Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2016; 5(5): 158-161
Received: 23-07-2016
Accepted: 24-08-2016

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Alternation of antimicrobial potential of mango peel and pulp after formalin treatment against six bacteria

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Abstract
The present study was carried out to investigate the comparative evaluation of antimicrobial properties of normal and formalin treated mango peel and flesh. The pure and chemical free mangoes are separated into two groups, normal and formalin treated. Then upon ethanol extraction zone on inhibition, MIC and MBC are determined. The test was carried out against six pathogenic bacteria using agar disc diffusion method. The extracts exhibited reasonable antibacterial activities against two gram positive (*Bacillus cereus, Staphylococcus aureus*) and four gram negative (*Escherichia coli, Shigella dysenteriae, Agrobacterium species, Shigella sonnei*) pathogenic bacteria. In comparison between peels and fleshes it is observe that fleshes contain more antibacterial action than peel and between normal and formalin treated extract, the later shows maximum activity. The present study clarifies that treatment with formalin elevates antimicrobial potential of mango extract.

Keywords: Mango, formalin, antimicrobial, MIC, MBC

1. Introduction
Food adulteration is basically lowering the quality of food for sale either by the admixture or by the substitution of inferior substances or by the removal of some valuable nutritious ingredient. Bangladesh is a developing country of the South Asian region, has been suffering from rampant food adulteration for the last few decades. Recent studies revealed that numerous deaths along with countless physical illnesses are occurring as the consequences of this ongoing food adulteration. There are several ways by which food is adulterated such as formalin treatment for preservation, addition of calcium carbide [1] for acceleration of ripening process, inclusion of DDT powder in sutki [2], mixing of toxic colorants which are used as textile dye [3], insecticide for pest control which ultimately destroy our natural eco-system [4], urea fertilizer to adulterate the *moori* and so on. Among the entire problem, in the recent year formalin treatment is a crucial problem. Supermarkets openly sell fruits, fishes and vegetables that have been treated with formalin to keep them fresh. A recent survey in 26 markets in Dhaka city by Save the Environment Movement (SEM) during June 1 to 10, 2013; it is found that around 94 percent of the mangoes and 100 percent blackberries and litchis are formalin-tainted (Formalin in fruits, *The Daily Star*, June 12; 2013). A similar result is found in the following year. Tests conducted by Poribesh Bachao Andolon (POBA) from June 1 and June 10, 2014; show that, all the black berries and 95 percent litchis sold in 35 city kitchen markets of Dhaka city contained formalin (Almost all litchis formalin-trained, *The Daily Star*, *June 12; 2014*).
This widespread use of formalin in various foods is considered to be gravely dangerous for public health. Scientific scholars suggest that consumption of formalin directly through food can cause different types of cancers [5] especially the lung cancer. Mango is a very popular fruit in Bangladesh and grown in large scale in Rajshahi. During the mango season tons of tons mango was distributed throughout the country from the local suppliers. But local suppliers use formalin spray to protect the mango from denatured and help to keep it hard for a long time i.e. Formalin works as a preservative. Though formalin applies on skin of mango and we did not eat the skin/ peel of mango but it may penetrate the skin and induce into the flesh of mango.
During the last 20 years, it has been reported that human infections are increasing at an alarming rate, especially in tropical and subtropical developing countries [6]. This is partly due to the indiscriminate use of antimicrobial drugs and the development of microbial resistance to some of the synthetic drugs [7].

The fact that microorganisms nowadays tend to develop resistance towards drugs, coupled to the undesirable side effects of certain antibiotics offer considerable potentials for the development of new effective antimicrobial agents; medicinal plants being a prolific source. As many microorganisms developed resistance due to the indiscriminate use of antibacterial drugs, there is a need to develop new molecules with minimum side effects [8]. Therefore investigation of the chemical compounds within medicinal plants has become desirable. Various plant extracts possess bacteriostatic and bactericidal effects due to secondary metabolites they contain, namely alkaloids, tannins, flavonoids, and phenolic compounds [9]. There was lots of work on antimicrobial properties of Mango [10, 12] but there was no study have been reported what are the effects of formaldehyde on antimicrobial activity on mango and after treatment of formalin, the evaluation of antimicrobial activity was our target.

