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Full Length Research Paper

Antifungal and antibacterial activity of the different parts of mature *Benincasa hispida* against various microbial infectious agents

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In this study we assayed on antimicrobial activity of peel, pulp, waxy coating of *Benincasa hispida*. Various parts of the plant are reported as antibacterial agents worldwide. Leaves, flower, root, seed are the most studied elements as antibacterial agent. Some fruits are also proved as antibacterial agent and used as herbal medicine as well as nutritional supplements during disease. Different parts of mature and immature winter melon fruits were extracted with different organic solvents viz., methanol, ethyl acetate and chloroform. These extracts were subjected to test against selected pathogenic bacterial (*Bacillus subtilis* IFO 3026, *Sarcina lutea* IFO 3232, *Xanthomonas campestris* IAM 1671, *Escherichia coli* iw 3310 IFO 3007, *Pseudomonas denitrificans* KACC 32026, and fungal (*Fusarium oxysporum*, *Aspergillus niger* KTCC 1700, *Collectotrichum melongenae*) strains using the well diffusion method. The waxy coating and peel of mature fruit of *B. hispida* extracts has strong antibacterial activities than immature fruit. In these cases, the minimum inhibitory concentration was 128 µg/ml. Ethyl acetate and chloroform extracts of waxy coating of *B. hispida* showed inhibition rate against fungal infection 43% and 33%, respectively against *F. oxysporum* but there is no inhibition against *A. niger, C. melongenae.* These results suggested that. So, the *B. hispida* would be a potential source that may help to develop natural antimicrobial agents.

Key words: Antibacterial, a fruit, winter melon, infectious, extracts, minimum inhibitory concentration (MIC).

INTRODUCTION

The common agricultural products are rice, wheat, jute, tea, cotton, sugarcane, flower, vegetables, fishes and seed development, livestock, horticulture are main agricultural sectors . Microbial contamination is one of the most alarming causes of agricultural production. According to the Food and Agriculture Organization (FAO), pests and diseases are responsible for about 25% of crop loss (Sheahan and Barrett, 2017). To solve this issue, new methods are needed to detect diseases and pests early such as novel sensors that detect plant

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odours, and spectroscopy and biophotonics that are able to diagnostic plant health and metabolism (Martinelli et al., 2015). Globally, plant extracts are employed for their antibacterial, antifungal and antiviral activities. It has been reported that more than 400,000 species of tropical flowering plant showed medicinal properties and the usages of medicinal flowering plant make the folk medicine cheaper the other medicine (Yuan et al., 2016). *Benincasa hispida* (Thunb.) which commonly called as winter melon belongs to the *cucurbitaceae* family. It is a popular vegetable crop, especially among Asian communities both for nutritional and medicinal purposes (Al-Snafi, 2013; Arora and Kaushik, 2016). Phytochemical screening of various extracts of fruits indicate the major constituents of the fruit are triterpenoids, flavonoids, glycosides, saccharides, carotenes, vitamins, ß-sitosterin, and uronic acid (Mandal et al., 2012).

Extracts of winter melon may be a natural drug with anti-ulcer activity and as well as antioxidant property (Lee et al., 2005). It contains terpenes, flavonoids, glycosides and sterols which have antioxidant effects (Shetty et al., 2008). Seed extract of winter melon howed anti-angiogenic property through inhibition of endothelial cell proliferation (Wen et al., 2008). The methanolic extracts of *B. hispida* showed excellent protection against histamine-induced bronchospasm probably through an antihistamine activity that is H1 receptor-antagonism (Simons and Simons, 2008). The ethanol extract of *B. hispida* have antinociceptive and antipyretic activity and used as herbal medicine against fever and pain conditions (Rahmatullah et al., 2012; Sharma et al., 2014). The antioxidant property of winter melon may be beneficial in the management of colchicene-induced rat model of Alzheimer's disease (Yagnik et al., 2009). The hypoglycemic effects of *B. hispida* waxy coating may be used to prevent diabetes mellitus (Gu et al., 2013).The methanol extract of winter melon fruit showed significant inhibition against fungus namely *Candida albicans* (Xing et al., 2012) also methanolic and petroleum ether extracts showed significant inhibition of carrageenan-induced paw edema, histamine induced paw edema and cotton pellet-induced granuloma in a rat model (Ashok et al., 2010; Elhassan et al., 2020; Patil and Patil, 2017). The ethanol extract of seeds showed an anti-urolithiatic effect with reduction in stone forming constituents in the urine and decreased kidney retention that reduced the solubility product of crystallizing salts (Juliana et al., 2018; Meshram et al., 2016; Tatiya et al., 2017). The seeds of *B. hispida* having bioactive peptide, Hispidalin showed inhibitory effects against human bacterial and fungal pathogens (Salas et al., 2015; Sharma et al., 2014) .The aqueous extract of stem of winter melon for hypoglycemic effect in alloxan-induced diabetic rabbits showed significant dose-dependent reduction in blood glucose levels (Jhonatas et al., 2019; Khan et al., 2012).

In this study the authors tried to analyse antimicrobial activity of the different parts of mature and immature winter melon fruits. The different parts were extracted with different organic solvents viz, methanol, ethyl acetate and chloroform. These extracts were subjected to test against selected pathogenic bacterial and fungal strains using the well diffusion method. The potentially of the different organic extract of *B. hispida* will be assayed to find strong antimicrobial activities. If they found *B. hispida* would be a potential source that may help to develop natural antimicrobial agents for the control of plant pathogens.

