits and food products

Utkarsh Tyagi[1*], Surya Prakash Dwivedi[1] and Sovan Bagchi[2]

ABSTRACT

Processed food products can be contaminated by a variety of pathogenic and spoilage microbes. Those are the causative agents of foodborne diseases and cause significant economic losses in the food industries and human health. This was the basis of the present study, carried out with an objective to investigate the antibacterial and antifungal potentials of *Mangifera indica* bark extract. The antimicrobial activity of plant extracts and phytochemicals was evaluated with antibiotic susceptible and resistant microorganisms. An attempt was made in this study to evaluate these properties to prolong the shelf life of fruits and vegetables at the time of transportation and storage. Parts of the Mango plants have multifarious uses as traditional and asthetic values as well as medicinal implications. Antioxidant property was also evaluated by DPPH method. Methanolic extract of mangifera bark was used to evaluate the properties of phytochemicals and their role as an effective antioxidant and antimicrobial agent. The antibacterial activity was tested against one Gram-positive—*Staphylococcus aureus, and* three Gram-negative—*Escherichia coli, Pseudomonas aeruginosa*, *Salmonella* human pathogenic bacteria; and two fungal strains— *Candida albicans* and *Aspergilus niger*. After evaluation, methanolic extract of *Mangifera* bark the presence of phytochemicals like, alkaloids, glycosides, phenolic compounds, tannins and steroids. Zone of inhibition of extracts were compared with that of different standards like oxytetracycline, levofloxacin for antibacterial activity and fluconazole for antifungal activity. The results showed that the remarkable zone of inhibition against the bacterial growth ranged from 14 to 15 mm for pathogenic strain of bacteria. The zone of inhibition was observed first time of around 13 mm against the fungal strain. After the preliminary screening for antimicrobial activity by agar well diffusion method, MIC & MBC were also determined against above mentioned bacteria ranged from 3.125 to 6.25 µg/mL. However MBC ranges from 1.562 to 3.125 µg/mL. The parameter IC_{50} (efficient concentration‖ value) is used for the interpretation of the results from the DPPH method and the IC_{50} value for *Mangifera indica* is 94.04 µg/ml. Therefore, this plant can be used to isolate bioactive natural products and can be employed to control the food spoilage and to improve the quality of fruits and food products and may also play a vital role for the improvement of quality products for food industries.

Keywords: *Mango bark, Antioxidant, Antimicrobial, Phytochemicals, IC50, MIC, MBC*

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INTRODUCTION

Processed Food products are high in demand for consumption. Energy foods and designer foods are the major source of energy, but they can be contaminated by a variety of pathogenic and spoilage microbes, the former causing food borne diseases and the latter causing significant economic losses for the food industry due to undesirable effects on the food properties. Chemical additives have been extensively used to prevent the survival and proliferation of microorganisms, but their

safety and impact on human health are under discussion. In packaged foods, growth and survival of common spoilage and pathogenic microorganisms such as *Listeria monocytogenes, Escherichia coli* O157, *Salmonella, Staphylococcus aureus, Bacillus cereus, Campylobacter, Clostridium perfringens, Aspergillus niger*, and *Saccharomyces cerevisiae* are affected by a variety of intrinsic factors, such as pH and presence of oxygen or by extrinsic factors associated with storage conditions, including temperature, time, and relative humidity (Singh *et al.,* 2003; Lòpez-Malo *et al.,* 2005; Rydlo, 2006). The prevention or inhibition of microbial growth in foods is of atmost importance for the current globalized food production. Hence, there is still the need for new processing methods, or some natural antimicrobials which are able to reduce or eliminate foodborne pathogens and spoilage bacteria to prevent the economic losses of food industry and human health. Traditionally crude plant extracts are used as herbal medicine for the treatment of infectious diseases because of the presence of phytochemical. The phytochemicals work in the human system and due to their therapeutic properties cure many ailments which cannot be cured by the modern drugs (Rahman *et al*., 2001). The antimicrobial properties of natural substances such as plant essential oils and extracts have been extensively studied with promising results (Sakkas *et al.,* 2016). A natural source for developing antimicrobials includes plants and their parts and these could be used to control the diseases caused by pathogenic microbes (Gullo *et al.,* 2005). Experiments based on scientific principles to study the antimicrobial properties of plant components were first documented in the late 19th century (Zaika, 1988). Therefore, antibacterial agents are needed to be developed and employed to control multi-drug resistant bacteria. The present study was aimed at evaluating the antimicrobial activities, MIC, MBC, phytochemical analysis and antioxidant activity of the methanol bark extract of *Mangifera indica* against the pathogenic microorganisms such as *Salmonella sp., Staphylococcus aureus, Pseudomonas aerogenosa, Escherichia coli, Candida albicans and Aspergilus niger.*

