



Antibacterial Activity of Different Citrus Fruits

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Abstract: Medicinal plants are the natural resources in developing of new drugs. The antibacterial activity of natural products from medicinal plant are applicable for the treatment of bacterial, fungal and viral disease and these are recent technical advancements taken place in this area. The genus Citrus has a number of species and hybrids that are well established for their pharmaceutical and economic importance. The peel extracts of Kagja Lemon (C₂), South African Malta (C₅), and Dargiling Orange (C₈) show very high antibacterial activity on *B. cereus*, both methanol and ethyl acetate has and in this case MIC is equal to MBC where the inhibitory concentration is 31.25 µg/ml. The other extracts exhibit moderated activities on *B. cereus*. The methanolic extract shows very potent activities against *S. aureus* but reduces activity on ethyl acetate extracts. The Kagji Lemon (C₁), Kagja Lemon (C₂), Elachi Lemon (C₃), and Batabi Lemon (C₄) extracts have inhibitory concentration of 31.25 µg/ml on *S. aureus* whereas the ethyl acetate extracts have inhibitory concentration of 250-750 µg/ml.

Key words: Citrus fruit, antibacterial, MIC, MBC, *B. cereus*, *S. aureus*

INTRODUCTION

Medicinal plants are the natural resources in developing of new drugs (Shakya AK et al., 2012). Nature has been a source of medicinal agent for thousands of years and an impressive number of modern drugs have been isolated from natural sources, based on their use in traditional medicine (Chaudhari TB et al., 2012). This is due to increased source awareness of the limited ability of the synthetic pharmaceutical products to control major diseases. The basic molecular and active structures for synthetic fields are provided by rich natural sources (Preeti R et al., 2010). Medicinal plants are used for the ailment of several microbial and non-microbial originated diseases due to their valuable effects in health care (Akroum S et al., 2009). The affordability, reliability, availability and low toxicity of medicinal plants in therapeutic use has made them popular and acceptable by all religions for implementation in medical health care all over the world. Plants are indeed the first material used in alternative medicine type of remedy against many diseases (Amjad K et al., 2005). Herbal medicine has been widely used as an integral part of primary health care in many countries (Akinyemi KO et al., 2005). Medicinal plants may constitute a reservoir of new antimicrobial substances to be discovered. The World Health Organization (WHO) reported that 4 billion people (80% of the world's population) use herbal medicines for some aspect of primary healthcare (Fabricant DS and Farnsworth NR, 2001). In developing countries, 80% of people use traditional medicines which are based on plant products. Thus many studies have been conducted on medicinal plants. Currently 80% of the world population depends on plant derived medicine for the first line of primary health care for human alleviation because it has no side effects (Chaudhari TB et al., 2012). Plants are important source of medicines and presently about 25% of pharmaceutical prescriptions in the United States contain at least one plant derived ingredient (Pandey M et al., 2011). In the last century,

roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources (Hasan SZ et al., 2009; Vemra S and Singh SP, 2008).

Bacterial infections are one of the prominent causes of health problems, physical disabilities and mortalities around the world. Medicinal plants are a rich source of antimicrobials and provide a safer and cost effective way of treating bacterial infections. The antibacterial activity of natural products from medicinal plants is applicable for the treatment of bacterial, fungal and viral diseases. Medicinal plants have been recognized as potential drug candidates because they possess drug like properties. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased (Bernhoft A, 2010). Secondary metabolites or phytochemicals such as phenols, flavonoids, alkaloids, terpenoids, and essential oil have proved to be responsible for the antimicrobial activity of plants (Hwang EI et al., 2001). Phenol and polyphenol group of compounds consist of thousands of diverse molecules with heterogeneous structure with common feature of having one or more phenol ring. Several workers have reported that phenolic compounds such as gallic acid, coumarins, polyphenols, caffeic acid, cinnamic acid, pyrogallol, eugenol show antimicrobial activity against virus, bacteria and fungi (Saify ZS et al., 2005).

Of all the plant spread by nature upon the surface of the globe, there are none more beautiful than those we know under the name of citron, lemon, and orange trees which botanists have included under the technical and generic name Citrus. No other equals them in beauty of leaf, delightful odor of flower, or splendor and taste of fruit. These brilliant qualities have made the Citrus a favorite in all countries. The genus Citrus, belonging to the Rutaceae family, comprise of about 140 genera and 1,300 species. *Citrus sinensis* (Orange), *Citrus paradise* (Grapefruit), *Citrus limon* (Lemon), *Citrus reticulata* (Tangerine), *Citrus grandis* (Shaddock), *Citrus aurantium* (Sour orange), *Citrus medica* (Citron), and *Citrus aurantifolia* (Lime) are some important fruit of genus Citrus (Singh U et al., 1983; Anwar F et al., 2008). Citrus are well known as one of the world's major fruit crops that are produced in many countries with tropical or subtropical climate. Brazil, Japan, China, Mexico, Pakistan, and countries of the Mediterranean region, are the major Citrus producers (Mahmood MA 2005; Khan SRA 2005). The Citrus fruits and their by-products are of high economic and medicinal value because of their multiple uses, such as in food industry, cosmetics and folk medicine (Silalahi J, 2002). In additions to large scale consumption as fresh fruits, the fruits are mainly processed to produce juice. The waste of Citrus processing industry left after juice extraction, such as peels, seeds and pulps, corresponding to about 50% of the raw processed fruit, can be used as a potential source of valuable by products (El- Adawy TA et al., 1999). Specifically, the Citrus peels, commonly treated as agro-industrial waste, are a potential source of valuable secondary plant metabolites and essential oils (Andrea V et al., 2003).