MATERIALS AND METHODS

Materials

The phytochemical analysis showed that different solvents are responsible for extracting different components from the plants. So, several solvents of different polarity are used to extract different plant components. Chloroform, Ethyl Acetate and Methanol were selected according to their degree of polarity. The different types of media were used during experiment Mueller-Hinton Agar (MHA), Lactose Broth, Potato Dextrose Agar (PDA) (Mahboob et al., 2019).

Microorganisms

Five different pathogenic bacteria and three fungi were collected from the microbiology laboratory of the department of Biotechnology and Genetic Engineering, Islamic University, Kushtia. The following strains were used as test strain *Bacillus subtilis* IFO 3026, *Sarcina lutea* IFO 3232, *Xanthomonas campestris* IAM 1671, *Escherichia coli* iw 3310 IFO 3007, *Pseudomonas denitrificans* KACC 32026, *Fusarium oxysporum*, Aspergillus *niger* KTCC 1700 and *Collectotrichum melongenae.*

Sample collection

The healthy, disease free, fresh immature and mature fruits of *B. hispida* were safely collected from farmers land. Various parts of *B. hispida* waxy coating, peel of mature and immature fruits were used.

Plant extract preparation

The fleshy surface, wax gourd of *B. hispida* dried under the shade and then powdered with mechanical grinder. It was later stored in air tight containers protected from direct sunlight for further experiments. The fine grinded powder was mixed (10 gm/100 ml) separately with chloroform, ethyl acetate and methanol. The samples with solvent were placed in rotatory shaker for 24 h at (30-36ºC) (Elnaggar et al., 2019; Ikeda et al., 2007; Sasidharan et al., 2011).The extracts of plant material were filtered more than three times and performed by passing the extracts through filter paper. The extracts with different solvents then allowed to air dry after filtration to concentrate.

Antimicrobial Assay

The crude organic extracts of selected solvents were used *In vitro* screening of antibacterial and antifungal activity. The screening of antibacterial and antifungal activity is a typical microbiological assay which is performed with culture of microorganisms. One of the most commonly used methods of bacterial assay is the well diffusion method (Mohsenipour and Hassanshahian, 2016; Valgas et al.,

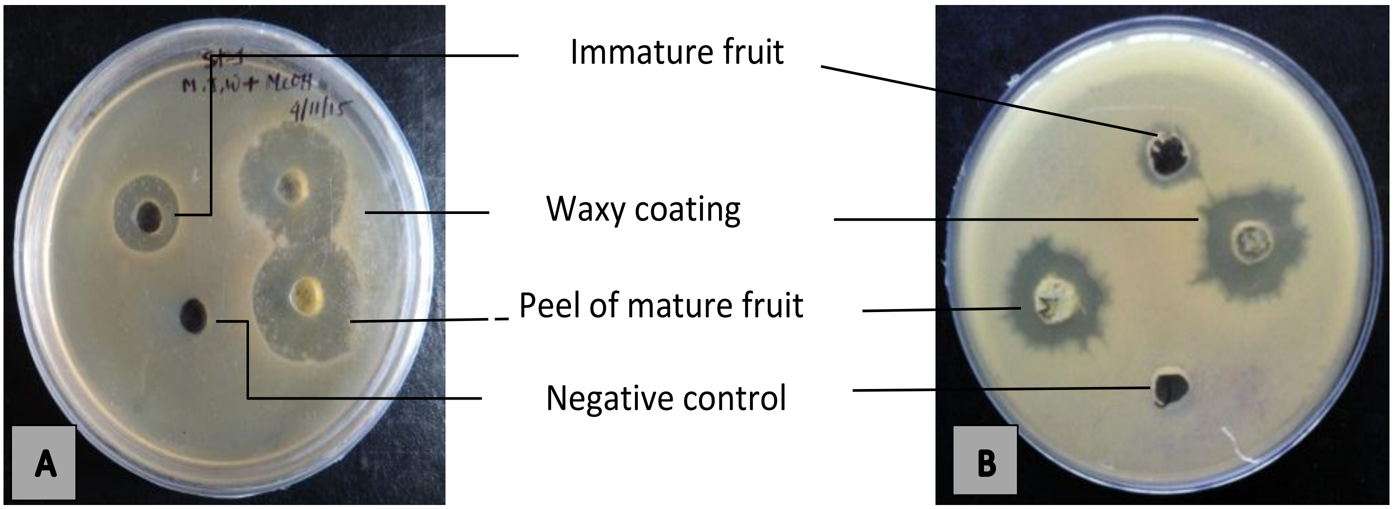

Figure 1. Antibacterial activity of methanol extract of *B. hispida* different parts against *B. subtilis* IFO 3026 (A), *E. coli coli* iw 3310 IFO 3007 (B). **N.B:** Negative control-Methanol (only solvent).

2007). The antimicrobial activity was evaluated by measuring the zone of inhibition expressed as mm against test organisms.

The antibacterial activity of plant extract was carried out by well diffusion assay using 100 µl of inoculum suspension of bacteria. Then each well was loaded with 100 µl of the different concentrations of extracts. Negative controls were used to screen the activity of solvents which may have effect on antimicrobial activity, for this the same solvents which were used to dissolve the plant extracts employed as negative control. Positive control was used to measure the antibacterial sensitivity of the test strain as well as it can confirm the effectiveness of the methods. Streptomycin (10µg/disc from Banex Ltd., USA) was used as positive reference standard to determine the sensitivity of the tested strain in each bacterial species.