MATERIALS AND METHODS

Collection of plant material

Bark of *Mangifera indica* was collected from herbal garden of IFTM University, Moradabad district of Uttar Pradesh, India. This plant was identified and confirmed with the authentic sources. Fresh barks were washed thoroughly under running tap water and dried under shade. They were then finely ground to a powder in an electric blender. Part was extracted with methanol using soxhlet apparatus (Elgorashi EE, *et al*, 2004). After removal of solvents under reduced pressure, extracts were stored at -20°C for further use. Then the extracts were used for phytochemical analysis, antibacterial and antifungal activity.

Phytochemical analysis

Alkaloids (Mayer's Test)

Mayer's test was performed for the presence of alkaloids. 1 ml plant extract was taken and 3 ml of ammonia solution was added. The solution was allowed to stand for few minutes to evaluate free alkaloids. 10 mL of chloroform was added to the test tube, shaken by hand and then filtered. The chloroform was evaporated from the crude extract by water bath. 3 mL of Mayer's reagent was added. A cream colour precipitation was obtained immediately, which showed the presence of alkaloids (Salehi-Surmaghi *et al.*, 1992).

Amino Acids (Ninhydrin Test)

For amino acid detection acetone extract was treated with 0.25% w/v ninhydrin reagent and boiled for a few minutes. Formation of blue colour indicates the presence of amino acid.

Flavonoids (Alkaline Reagent Test)

For the presence of Flavonoids in plant extract**,** 1 ml plant extract was taken in a test tube and a few drop of dilute NaOH solution was added. An intense yellow colour was appeared in the test tube. It became colourless when on addition of a few drop of dilute acid that indicated the presence of flavonoids (Shah *et al.*, 2014).

Glycosides (Keller - Killiani Test)

For the qualitative analysis of Glycosides, test solution was treated with a few drops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides (Rahman_Gul *et al*, 2017).

Phenols (Ferric Chloride Test)

For the presence of phenolic compounds **e**xtracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols (Shah *et al.*, 2014).

Proteins (Xanthoprotic Test)

Presence of protein content was confirmed by this method._The extracts were treated with a few drops of conc. HNO_3. Formation of yellow color indicates the presence of proteins.

Saponins (Froth Test)

Presence of saponins was confirmed by Froth test. Extracts were diluted with distilled water to 20mL and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Steroids (Liebermann Burchard Test)

1 ml extract was taken in a test tube and dissolved in chloroform (10 ml). Equal volume of concentrated sulphuric acid was added to the test tube by sides. The upper layer in the test tube turns into red and sulphuric acid layer show yellow with green fluorescence. It indicates the presence of steroids (Savithramma, 2011).

Tannins (Gelatin Test)

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Carbohydrates (Molisch's test)

2ml of sample was treated with a few drops of Molisch's Reagent. Then 1ml concentrated H_2SO_4 was added slowly along the side of the test tube. A red violet ring was formed at the junction of two layers.

Antibacterial Activity

Agar well diffusion method (Azam *et al*., 2012) was employed in present study. Nutrient Agar media (for bacteria) and Potato Dextrose Agar media (for fungus) were prepared and autoclaved at 121^0 C and 15 psi. The media were then poured in sterile petri plates (in the Laminar Air Flow Cabinet) and were allowed to solidify. After solidification, microbial broth (100µL) was spread over the media via sterile glass rod spreader and was left to set for ten minutes. Each plate had two wells, one of positive control (antibiotic), the other one of MI sample. Then wells were punctured on the plate using micropipette tips and then 20µL of the solution was loaded in different respective wells. The plates were left for 15-20 minutes in LAF and then sealed and incubated at 37ºC for 12-14 hrs. After incubation the plates were observed and their clear zones around wells called as zone of inhibition was measured in mm.