MATERIALS AND METHODS

Collection and identification of the plant sample

Ten different varieties of unripe raw citrus fruits named as Kagji Lemon (*Citrus microcarpa*), Kagja Lemon (*Citrus longilimon*), Elachi Lemon (*Citrus aurantifolia*), Batabi Lemon (*Citrus aurantifolia*), South African Malta (*Citrus pyriformis*), Egyptian Malta (*Citrus sinensis*), Chaina Orange (*Citrus jambhiri*), Dargiling Orange (*Citrus reticulata*), China Lemon (*Citrus aurantium*) and African Orange (*Citrus medica*); were collected from the local market of sylhet district, Bangladesh. The fruits was collected pure state as it does not contain foreign chemicals.

Preparation and extraction of the sample

The fruits was washed thoroughly after collection by in distilled water and then sun dried. After washing the peels are separated and oven dried at 55°C and then ground into coarse powder and preserved at room temperature (RT) for future use. The sample was extracted by mehanol and ethyl acetate and the extract was then filtered through Whatman No.1 filter papers. The filtrate was then concentrated by rotary evaporator at 50°C under reduced pressure. The samples are given code name. [C₁: Kagji Lemon, C₂: Kagja Lemon C₃: Elachi Lemon, C₄: Batabi Lemon, C₅: South African Malta, C₆: Egyptian Malta, C₇: Chaina Orange, C₈: Dargiling Orange, C₉: China Lemon and C₁₀: African Orange].

Source of Test Microorganisms

Pure cultures of pathogenic bacteria *Bacillus cereus* and *Staphylococcus aureus* were collected from the Department of Pharmacy, Rajshahi University, Bangladesh for the research work. The collected organisms were subcultured in nutrient agar media and stored in nutrient agar slants at 4°C until needed for use.

***In Vitro* Antimicrobial Screening**

The antibacterial screening of an agent is essential to ascertain its spectrum against various types of pathogenic organisms. Antibacterial action of any plant sample can be ensured by witnessing the growth response of various microorganisms to the plant extract, which is placed in contact with them. Antibacterial property was observed by determining minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) value.

Minimum inhibitory concentration

The lowest concentration of the compounds or extracts essential to inhibit the multiplication of the organism is referred to as minimum inhibitory concentration (MIC) (Andrews JM, 2001; Rasooli I and Abyanek MR, 2004). Here "Serial dilution technique" (Ronald R, 1982) was followed using nutrient broth media. The test bacteria grown at 37°C in nutrient agar medium, were diluted in sterile nutrient broth medium in such a manner that the suspension contained about 10⁷cells/ml. Here nine different concentrations (31.25, 62.5, 125, 250, 500,750, 1000, 1500 & 2000 µg/ml) of extracts are used for determination of MIC. 1 ml of nutrient broth medium was transferred to each of the test tubes. Next the test tubes were cotton plugged and sterilized in an autoclave for 15 minutes at 121°C and 15-lbs/sq. inch pressure. After cooling, 1 ml of the sample solution was added to the 1st test tube and mixed well and then 1 ml of this content was shifted to the 2nd test tube. The content of the 2nd test tube was shacked well and then again 1 ml of this mixture was moved to the third test tube. The entire process of serial dilution was carried up to the 9th test tube. Then 10 µl of properly diluted inoculums was added to each of the six test tubes and mixed well. Finally, 1 ml of the sample was added to each of the test tubes and all the test tubes were incubated at 37°C for 18 hours.

Minimum bactericidal concentration

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 (Parvez GMM et al., 2016). 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 24 hours. 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.

RESULTS AND DISCUSSIONS

Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted (Rios JL et al., 2005). In recent years, emergence of bacterial resistance against multiple antibiotics has accelerated dramatically. Community- and hospital-acquired pathogens and larger part of them are multi-drug resistant bacteria (Lai CC et al., 2011; Solórzano-Santos F and Miranda-Novales MG, 2012). So, there has been growing interest in substitution of synthetic antimicrobial agents by natural ones has fostered research on vegetable sources and screening of plant materials in order to identify new compounds or test natural chemicals already known for important activities that have not been discovered so far (Ait-Ouazzou A et al., 2011; Lv F et al., 2011; Badawy MEI and Abdelgaleil SAM, 2014).