The crude sample which showed the best potentiality against the selected organisms used *In vitro* screening of minimum inhibitory concentration (MIC). In microbiological research, minimum inhibitory concentration (MIC) is the lowest concentration in which the extract showed inhibition of the visible growth of a microorganism after incubation. In this study, it was determined by the serial dilution technique (Meenakshi and Anoop, 2019). The process of serial dilution was continued up to nine tubes. It contained 16384 µg ml^{-1} in the first tube and 64 µg ml^{-1} in the last test tube.

The antifungal activity of plant extract was carried out by well diffusion assay using the mycelia from a 24-h culture. Each of them was well loaded with 100 µl of the different concentrations of extracts. Negative controls were used for the same solvents as was discussed earlier. The plates were incubated at 27 ± 2 ^{0} C for 48-72 h. The antifungal activity was evaluated by calculating Growth Inhibition Rate (%) against test organisms (Bhalodia and Shukla, 2011). Each test in this experiment was replicated four times.

Data analysis

Analysis of variance of antimicrobial activities of extracts from winter melon was assayed using SPSS-16 Program. Total antimicrobial activities were expressed as the mean ± S.D. (n=4). Significance of difference was calculated by Duncan's new multiple range test and results with P<0.05 were considered statistically significant.

RESULTS

Agar well diffusion method was used to test antibacterial efficacy of crude aqueous extracts of waxy coating, peel (mature fruit) and immature fruit of winter melon. In this case 100 µl aqueous methanol extract of different sample of *B. hispida* were applied in each well. The wells of 9 mm diameter were prepared in pre-inoculated Mueller-Hinton Agar (MHA) plates with the test organism.

Methanol extract

At the concentration of 4.096 µg/µl (applied 100 µl), the methanol extract of waxy coating showed zone of inhibition against *B. subtilis* IFO 3026, *S. lutea* IFO 3232, *X. campestris* IAM 1671, *E. coli* iw 3310 IFO 3007, *P. denitrificans* KACC 32026 were 15.6 mm, 16.4 mm, 15.6 mm, 16.8 mm, 15.7 mm, respectively. Peel extract of mature fruit produced zone of inhibition against these strains 15.3 mm, 14.9 mm, 13.2 mm, 16.2 mm and 13.5 mm, respectively whereas immature fruit extract showed inhibition in the diameter 11.5 mm, 11.2 mm and 12.1 mm against *S. lutea* IFO 3232, *X. campestris* IAM 1671, *E. coli* iw 3310 IFO 3007 but *B.* subtilis, P. *denitrificans* were not inhibited by methanol extract of immature fruit (Figures 1 and 2).

Ethyl acetate extract

At the concentration 4.096 µg/µl (applied 100 µl), ethyl acetate extract of waxy coating of winter melon, the zone of inhibition against *B. subtilis* IFO 3026, *S. lutea* IFO 3232, *E. coli* iw 3310 IFO 3007, and *P. denitrificans* KACC

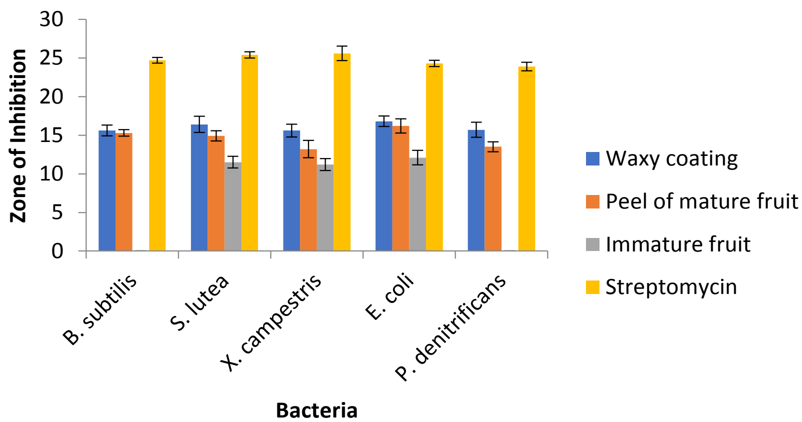

Figure 2. Comparative antibacterial activity of commercial antibiotic and methanol extract of *B. hispida* against selected pathogenic bacteria.

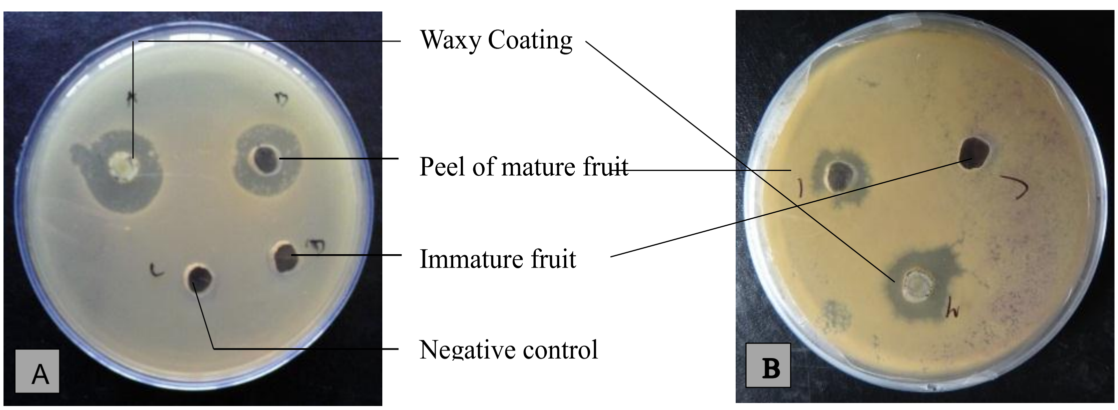

Figure 3. Antibacterial activity of ethyl acetate extract of *B. hispida* different parts against *B. subtilis* IFO 3026 (A) and *P. denitrificans* KACC 32026 (B). Negative control- Ethyl acetate (only solvent).