2. Materials and Methods

2.1 Collection and identification of the plant sample

About 10 kg of raw mango was collected from the garden of Rajshahi, Bangladesh in July 2013. The mango was collected as pure state as it does not contain any foreign chemical.

2.2 Preparation and extraction of the sample

After collection, the mango was washed thoroughly in distilled water and shed dried. The mango was divided into two groups, one group is treated with formalin for 7 days and other group was kept as normal. The formalin solution was sprayed by the spray gun. Both groups of mangoes were peeled off and peels and flesh is oven dried at 55 °C. The peels and flesh of each group are then ground into coarse powder by a grinding machine in the Department of Pharmacy, University of Rajshahi, Bangladesh and stored at room temperature (RT) for future use. About 150 gm of normal mango peel (NP), formalin treated mango peels (FP), normal mango flesh/pulp (NF) and formalin treated mango flesh/pulp (FF) was taken in plastic cap containing glass bottle and extracted by ethanol under sonication bath (Trans sonicator, T-60). The sample is extracted by three times to get the maximum extract. The mixture (sample + solvent) was filtered through Whatman No.1 filter papers. The filtrate was then concentrated with a rotary evaporator under reduced pressure at 50 °C to obtain brownish mass of peels and reddish mass of flesh.

2.3 *In Vitro* antimicrobial Screening

The antibacterial screening of an agent is essential to ascertain its spectrum against various types of pathogenic organisms. Antibacterial activity of any plant can be detected by observing the growth response of various microorganisms to the plant extract, which is placed in contact with them. Antibacterial activity was observed against six strains of both Gram-positive and Gram-negative bacteria. In general, antibacterial screening is undertaken in two phases. A primary qualitative assay to detect the presence or absence of activity and a secondary assay which quantities the relative potency, expressed as the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) value.

2.4 Disc Diffusion Method

Disc diffusion method was used to determine the antimicrobial activity of the crude extracts and its fractions [13] against the microbial strains gram positive bacteria then gram negative bacteria. Disc diffusion assay method is based on the ability of antibiotics to diffuse from a confined source through the nutrient agar gel and create a concentration gradient. If the agar is seeded or streaked with a sensitive organism, a zone of inhibition will result where the concentration exceeds the MIC for the particular organism. In this method, measured amount of the test samples is dissolved in definite volumes of solvent to give solutions of known concentrations (µg/ml). Then sterile filter paper (BBL, Cocksville U.S.A) discs having 5 mm in diameter are impregnated with known amounts of the test substances and dried. These test material discs as well as standard antibiotic discs are placed on plates containing a suitable medium (nutrient agar) seeded with the test organisms. These plates are kept at 4 °C for 24 hours to allow maximum diffusion. The plates are then kept in an incubator at 37 °C for 12-18 hours to allow the growth of the organisms. If the test material has antibacterial activity, it will inhibit the growth of microorganisms, giving clear, distinct zone called "Zone of Inhibition". The antibacterial activity of the test agent is determined by measuring the diameter of the zone of inhibition in term of mm.

2.5 Test materials used for the study

In our present study, the antibacterial activity of the NP, FP, NF and FF was investigated in comparison with a standard antibiotic ampicillin (40 µg/discs). The sample concentration is 400µg/ml.

2.6 Test organisms

Both Gram-positive and Gram-negative bacterial strains taken for the test were listed in the following table 1. These organisms are available in the Microbiological Research Laboratory at the Department of Pharmacy, Bangladesh.

Table 1: List of tested bacteria.

