Antimicrobial Activity of Ethnobotanical Plant Psidium Guajava

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Abstract

The present work demonstrates the antimicrobial potential of Psidium guajava leaves extract by using various solvents. The results indicate that water and acetone are better than methanol and ether for the extraction of the antibacterial properties of guava. The results also indicate that the plant extracts have antibacterial effect on both Gram positive and Gram-negative bacteria, that is, can be used as broad spectrum antibacterial compounds. The observed inhibition of Gram-positive bacteria Staphylococcus aureus and Gram negative bacteria E.coli suggests that guava possesses compounds containing antibacterial properties that can effectively suppress the growth when extracted using acetone and water as solvent. Comparisons with related data from the literature indicate that according to the different methodologies of studies on antibacterial activity, the most diverse outcomes can be obtained. This study provides scientific insight to further determine the antimicrobial principles and investigate other pharmacological properties of guava. On the basis of the present finding, P. guajava leaves possess capabilities of being a good candidate in the search for a natural antimicrobial agent against infections and/or diseases caused by S. aureus and E.coli.
INTRODUCTION

Antibiotic resistance has become a global concern. In recent years there is increasing incidence of multiple resistances in human pathogenic microorganisms, largely due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases (Westh et al., 2004). This has forced scientist to search for new antimicrobial substances from various sources like medicinal plants. Search for new antibacterial agents should be continued by the screening of many plant families (Parekh and Chanda, 2007).

Traditional medicines use a variety of herbal preparations to treat different kinds of ailments, including microbial infections. Due to the indiscriminate use of antimicrobial drugs the microorganisms have developed resistance. Additionally, using antibiotics is sometime associated with adverse effects. Therefore, herbal medicine will be an alternative for the treatment of bacterial infections and may decrease such problems.

Psidium guajava is a fruit-bearing tree commonly known as guava, which belongs to the family Myrtaceae. Guava grows nearly throughout India up to 1500 m in height and is cultivated commercially in almost all states, the total estimated area being 50,000 hectares. The important guava-growing states in India are Uttar Pradesh, Bihar, Maharashtra, Assam, West Bengal and Andhra Pradesh. Cultivated varieties grow about 10 m in height and produce fruits within 4 years. Wild trees grow up to 20 m high and are well branched. The tree can be easily identified by its distinctive thin, smooth, copper-colored bark that flakes off, showing a greenish layer beneath. Guava trees have spread widely throughout the tropics because they thrive in a variety of soils, propagate easily and bear fruits quickly. The fruits are enjoyed by birds and monkeys, which disperse guava seeds and cause spontaneous dumps of guava saplings to grow throughout the rainorest(wealth of India,2003).

The leaves and bark of guava tree have a long history of medicinal uses. In India, decoction of the leaves and bark of guava is used to cure diarrhea, dysentery, vomiting and sore throats, and to regulate menstrual cycles. The tribes of the Amazon use leaf decoction for mouth sores, bleeding gums, as douche for vaginal discharge and to tighten and tone up vaginal walls after labor. They are also an excellent source of fiber, potassium and vitamin A.

The guava (Psidium guajava) is a phytotherapic plant used in folk medicine that is believed to have active components that help to treat and manage various diseases. The many parts of the plant have been used in traditional medicine to manage conditions like malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, sore throat,
inflamed gums, and a number of other conditions. This plant has also been used for the controlling of life-changing conditions such as diabetes, hypertension, and obesity.

The genus \textit{Psidium} belongs to the family Myrtaceae, which is considered to have originated in tropical South America. Guava crops are grown in tropical and subtropical areas of the world like Asia, Egypt, Hawaii, Florida, Palestine, and others. The genus \textit{Psidium} comprises approximately 150 species of small trees and shrubs in which only 20 species produce edible fruits and the rest are wild with inferior quality of fruits. The most commonly cultivated species of \textit{Psidium} is \textit{P. guajava}, which is the common guava. Other species are utilized for regulation of vigor, fruit quality improvement and resistance to pest and disease. Guava fruit today is considered minor in terms of commercial world trade, but it is widely grown in the tropics, enriching the diet of hundreds of millions of people in those areas of the world.

Antibacterial screening has been done selectively by many researchers in guava essential oil and solvent extract. The mechanism by which they can inhibit the microorganisms can involve different modes of action. It has been reported that these oils and extracts penetrate the lipid bilayer of the cell membrane, rendering it more permeable, leading to the leakage of vital cell contents. Sanches et al. evaluated the antibacterial activities of guava against gram-positive and gram-negative bacteria testing ethanol and water extract of \textit{P. guajava} leaves, stem, bark and root, and aqueous extract against \textit{Staphylococcus aureus} were found to be more active by using ethanol and water extract than with just aqueous extract. Sacchetti et al. reported that the oil showed a strong resistance against \textit{Yarrowia lipolytica} which is a pathogenic yeast. Vieira et al. have also reported the antibacterial effect of guava leaves extracts and found that they inhibited the growth of the \textit{S. aureus}. Gnan and Demello testing guava leaf extract found good antimicrobial activity against nine different strains of \textit{Staphylococcus aureus}. The antibacterial activity of guava leaf extract was tested against acne developing organisms by Qa'dan et al. concluding that the leaf extracts may be beneficial in treating acne especially when they are known to have anti-inflammatory activities.