Antibacterial effects of various citrus peels have been demonstrated in the literature (Lawal D et al., 2013, Ramachandra YL et al., 2013, Dhanavade MJ et al., 2011). Dubey D et al. (2011) showed potent antibacterial activity (against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Shigella flexneri*, *Bacillus subtilis* and *Escherichia coli*) of extract from fruit of Orange peels. Cuishnie TP and Lamb AJ concludes that the phenolic compounds in citrus peels are responsible for antimicrobial activity (Cushnie TP and Lamb AJ, 2005). Tumane PM et al., (2014) studied the antibacterial activity of *Citrus aurantium* (sour orange) and *Citrus medica* (lemon) methanolic fruit peel extracts. The ethyl acetate extracts of the citrus peels exhibited more effect against foodborne bacteria (Chanthaphon S et al., 2008).

It found that all the extracts possess very high antimicrobial activity. The Kagji Lemon peel (C₁) shows very good activity against *S. aureus* on methanolic extract where it inhibits at 31.25 µg/ml concentration. The extracts of Kagja Lemon (C₂), South African Malta (C₅), and Dargiling Orange (C₈) on both methanol and ethyl acetate have very high antibacterial activity on *B. cereus* and in this case MIC is equal to MBC. The four extracts Elachi Lemon (C₃), Batabi Lemon (C₄), Egyptian Malta (C₆) and China Lemon (C₉) have moderate activity against *B. cereus*, where the inhibitory concentration lies between 62.5 µg/ml to 125 µg/ml while Chaina Orange (C₇) and African Orange (C₁₀) extract have week activity against *B. cereus*. Chanthaphon S et al., (2008) found MIC value around 1000 µg/ml of ethyl acetate extract against *S. aureus*. Similar result was published by Abdel-Salam AF and Mostafa FAA. (2014).

Table 1: MIC and MBC value of extracts against *B. cereus*.

Sample (Methanol Extract)	MIC µg/ml	MBC µg/ml	Sample (Ethyl Acetate Extract)	MIC µg/ml	MBC µg/ml
C ₁ M	500	500	C ₁ E	125	125
C ₂ M	31.25	31.25	C ₂ E	31.25	31.25
C ₃ M	125	125	C ₃ E	62.5	62.5
C ₄ M	62.50	62.50	C ₄ E	31.25	31.25
C ₅ M	31.25	31.25	C ₅ E	31.25	31.25
C ₆ M	125	125	C ₆ E	62.50	62.50
C ₇ M	750	750	C ₇ E	250	250
C ₈ M	31.25	31.25	C ₈ E	31.25	31.25
C ₉ M	62.50	62.50	C ₉ E	31.25	31.25
C ₁₀ M	250	250	C ₁₀ E	250	>250

Where M= Methanol and E=Ethyl Acetate.

The methanolic extract of C₁, C₂ and C₃ extract exhibit very high activity against *S. aureus* while MIC and MBC are 31.25 µg/ml but ethyl acetate extract shows weak activity on same organism (Table 2). The Egyptian Malta (C₆) exhibits reverse trend where ethyl acetate extracts are more potent against *S. aureus*. The China Lemon (C₉) peel extract shows the least activity among all the extracts. Chanthaphon S et al., (2008) found MIC value of 560 µg/ml to 1000 of ethyl acetate extract against *B. cereus*. Similar types of activity were found in orange peel by ethyl acetate extract where MIC=MBC and the concentration is 200 µg/ml (Abalaka ME and Bello AO, 2016). Johan S et al. (2007) found hexane extract of various citrus peels has MIC value lies between 500-2000 µg/ml on *S. aureus*. Ajithkumar INP and Panneerselvam R. (2012) determined MIC value of 22.5 µg/ml of citrus peel extracted by methanol against *S. aureus*. In 2013, Shafiq SA et al. finds MIC value of 100 µg/ml of citrus peels against *S. aureus* (Shafiq SA et al., 2013).

Table 2: MIC and MBC value of extracts against *S. aureus*.

Sample (Methanol)	MIC µg/ml	MBC µg/ml	Sample (Ethyl Acetate)	MIC µg/ml	MBC µg/ml
C ₁ M	31.25	31.25	C ₁ E	250	250
C ₂ M	31.25	31.25	C ₂ E	750	750
C ₃ M	31.25	31.25	C ₃ E	750	750
C ₄ M	31.25	31.25	C ₄ E	500	>500
C ₅ M	62.50	62.50	C ₅ E	62.5	62.5
C ₆ M	62.50	62.50	C ₆ E	31.25	>31.25
C ₇ M	250	250	C ₇ E	62.5	62.5
C ₈ M	500	500	C ₈ E	31.25	31.25
C ₉ M	500	500	C ₉ E	1000	1000
C ₁₀ M	31.25	31.25	C ₁₀ E	31.25	31.25

Where M= Methanol and E=Ethyl Acetate

CONCLUSION

The antibacterial activity of all the citrus fruit peel may be due to the presence of antioxidant bioactive components in its fruits peel. So, by future studies, it is possible to clear out the exact chemical nature of the compounds responsible for their action against the respective bacteria. If this goal is achieved soon, we can design formulations with less expense and great accuracy of specific chemical compounds from these plant parts, or we can attempt for their synthetic analogues. Toxicity studies should also be done to determine safety.

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