Determination of MIC

The MIC values were **determined by broth dilution assay**. Sterile plant extracts were serially diluted to obtain a concentration range from 25 µg/mL to 0.781 µg/mL. Then, 0.1 mL of each concentration was added to 9ml of nutrient broth (for bacteria) or Potato dextrose broth (for fungi) containing 0.1mL of standardized test organism of bacterial/fungal cells. Tubes with medium only were set as controls for sterility of the medium. Test tubes were evaluated for the presence or absence of visible turbidity in the broth after the incubation period. The lowest concentration (highest dilution) of the extract preventing appearance of turbidity (growth) was considered and recorded as the MIC.

Determination of MBC

From the tubes showing no visible sign of growth/turbidity in MIC, 0.1 mL of the sample was inoculated onto sterile nutrient agar using the spread plate method. The plates were then incubated at 37°C for 24 hours. The least concentration that did not show growth of the test organism was considered as the MBC.

Antioxidant Assay

The antioxidant activity of the extract was checked by DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity. 0.3 ml of different concentration (100, 200, 300, 400 and 500µg/mL) of sample was taken and made up to 0.4 mL with distilled water. To this added 0.6 ml of 100 M DPPH reagent in methanol was added. The reaction mixture was incubated for 20 minutes under dark and the reading was taken at 517 nm. The decrease in absorbance at 517 nm was taken as the antioxidant capacity of the sample.

RESULTS

The methanol extract of bark of *M. indica* showed various levels of antibacterial activity when tested by agar well diffusion method. The antibacterial susceptibility of the methanol extract of bark of *M. indica* on various microorganisms like *Pseudomonas aerugenosa, Staphylococcus aureus, Escherichia coli, Salmonella spp.* The antibacterial activity of *M. indica* against, *Pseudomonas aerugenosa* and *Staphylococcus aureus* showed 14 and 15 mm. zone of inhibition respectively and compared with the positive control oxytetracycline used for the test *(Table 1)*. The antibacterial activity against *E.coli* and *Salmonella* showed no zone of inhibition *(*Table 2).

Table 1. Positive controls used for different microbes.

Name of Microbes	+ control used (ppm)
P. aeruginosa	Oxytetracycline (500 ppm)
Salmonella	Oxytetracycline (500 ppm)
E. coli	Levofloxacin (500 ppm)
S. aureus	Oxytetracycline (500 ppm)
C. albicans	Fluconazole (5000 ppm)
A. Niger	Fluconazole (7000 ppm)

Table 2. Antibacterial activity of methanol bark extract of M. indica.

Test Microorganism	*M. indica* (mm)	+ve Control (mm)
P. aeruginosa	14	24
S. aureus	15	32
E. coli	-	25
Salmonella	-	16

The antifungal activity of methanol extract of bark of *M. indica* against on *Candida albicans* showed no zone of inhibition while the extract against on *Aspergillus Niger* showed zone of inhibition i.e. 13 mm (Table 3*)*.

Table 3. Antifungal activity of methanol bark extract of M. indica.

Test Microorganism	*M. indica* (mm)	+ve Control (mm)
Candida albicans	-	23
Aspergillus niger	13	20

The MIC & MBC also determined against above mentioned bacteria ranged from 3.125 to 6.25µg/mL. However MBC ranges from 1.562 to 3.125 µg/mL (Table 4).

Table 4. Determination of MIC (mg/mL) and MBC (mg/mL) values for the extracts against test organism.

Extract	MIC (µg/ml)			MBC (µg/ml)		
	(a)	(b)	(c)	(a)	(b)	(c)
M. indica	3.125	6.25	3.125	1.562	3.125	1.562

Where (a) is *P. aeruginosa,* (b) is *S. aureus and* (c) is *C. albicans*

Antimicrobial activity of methanol extract of *M. indica* with positive control (Oxytetracycline) against *P. aeruginosa*, *S. aureus* and *A. niger* were graphically represented in (Figures 1-3) respectively. The phytochemical analysis were done for MI extract using different biochemical analysis in which tests for Alkaloids, Glycosides, Phenols, Steroids and Tannins were found positive.