32026 produced 15.7 mm, 14.4 mm, 16.5 mm, and 16.9 mm, respectively. In case of mature fruit extract, the zone of inhibition showed 14.8mm, 13.2 mm, 15.6 mm, and 15.2 mm against tested bacterial strains *B. subtilis* IFO 3026, *S. lutea* IFO 3232, *E. coli* iw 3310 IFO 3007, and *P. denitrificans* KACC 32026, respectively. *X.* campestris IAM 1671 did not shown inhibition zone when the waxy coating and peel extract were applied in the well. Immature fruit extract exhibited inhibition zone in the diameter of 11.5 mm and 12.1 mm against *S. lutea* IFO 3232, and *E. coli* iw 3310 IFO 3007, respectively. In this study, the most sensitive strain was *P. denitrificans* KACC 32026 with the inhibition zone of 16.9 mm produced by waxy coating of *B. hispida* (Figures 3 and 4).

Chloroform extract

Chloroform extract of waxy coating of *B. hispida* produced inhibition against *B. subtilis* IFO 3026, *S. lutea* IFO 3232, and *E. coli* iw 3310 IFO 3007. At the concentration 4.096 µg/µl (applied 100 µl), the diameter of zone of inhibitions were found 14.6 mm, 14.4 mm, and 16.4 mm, respectively. Peel extract of chloroform also produced inhibition zones against these strains in the diameter of 12.7 mm, 12.5 mm, and 13.4 mm, respectively. *X. campestris* IAM 1671 and *P. denitrificans* KACC 32026 did not show any sensitivity with these extracts. Immature fruit extracts showed small zone of inhibition in the diameter of 10.2 mm, and 10.1 mm against *S. lutea* IFO 3232, and *E. coli* iw 3310 IFO 3007, respectively but did not produce any inhibitions against other bacterial strains (Figures 5 and 6).

Comparative study of different crude extracts of waxy coating of *B. hispida* against tested pathogenic bacteria

Different concentration of crude extracts of waxy coating

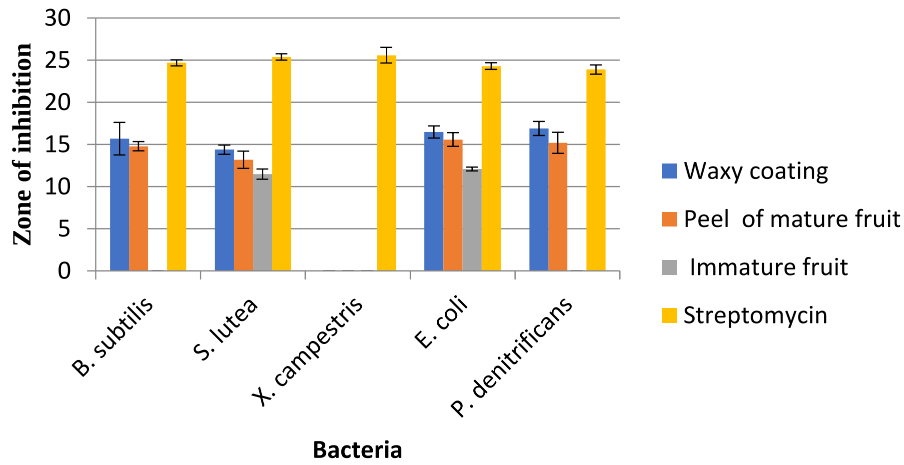

Figure 4. Comparative antibacterial activity of commercial antibiotic and ethyl acetate extract of *B. hispida* against selected pathogenic bacteria.

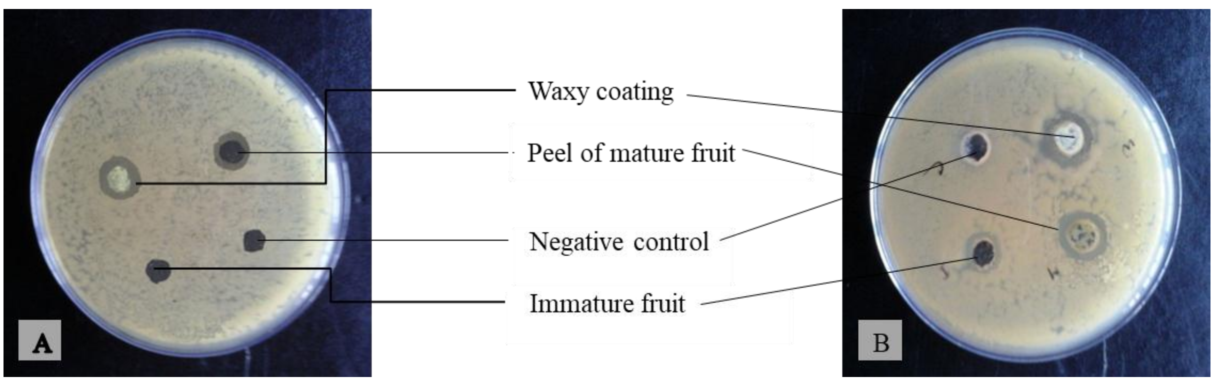

Figure 5. Antibacterial activity of chloroform extract of *B. hispida* different parts against *B. subtilis* IFO 3026 (A) and *S. lutea* IFO 3232 (B). Negative control- Chloroform (only solvent).

of *B. hispida* was examined. It is found that methanol and ethyl acetate extract of waxy coating showed better antibacterial activity than chloroform extract. It was found that *E. coli* iw 3310 IFO 3007 showed minimum inhibitory concentration (MIC) of Methanol and ethyl acetate extract at lower extent than other bacteria (Figures 7 and 8).

