ORIGINAL RESEARCH

ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACT OF STRAWBERRY FRUITS (*FRAGARIA ANANASSA*)

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ABSTRCT: The ethanolic extract of strawberry (Fragaria. ananassa.) fruits were analyzed for total antimicrobial activity against four different strains of Staphylococcus aureus, Streptococcus pneumoniae, Proteus.vulgaris and E.Coli. In addition, fruits were analyzed for Antifungal activity against Asparagillus.Niger and Candida albicans.. Total anthocyanin content ethanolic extract of strawberries have enough antimicrobial spectra and there was no inhibition against fungi Aspergillus niger and Candida albicans. The ethnolic extracts were also analysed by U.V.Spectrophotometer for the determination of anthocynin content. Total anthocyanin content of increased with maturity of fruits. The results showed a linear correlation between ethanolic extract of fruits for its antimicrobial activity. Moreover ripen berries have more activity than the normal unripen fruits. . However, there was no inhibition against fungi Aspergillus niger and Candida albicans. The minimum inhibitory concentration values were 12.00, 12.50, and 11.00 mm ($P<0.05$) against S.aureus, Streptococcus pneumoniae, Proteus.vulgaris and E.coli respectively. The activity was tested against three different concentrations such as 1000, 800,500 & 100ug/ml. The minimum inhibitory concentration was also found at ethanolic extract at 100ug/ml.

KEYWORDS: Antimicrobial activity; Strawberry, MIC, U.V. Spectrophotometer, Anthocyanin.

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INTRODUCTION

The octoploid cultivated strawberry (*Fragaria ananassa*) is a financially important fruit that has a short post-harvest shelf life owing to its rapid softening during ripening. As fruit texture is of commercial importance for consumer acceptability and for its harvest and transportation, there is great interest in controlling the textural qualities of this fruit. This has prompted research on the biochemical and molecular biology of the cell-wall polysaccharides synthesis and their degradation[1] .Fruit ripening is a genetically programmed and irreversible phenomenon involving a series of changes that lead to the development of a soft and edible ripe fruit. The chief textural changes resulting in fruit softening are due to enzyme-mediated alterations in the structure and composition of cell walls that lead to partial or complete solubilization, de-esterification, and depolymerization of cell-wall polysaccharides, accompanied by a loss of neutral sugars and galacturonic acid [2,3,4] . Strawberry seeds, or more precisely achenes, are the main source of the fruit's bioactive antioxidant compounds, despite being a very small fraction of the fruit.

High concentrations of reactive oxygen species (ROS) are dangerous for living cells, and have been linked with a number of diseases. Some bioactive compounds like vitamin C, β-carotene and some phenolic compounds – like flavonoids and anthocyanins – can neutralise ROS and may improve human health. The spoilage and poisoning of foods by microorganisms is also a problem that has not yet been brought under adequate control despite the range of robust preservation techniques available. As a result, there has been an increasing interest in different medicinal and dietary plants for their antioxidant and antimicrobial potential because the antioxidant compounds are related to human health, as well as to pharmaceutical and food industries. Moreover, the use of plant extracts, with known antimicrobial properties, can be of great significance in therapeutic treatments. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant[5]. Oxidation is one of the major causes of chemical spoilage, resulting in rancidity and/or deterioration of the nutritional quality, color, flavor, texture, and safety of foods. Strawberries are popular due to their desirable sweet taste and attractive aroma, with smooth texture and red color. There were more than 600,000 acres and 3.9 million tons of strawberries produced worldwide in 2005[6]. Strawberries are a very good source of dietary fiber, iodine, and folate and are a good source of copper, potassium, biotin, phosphorus, magnesium, vitamin B6, and omega-3 fatty acids [7]. Moreover, they are a rich source for ascorbic acid and contain a diverse range of polyphenols [8,9,10] indicated that strawberries could be beneficial for pharmaceutical applications or food supplements.

The aim of this research was to determine the antimicrobial potential of ethanolic extracts of strawberries against S.aureus, Streptococcus pneumoniae, Proteus.vulgaris and E.coli respectively.