Figure 1. Graphical representation of antibacterial activity of methanol extract of *M. indica* with positive control (Oxytetracycline) against *P. aeruginosa*.

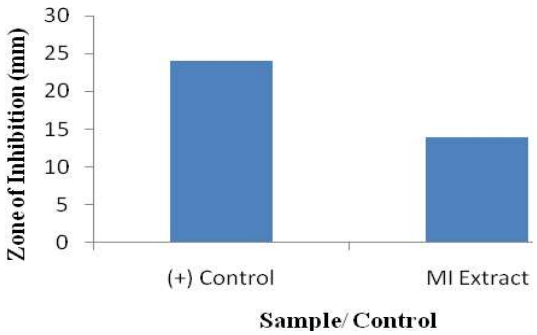


Figure 2. Graphical representation of antibacterial activity of methanol extract of *M. indica* with positive control (Oxytetracycline) against *S. aureus*.

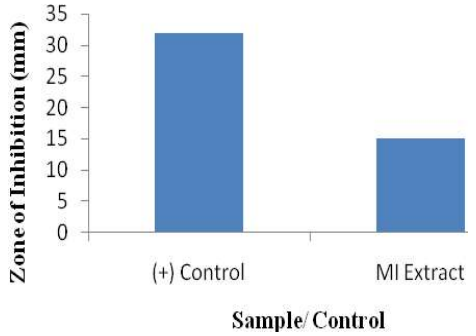


Figure 3. Graphical representation of antifungal activity of methanol extract of *M. indica* with positive control (Fluconazole) against *A. niger*.

However, Amino Acids, Flavonoids, Proteins, Saponins and Carbohydrates were not found in MI extract (Table 5). The antioxidant activity of the extract was checked by DPPH (2, 2-diphenyl-1- picrylhydrazyl) radical scavenging activity. 0.3 mL of different concentration (100, 200, 300, 400 and 500µg/mL) of sample was taken and made up to 0.4 mL with distilled water. To this added 0.6 mL of 100 M DPPH reagent in methanol. The reaction mixture was incubated for 20 minutes under dark and the reading was taken at 517 nm. The decrease in absorbance at 517 nm was taken as the antioxidant capacity of the sample.

Table 5. Table below displays the result of phytochemical tests for the extract.

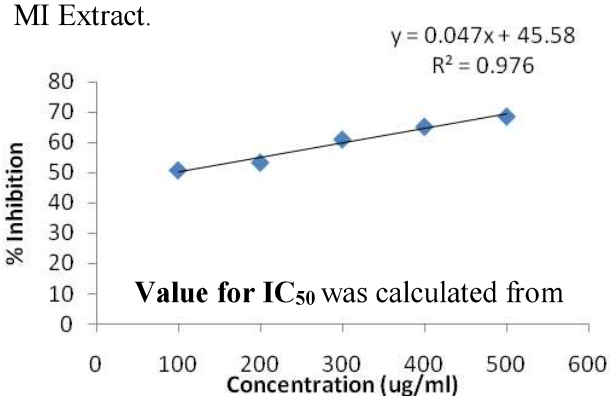
Name of the test	MI Extract
Alkaloids (Mayer's Test)	Positive
Amino Acids (Ninhydrin Test)	Negative
Flavonoids (Alkaline Reagent Test)	Negative
Glycosides (Keller - Killiani Test)	Positive
Phenols (Ferric Chloride Test)	Positive
Proteins (Xanthoprotic Test)	Negative
Saponins (Froth Test)	Negative
Steroids (Liebermann Burchard Test)	Positive
Tannins (Gelatin Test)	Positive
Carbohydrates (Molisch's test)	Negative

150ul of DPPH solution was mixed in 3ml ethanol and absorbance was taken immediately at 517nm for control reading. The absorbance for control was 0.118.Methanol was used as blank. Value of percent inhibition was calculated from the given formula and a graph was plotted. The results found were arranged and depicted in Table 6 and their orresponding graph was shown in Figure 4. The R^2 value was found 0.9762, which shows the significant data sets.