Gram-positive	Gram-negative
1. *Bacillus cereus*	1. *Escherichia coli*
2. *Streptococcus aureus*	2. *Shigella dysenteriae*
	3. *Agrobacterium species*
	4. *Shigella sonnei*

2.7 Minimum inhibitory concentration (MIC)

The lowest concentration of the compounds or extracts required to inhibit the growth of the organism is referred to as minimum inhibitory concentration (MIC). Here "Serial dilution technique" [14] was followed using nutrient broth media. The test bacteria *E. coli*, grown at 37 °C in nutrient agar medium, were diluted in sterile nutrient broth medium in such a manner that the suspension contained about 10^7cells/ml. Here six different concentrations (31.25, 62.5, 125, 250, 500 & 1000µg/ml) of extracts are used for determination of MIC. The control test tube containing medium only was used to confirm the sterility of the medium. All the test tubes were incubated at 37 °C for 18 hours.

2.8 Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) is determined by sub-culturing the contents of the tubes of MIC showing no growth onto antibiotic-free liquid medium and examining for bacterial growth. Into each of the test tubes of MIC, 5 ml of nutrient broth medium were added. This was done to eliminate the effect of antimicrobial agent. The test tubes were then incubated at 30 °C for 24hrs. If growth of

bacteria is observed in the MIC tubes, it indicates the presence of bacteriostatic agent and in this case the MBC>MIC. No growth of bacteria in the tubes after dilution indicates the presence of bactericidal agent and in this case, MIC=MBC.

3. Results and Discussion

Literature shows the presence of phenolic and flavonoids in mango [15, 18] which is responsible for antimicrobial activity. It is observed that, the mango pulp usually exerts more antibacterial activity than mango peel which is also resembles of the previous data [19, 20]. In case of formalin treatment it also exhibits similar trend. Treatment with formalin usually increases antimicrobial activity of both mango peels and pulps/fleshes but there is exception in case of fleshes upon *Shigella sonnei* and *Shigella sonnei*. Normal mango flesh/pulp (NF) shows maximum activity against *Shigella sonnei* and *Bacillus cereus* where it inhibits about 25 and 20 mm diameter area. Formalin treated flesh/pulp (FF) gives maximum activity against *Agrobacterium species* and *Escherichia coli* where its inhibition zone is, 32 and 24 mm respectively while the standard antibiotic ampicillin exerts antimicrobial activity which more than the extracts of peels and fleshes in all cases.

In MIC and MBC studies it is found that all the extracts inhibits bacterial growth at concentration of 250 µg/ml and among the three extracts normal mango peel (NP) kills the bacteria and gives bactericidal effects while other extracts at 250 µg/ml gives bacteriostatic effects, that is it inhibits the growth but not to the kills of the cell.

Table 2: *In vitro* antibacterial activity of normal and formalin treated mango peel and pulp.

Bacteria	Diameter of the zone of inhibition (mm)				
	Ampicillin	NP	FP	NF	FF
Shigella sonnei	28	11	12	25	14
Bacillus cereus	23	11	11	20	21
Agrobacterium species	34	11	13	14	32
Escherichia coli	27	14	13	18	24
Streptococcus aureus	25	11	10	17	20
Shigella dysenteriae	22	12	13	18	13

Table 3: MIC and MBC value of normal and formalin treated mango peel and pulp against *E. Coli.*

Sample	MIC µg/ml	MBC µg/ml
NP	250	250
FP	250	>250
NF	250	>250
FF	250	>250

4. Conclusion

The plant-based formulations are a viable option that could be useful in reducing the side effects associated with conventional antibiotic treatment [21]. Plant-based formulations would also increase the number of compounds that are used to limit infections [22] and might function synergistically with current therapies [23]. This is the first study about the MIC and MBC value of mango peel and pulp against *E. coli*. Although literature review only provides information about the MIC and MBC value of ripe mango seed [24, 25] but there is no data about the antimicrobial property of mango pulp. A further study is needed about the mechanism of inhibition of microbial species and the synergistic effects of formalin.

5. Acknowledgements

The authors are thankful to the Ministry of Education, Bangladesh for providing financial support for this research work. The authors are also grateful to Department of pharmacy, Rajshahi University for the supply of facilities to conduct the experiment.

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