MATERIALS AND METHODS

Extract preparation

Dried samples of three kinds of strawberries were supplied by NCBS, Bangalore. Each sample (100 g) was extracted with 1,000 mL of 70% ethanol at room temperature for 24 h. The extraction process

was repeated three times. The extracted materials were filtered with Whatman No. 3 filter paper (Whatman International Ltd., Kent, UK), concentrated with a rotary evaporator . The extracts were dissolved in dimethyl sulfoxide (DMSO) for analysis.

Measurement of total anthocyanins

Total anthocyanin was measured using the pH differential method indicated by Giusti and Wrolstad [11] . Two flasks were filled with 1 mL of extract each. The first flask was diluted with 4 mL of pH 1.0 buffer (potassium chloride, 0.025 M) and the second one diluted with pH 4.5 buffer (sodium acetate, 0.4 M), separately. Absorbance was measured at 520 and 700 nm in pH 1.0 and 4.5 buffers. A molar extinction coefficient of 26,900 L/cm/mol and a molecular weight of 449.2 were used for anthocyanin calculations. Results were expressed as mg of cyanidin 3-glucoside equivalents (mg CE) per g dry weight.

Antimicrobial test using the disc diffusion method

The samples were dissolved in DMSO and filtered through 0.45 µm Millipore membrane filters. The microbial strains used in the experiment were purchased as lyophilized samples from the National collection of Industrial Micro-organisms, Pune. Gram-positive bacteria [Staphylococcus aureus subsp. (NCIM 2901)and Streptococcus pneumoniae(NCTC 12977)], Gram-negative bacteria Proteus.vulgaris NCIM 2813.and Escherichia coli (NCIM 2568) , and fungi [Aspergillus niger (NCIM 1207) and Candida albicans (NCIM 3628)] were used for antimicrobial tests of the extracts. The obtained strains were inoculated with trypticase soy broth and incubated at 37°C for 24 h. The antimicrobial test was then carried out using the disc diffusion method[12]. One hundred µL of each bacterial suspension contained 10^8CFU/mL, and each fungal culture was standardized to 10^5 CFU/mL and was spread on nutrient agar medium. Then, 10 to 80 µL (with 10 mg/mL stock samples) per disk was impregnated into 8 mm diameter sterile discs and the disks were allowed to dry for 24 h in the dark at room temperature. The impregnated discs were placed on the inoculated agar and incubated at 37°C for 24 h for clinical bacterial strains and 96 h for fungal strains. Antimicrobial activity was evaluated by measuring the zone of inhibition against the tested organisms. Each assay in this experiment was repeated two times.

Determination of the minimum inhibitory concentration (MIC)

The MIC values were defined as the lowest concentration of the extracts that inhibited the growth of the micro-organism. MIC of extracts was evaluated with resazurin based microtiter dilution assay as follow: under aseptic conditions, 96 well microtitre plates (Tarsons Products Pvt. Ltd., Kolkata, India) were used, and the first wells of the microtiter plate were filled with 100 µL of test materials (from 1,000 µg/mL extract stock solution). The 2nd to 10th wells of the microtitre plates were filled with 50 µL of sterile water. Two fold serial dilution (throughout 2nd to 10th wells) was achieved by transferring 50 µL test material from the first wells to the subsequent wells (the next well) of the same row so that each well had 50 µL of test material in serially descending concentrations. From the 10th wells, 50 µL was

removed. The working solution of extracts (100 µg/mL) was diluted out across a 96-well in a two-fold serial dilution to give final testing concentrations of 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.195, and 0.098 µg/mL. Each microtiter plate had a set of 2 controls: (a) a control with test organism without test extract as positive control (11th wells) and (b) a control with all solutions except test organism (12th wells) to confirm that no contamination occurred while preparing the plate. Then, a volume of 20 µL was taken from bacterial and fungal suspensions (test organisms) and added to each well. The plates were incubated in a temperature controlled incubator at 37°C for 24 h for bacteria and 48 h for fungi. After the period of incubation, 80 µL resazurin dyes was added and re-incubated for 2 h for color development. Finally, the color change in the well was observed visually. The inhibitory concentration was indicated by the blue coloration of the wells following the addition of resazurin. A change of color from blue to red indicated the presence of live micro-organisms. All the experiments were performed in triplicates. The average values were calculated for the MIC of test material.