Table 6. Absorbance and %inhibition for different concentration of test samples

Concentration (µg/ml)	Absorbance (517nm)	%Scavenging
100	0.058	50.84
200	0.055	53.38
300	0.046	61.02
400	0.041	65.25
500	0.037	68.64

Figure 4. Percentage Inhibition curve for the MI Extract.



The graph placing the value of y=50 and calculating for the value of x. Therefore, IC_{50} value for *Mangifera indica* is 94.04µg/mL.

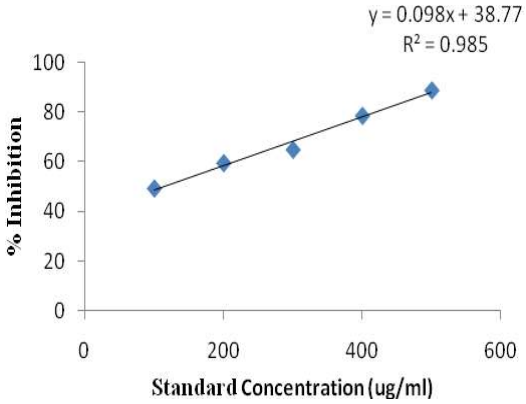
***L-ascorbic acid was taken as standard.**

Different concentrations of ascorbic acid were prepared (100, 200, 300, 400 and 500µg/ml) according to 1mg/mL of methanol. Immediate reading was taken at 517 nm from 1mg/ml solution for the purpose of control reading. The absorbance obtained for control was 0.205. The prepared concentrations were incubated for 30minutes and then reading was taken at 517nm at above concentration (Table 7). Value of percent inhibition was calculated and a standard graph was plotted (Figure 5).

Table 7. Absorbance and %inhibition for different concentrations of standard ascorbic acid

Concentration (µg/ml)	Absorbance (517nm)	%Scavenging
100	0.104	49.26
200	0.083	59.51
300	0.072	64.87
400	0.044	78.53
500	0.023	88.78

Figure 5. Percent Inhibition for the Standard Ascorbic Acid.



Value for IC_{50} was calculated from the graph placing the value of y = 50 and calculating for the value of x. Therefore IC_{50} value for l-ascorbic acid is 114.59µg/mL.

DISCUSSION

In the present study the methanol bark extract of *M. indica* shows antibacterial activity against pathogenic bacteria providing a leading role in microbial infections (Divyalashmi *et al.,* 2017). Antifungal activity of extract was found against *A. niger*. This indicates that the phytochemicals present in the extract have inhibitory action on the growth of *A. niger*. In the present study an attempt was made to characterize the phytochemicals, antioxidant and antimicrobial properties of methanolic bark extract of *M. indica* and their synergistic effect to prolong the shelf life of fruits and food stuff. The extract can be employed as a potent inhibitory agent against pathogenic bacteria strain. The result of phytochemicals showed the presence of different types of alkaloids, glycosides, steroids, phenols, tannin, steroids etc. These phytochemicals may be responsible for antibacterial activity. This suggests that these compounds could be responsible for the bactericidal and bacteriostatic property. The result of the present study gives credence to its use as potent remedy for infections and diseases caused by the pathogenic bacteria like *P. aerugenosa, S. aureus* taken under study. The antifungal activity of extract against *A. niger* would be an alternative remedy for candidacies. Antioxidant activity is one of the key properties for designer food on the basis of their role in human health especially due to presence of free radical scavenging activity and protection against oxidative stress and thus provides resistance towards bacterial infections among the processed food materials. Antioxidant properties play a pivotal role for the cure of heart disease and cancer**.** The IC_{50} value of *methanolic bark extract* of *Mangifera indica* is 94.04µg/ml concentration shows moderate level of scavenging activity of extract. Further studies can be done for effective inhibitory effects on *A.niger* by combination of other plant extract and their synergistic effects using nanotechnology. The antibacterial activity of *M. indica* demonstrated by mangifera that the bark extracts could be used to control bacterial growth among food materials and to develop quality food products with prolong shelf life along with antioxidant properties. The synergistic effect of antibacterial with antioxidant properties can be the basis to include bark extract of *M. indica* as food additive to prolonged self-life of food with scavenging properties of oxidative stress. The finding from this present study shows a remarkable property of mangifera bark extract and it could be exploit for possible phytogenic agent to control *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Aspergilus niger* infections in food materials.

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