Comparative study of different crude extracts of mature fruit (peel) of *B. hispida* against tested pathogenic bacteria

The comparative study of peel extracts of *B. hispida* mature fruit were evaluated against different test strains. It is indicated that methanol and ethyl acetate extract of mature fruit (peel) showed better antibacterial activity against *E. coli* iw 3310 IFO 3007 than chloroform extract. Ethyl acetate extract produced better inhibition zone against *P. denitrificans* KACC 32026 than methanol extract, whereas chloroform extract did not show inhibition zone against this bacteria and it was found that *E. coli* iw 3310 IFO 3007 showed minimum inhibitory concentration (MIC) of different extract at lower extent than other bacteria. Methanol extracts showed good results in case of MIC (Figures 9 and 10).

Comparative study of different crude extracts of immature fruit of *B. hispida* against tested pathogenic bacteria

The comparative study of immature fruit extracts of *B. hispida* along with standard antibiotic streptomycin. It is indicated that methanol, ethyl acetate and chloroform extract of immature fruit didn’t show better antibacterial activity against *B. subtilis* IFO 3026, *X. campestris* IAM 1671 and *P. denitrificans* KACC 32026, whereas showed small inhibition zone against *S. lutea* IFO 3232, *E. coli* iw

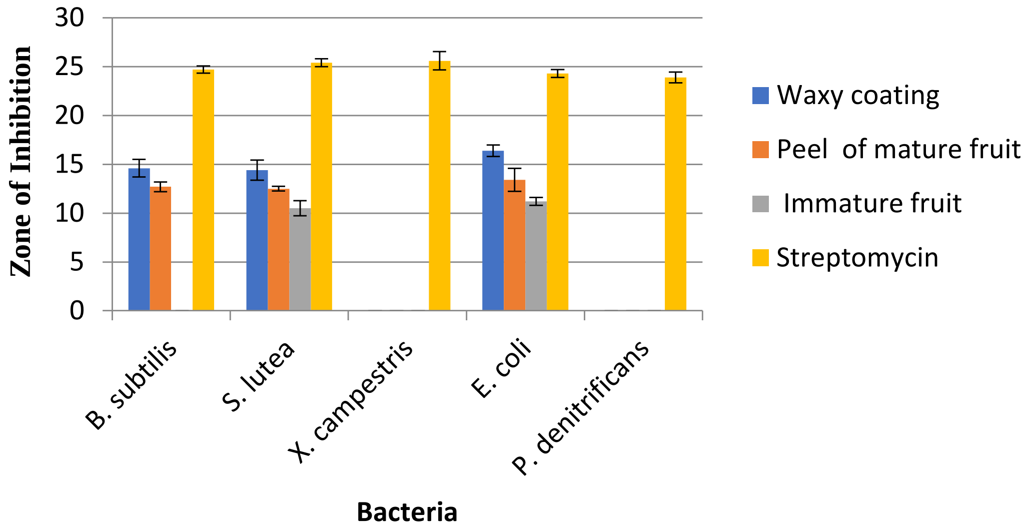

Figure 6. Comparative antibacterial activity of commercial antibiotic and chloroform extract of *B. hispida* against selected pathogenic bacteria.

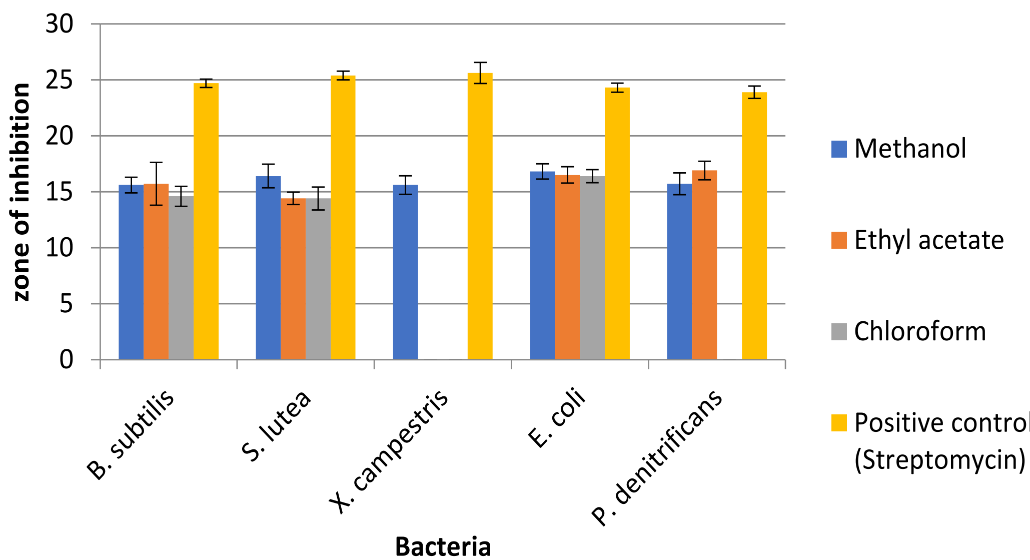

Figure 7. Comparative antibacterial activity study of different waxy coating extracts of *B. hispida* mature fruit.

3310 IFO 3007. It was found that methanol extract showed good results in case of MIC than ethyl acetate and chloroform extracts (Figures 11 and 12).