Disc diffusion antimicrobial activity of extracts

In present study, the antibacterial activity of extracts from strawberries against four strains of bacteria; two Gram-positive (S. aureus and S. pneumoniae) and two Gram-negative (Proteus.vulgaris.and E. coli) were evaluated using the disc diffusion assay. The inhibitory activity examined was at four concentration levels of samples: 1000, 800, 500, and 100 µg/disc as shown in Table1. The inhibitory activity of extracts showed a similar trend at different concentrations for the same test organisms, and their effectiveness increased as the sample concentration increased from 100 µg/disc. A comparison of the inhibitory effect among samples was done with 600 µg/mL concentration. Extracts from strawberries exhibited potent antimicrobial activity against Gram-positive bacteria, S. aureus, and the inhibitory activity was significantly different ($P<0.05$) from other bacteria. It is known that S. aureus is one of the most common Gram-positive bacteria causing food poisoning. Its source is not food itself but humans who contaminate foods after they have been processed. Extracts of straw berrry affected this bacterium. Based on their effectiveness, the growth inhibition zone diameter are given as 12.5, 12.0, and 11.5 mm, respectively. Similarly, the extracts were found to possess high antimicrobial activity against Gram-positive S. Aureus and proteus vulgaris.($P<0.05$) varying in their antimicrobial activities. Extracts significantly differed ($P<0.05$) in the inhibitory effect against the two Gram-negative bacteria tested. The strawberry extracts had the same inhibitory effect against E. coli with 10 mm zone of inhibition. The inhibition effect of extracts against E. coli was also significantly varying ($P<0.05$) from one to another. In the present study, extracts were observed to have a more potent inhibitory activity against Gram-positive bacteria than Gram-negative and were inactive against fungi. The reason for the difference in sensitivity between Gram-positive and Gram-negative bacteria may be related to their cell wall structure. The resistance of Gram-negative bacteria towards antibacterial substances may be due to the outer phospholipidic membrane carrying structural lipopolysaccharide components of a selective barrier to the hydrophilic solution. The antifungal effect of extracts was tested against two fungi

strains, A. niger and C. albicans, and the results are shown in Table1. Extracts of strawberries did not have inhibitory activity against the tested fungi; this calls for further study using concentrations above 800 µg/disc which was used in the present study. Moreover, their inhibitory activity was specially promising for potential against Gram-positive bacteria, S. aureus.

MIC of extracts

In microbiology, the MIC is the lowest concentration of an antimicrobial (like an antifungal, antibiotic, or bacter-iostatic) drug that will inhibit the viable growth of a microorganism after overnight incubation. The MICs for strawberry extracts against the examined bacterial strains and fungi are presented in Table 2. The results showed that MIC of different extracts of strawberry against bacterial strains ranged from 6.25 µg/mL to 8.00 µg/mL and no inhibition was observed for fungi up to the highest concentration, 60 µg/mL. In this study, the extracts of straw berry have more growth inhibitory activity for the two Gram-positive bacteria, S. pneumoniae and S. aureus, with MIC values of 7.25 and 12.5 µg/mL, respectively. MICs are defined as the lowest concentration of antimicrobial that will inhibit the visible growth of a micro-organism after overnight incubation. S.Aureus was found to be more susceptible to the action of strawberries; this bacterium can be inhibited at the same MIC values of 7.25 µg/mL concentration. Comparing the MIC values of extracts, the lowest value for the Gram-negative bacteria, E. coli, was at 6.25 µg/mL concentration. Extracts straw berry had the same MIC of 12.5 µg/mL for E. coli. For the other Gram-negative bacteria, proteu vulgaris., strawberries exhibited the same MIC values of 8.00µg/mL of extracts. In the present study, the MIC values at 7.25 µg/mL concentration could be achieved against S. pneumoniae by the extracts. For two bacteria strains, S. aureus and Proteus.vulgaris, the extracts could exhibit the same MIC values at 12.5 µg/mL concentrations. The difference in MIC of extracts against different strains of bacteria is indicative of the importance of selecting specific kinds of strawberry extracts for target bacteria. For fungi, it was not possible to determine MIC values, due to the absence of, or only weak, antifungal activities up to 50 µg/mL samples concentration used in the current study. However, extracts could have MIC values as the concentrations get stronger.

RESULT AND DISCUSSION:

In general, the Gram-positive and Gram-negative strains of bacteria tested appeared to be more sensitive to the extracts. However, this study also records a significant susceptibility of some of the examined fungal strains. This antimicrobial activity of ethanolic extract of the fruit may be due to dissolving fruit components in ethanol. The activity of extracts at the lowest concentration may be due to more permeability in the outer membrane of . Gram-positive and Gram-negative strains This permeability is 10–100 folds lower in fungal strains. From this study we showed that the strawberry has more antibacterial spectrathan the fungi.Many plant species are currently used as sources of nutritional additives because of their antioxidant and antimicrobial properties that increase immunity to some diseases. This work, as the first in vitro antimicrobial study has shown appreciable antimicrobial potential for this sample. These findings, candidate them as a good cases for more in-depth studies. Moreover, these cultivars

can be proposed in food industries, as flavor and preservative or in cosmetic-health industries as antimicrobial agents. We wish our future research lead to the identification and structure elucidation of biologically active molecules present in their extracts

Table 1: The antimicrobial activities of ethanol extracts from different kinds of strawberries against 6 microorganisms

Con.(µg/disc)	Test organisms					
	SA	SP	PV	EC	AN	CA
1000	12.52 ± 0.35^{Ac}	11.51 ± 0.71^{Ac}	12.25±0.35	12.00 ± 0.31^{Aa}	8.00±0.00	8.00±0.00
800	11.01 ± 0.35^{Ac}	8.04 ± 0.35^{Cc}	10.54 ± 0.01^{Bb}	11.03 ± 0.30^{Aa}	7.00±0.00	8.00±0.00
500	10.00 ± 0.01^{Bc}	9.01 ± 0.71^{Bb}	9.03 ± 0.35^{Bb}	12.53 ± 0.02^{Aa}	6.00±0.00	8.00±0.00
100	6.00±0.01	700±0.00	9.00±0.00	8.00±0.00	7.00±0.00	8.00±0.00
600	11.03 ± 0.21^{Bb}	12.52 ± 0.56^{Ab}	11.01 ± 0.25^{Ca}	8.50 ± 0.56^{Db}	7.00±0.00	8.00±0.00

Data represent mean±standard deviation.

Means in column (a–c) are significant different within the same concentration and microorganism.

A, Staphylococcus aureus; SP, Streptococcus pneumoniae; PV, Proteus vulgaris; EC, Escherichia coli; AN, Aspergillus niger; CA, Candida albicans.

Table 2: Minimum inhibitory concentration (MIC) values of ethanol extracts of strawberries against 6 microorganisms

Test organisms	MIC(µg/ml)
Staphylococcus aureus	**12.50±0.08**
Streptococcus pneumoniae	**12.25±0.25**
Escherichia coli	**6.25±0.24**
Proteus vulgaris	**7.50±0.06**
Aspergillus niger	**>50.00**
Candida albicans	**>50.00**

CONCLUSION:

The results obtained in the present investigation demonstrated that the fruits of Straw berry display in vitro antimicrobial activity. The traditional use of the fruits is applied to the skin as a cleansing cosmetic to soften and whiten it. The seeds of these plants are anti aging and anticancer effect. Hence the plant extracts possess compounds with antibacterial properties that can be used as very good antimicrobial agents.

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