Antifungal activity study

Agar well diffusion method was used to test antifungal efficacy of crude aqueous extracts of waxy coating, peel (mature fruit) and immature fruit of winter melon. Ethyl acetate extract of waxy coating and peel of mature fruit showed more positive results against *Fusarium oxysporum* than methanol or chloroform extract. Immature fruit extract did not show any considerable inhibition against this fungus (Figures 13 and 14).

DISCUSSION

The importance on the investigations of natural antimicrobials from plant extracts which can substitute synthetic chemicals may reduce harmful side effects due

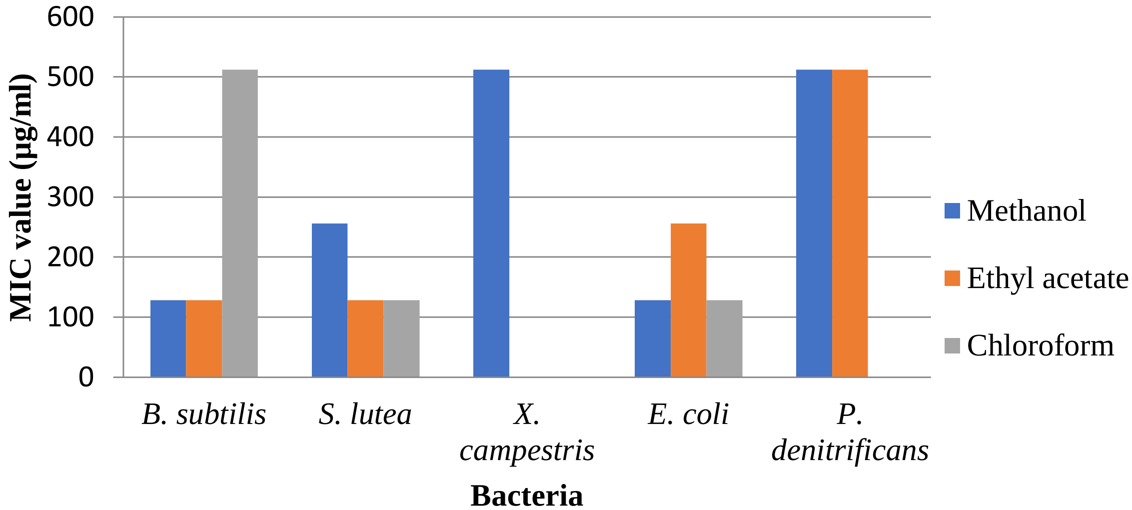

Figure 8. Comparative study of minimum inhibitory concentration (MIC) of different waxy coating extracts of *B. hispida* mature fruit against pathogenic bacteria.

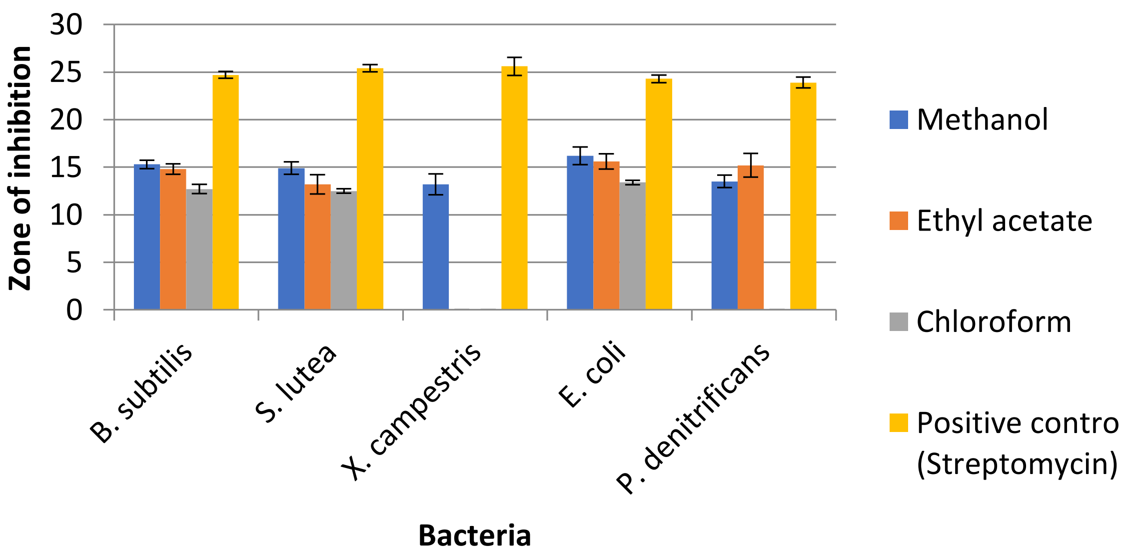

Figure 9. Comparative antibacterial activity study of different peel extracts of *B. hispida* mature fruit.

to potential toxicity of these commercially available drugs.

Now days multiple drug resistance become great threat of public health due to indiscriminate use of commercially available antimicrobial drugs. There is a significant role of phytochemical to mitigate societal health issues and to avoid drug resistance (Zhang et al., 2015; Karuppiah and Rajaram, 2012). Humans from the pre-historical times have used the herbs, spices, and plants as a natural sources of antimicrobial agents, although the levels and range of activity were not fully defined. Studies have pointed out that many drugs that are used in commerce have come from folk-use and use of plants by indigenous cultures (Cowan, 1999). The antimicrobial activity of the leaves of some wild Cucurbitaceae species against some human pathogenic microorganisms has investigated recently (Anyanwu and Okoye, 2017). The present study was conducted to find out the antimicrobial effects of different extracts of *B. hispida* belongs to *Cucurbitaceae* family.

In this study, different samples that are the waxy coating, peel of mature fruit, and immature fruit of *B. hispida* were extracted with methanol, ethyl acetate and chloroform. These extracts were evaluated againsttwo species of Gram-positive bacteria *B. subtilis* IFO 3026, *S. lutea* IFO 3232 and three species of Gram-negative

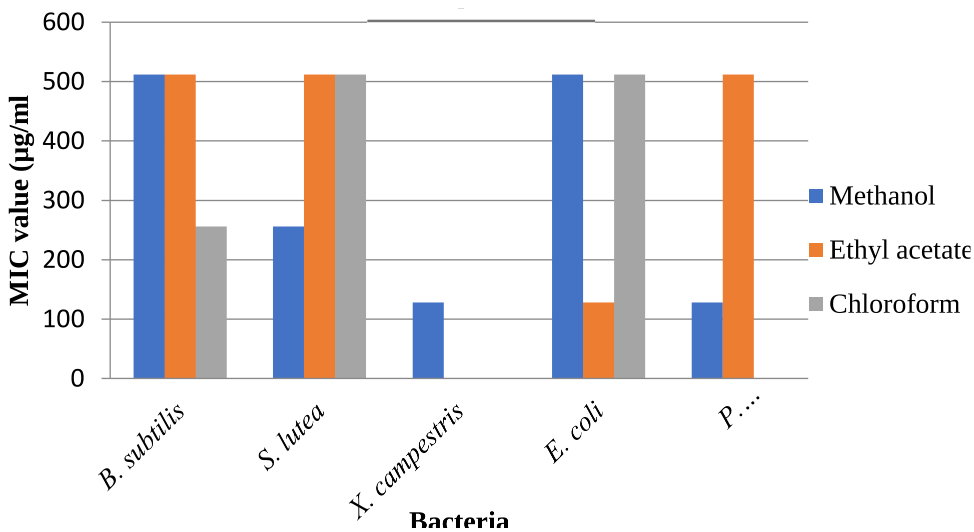

Figure 10. Comparative study of minimum inhibitory concentration (MIC) of different peel extracts of *B. hispida* mature fruit against pathogenic bacteria.

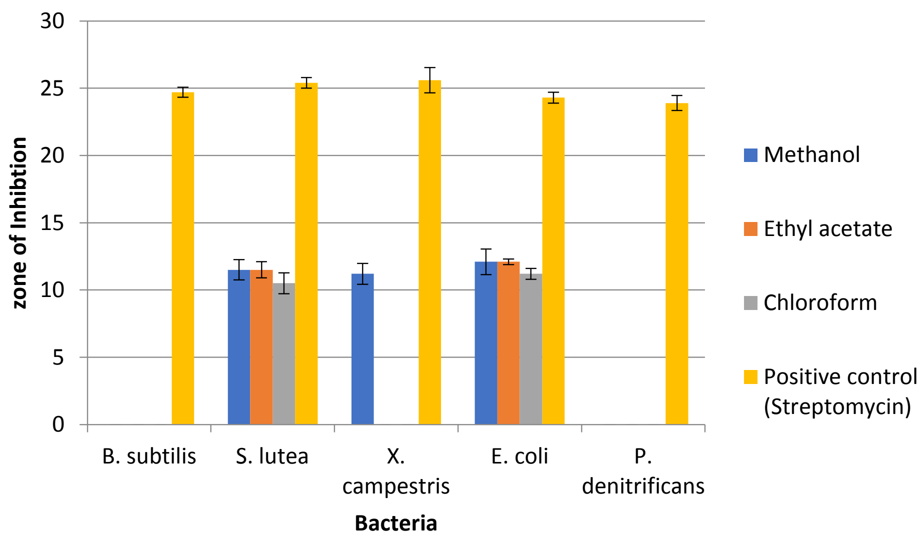

Figure 11. Comparative study of antibacterial activity of different immature fruit extracts of *B. hispida.*

bacteria, *X. campestris* IAM 1671, *Escherichia coli* iw 3310 IFO 3007, *P. denitrificans* KACC 32026. These organic extracts were also investigated against three pathogenic fungal species such as *F. oxysporum*, *A. niger* KTCC 1700, *C. melongenae.* The inhibition zone was the highest in case of standard antibiotic but methanol extract inhibited all bacterial strain efficiently than ethyl acetate or chloroform extract. Methanol extract has been reported effectiveness in many studies (Atef et al., 2019). This study explored a query about the reason of the effectiveness of methanol extract comparing other solvent extracts. Further study is required to find out the chemical composition of methanol extract and other extracts to search the responsible components of antimicrobial activity. *B. hispida* known as water melon is a common and popular fruits all over the world. These findings explore the health benefit of *B. hispida* which the authors take very frequently. So, *B. hispida* can be recommended antimicrobial agent.

Conclusion

Winter melon is used for the management of various

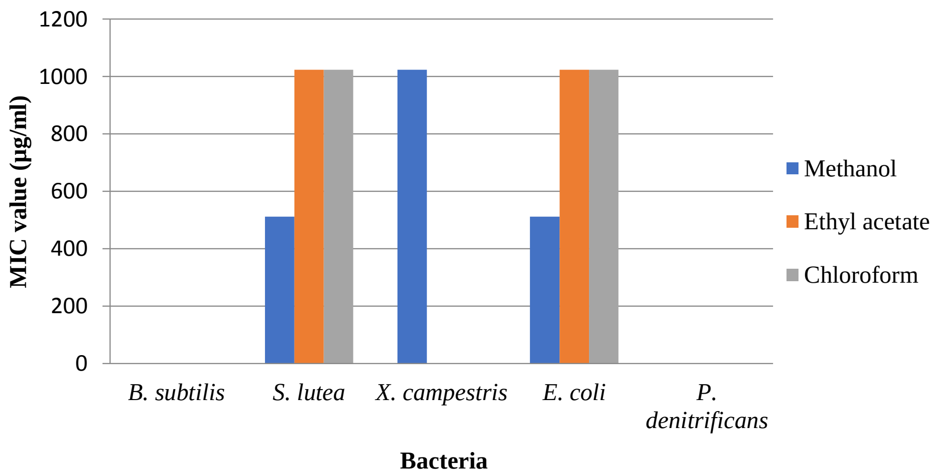

Figure 12. Comparative study of minimum inhibitory concentration (MIC) of different immature fruit extracts of *B. hispida* against pathogenic bacteria.

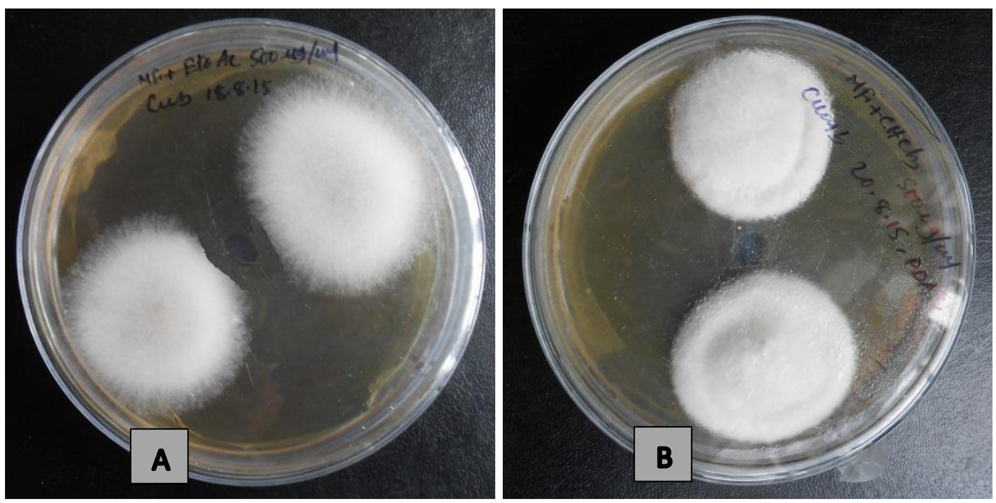

Figure 13. Antifungal activity of ethyl acetate (A) and chloroform (B) extracts of waxy coating of *B. hispida* mature fruit against pathogenic fungus *F. oxysporum.*

diseases and also used as antioxidant, styptic, anti-inflammatory, astringent, anthelmintic, aphrodisiac, demulcent, diuretic, febrifuge, and tonic agents. The results of the study revealed that the different extracts of waxy coating and peel of mature of *B. hispida* possess many active ingredients that inhibited the growth of selected pathogenic bacteria (*B. subtilis* IFO 3026, *S. lutea* IFO 3232, *X. campestris* IAM 1671, *E. coli* iw 3310 IFO 3007, *P. denitrificans* KACC 32026) . On the other hand, ethyl acetate extracts of waxy coating and peel of mature winter melon fruit showed maximum antifungal activity against *F. oxysporum*. So, it is concluded that waxy coating and peel of mature fruit has better antibacterial and antifungals activities. If it is possible to find out the reason of self-protective mechanism of mature fruits of *B. hispida,* there is enormous possibility to prevent immature fruit-rot of winter melon from various infections and also will help to develop antifungal and antibacterial agents.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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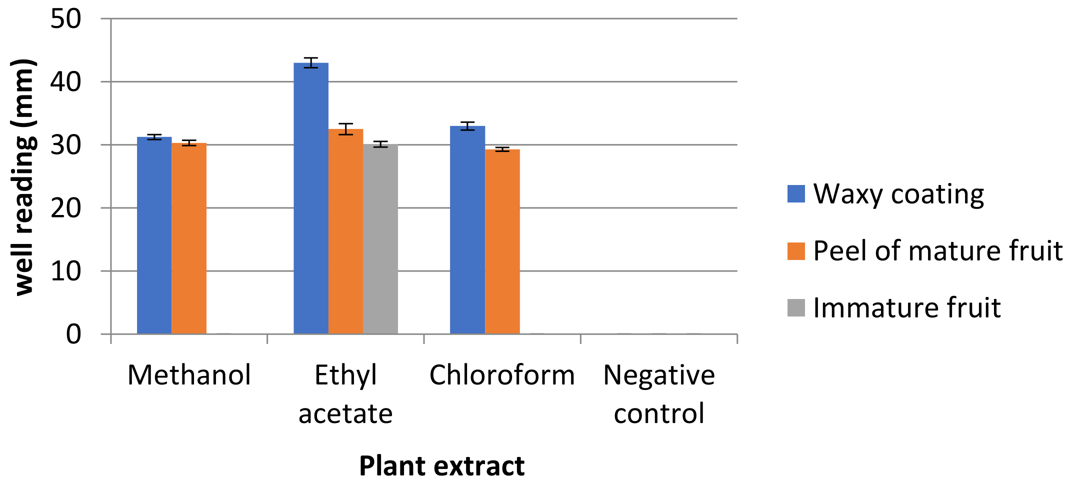

Figure 14. Growth Inhibition Rate (%) of different extracts of *B. hispida* against pathogenic fungi *F. oxysporum*.

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