Phytochemicals are nonnutritive chemicals produced by plants for their own protection, but they have been found to protect humans against diseases through recent research. Scientists have identified thousands of phytochemicals, although only small fractions have been studied closely and each one works differently. Begum et al. reported the isolation of two triterpenoids: guavanoic acid and guavacoumaric acid from the leaves of guava. Four flavonoids were isolated
and identified by Arima and Danno which were found to inhibit the growth of Salmonella enteritidis and Bacillus cereus. A study was done to evaluate the spasmolytic activity of guava leaf and was found that a compound called “aglycone quercetin” is responsible for spasmolytic activities, which is formed when flavonoids of guava leaves are hydrolyzed by the gastrointestinal fluids.

MATERIALS AND METHODS

EXTRACTION OF PLANT MATERIALS

The studied plant materials were collected from Karimganj College area in the month of August 2013. The plant samples were authenticated & the voucher specimens were prepared and deposited in the herbarium of the Deptt of Botany & Biotechnology, Karimganj College, Karimganj, Assam. The local usages of the plants were also recorded. Leaves & tender twigs of guava leaves was used. Fresh plant materials were thoroughly washed under tap water, pat dried with blotting paper and then extracted in organic solvents like methanol and acetone. Extracts were also made in distilled water. The volume ratio was maintained to 1 : 1. The extracts were kept in petriplates for 24 hrs to evaporate weight to the organic solvents. After 24 hrs, the resultant extracts were filtered and concentrated to dryness. A little autoclaved distilled water was added to the dried extracts (1ml) before addition.

MICROBIAL STRAINS USED AND THEIR PREPARATIONS

Microbial strains viz: Staphylococcus aureus (gram positive) and Escheria coli (gram negative) was obtained from the Department and were grown on nutrient broth (Hi – media) at 37°C for 18-24 hrs and were maintained on respective agar slant at 4°C.

PREPARATION OF INOCULUM

Bacteria were cultured over night at 37°C in nutrient agar (NA) media and used as inoculums.
ANTIMICROBIAL DISC DIFFUSION ASSAY

The dried extracts were dissolved in autoclaved distilled water to make a final concentration of 1 gram per ml. The standard disc – diffusion method was used to evaluate the antimicrobial activity (Murray et. al. 1995, NCCLS, 1999). Using sterilized johnson ear buds, the petridishes containing nutrient agar were seeded. Discs of 6 mm in diameter (Watman No-1 filter paper) were placed on seeded agar plates and impregnated with 10 micro litres of 0.5 gram/ml crude extracts of the studied samples. Gentamicin & Cephalexin discs were used as positive control, the solvents were used as negative control. Test plates were incubated at 37°C for 18-24 hours. Antimicrobial activity was evaluated by measuring the zone of inhibition against each organism. The experiments were conducted in triplicates and the data was tabulated.

RESULT

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Scientific Name</th>
<th>Family</th>
<th>Common Name (Bengali)</th>
<th>Parts Used</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Psidium guajava</td>
<td>Myrtaceae</td>
<td>Pyara</td>
<td>Leaves</td>
<td>It is used in diarrhea.</td>
</tr>
</tbody>
</table>

TABLE 1: TAXONOMIC TREATMENT OF THE USED PLANTS

ANTIMICROBIAL ASSAY

The antimicrobial disk diffusion assay using the ethno botanically important plants yielded quite interesting result. Many of the extracts, showed good inhibitory activity on both the bacteria tested. The result have been tabulated below, the table containing botanical name of the plant, extracts used and name of the bacteria used. The inhibitory zone has been measured in mm.
<table>
<thead>
<tr>
<th>Plants</th>
<th>Extracts</th>
<th>Zone of inhibition (mm) in <em>Staphylococcus aureus</em></th>
<th>Zone of inhibition (mm) in <em>Escherichia coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Psidium guajava</em></td>
<td>Acetone</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Ether</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Standard antibiotic</td>
<td>Gentamicin</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td></td>
<td>Cephalexin</td>
<td></td>
<td>_</td>
</tr>
</tbody>
</table>

TABLE 2: Zone of inhibition of extracts
DISCUSSIONS AND DATA INTERPRETATION

Herbal medicines are a valuable and reality available resources for primary health care and complementary health care system. Unfortunately many species of plants containing substances of medicinal value have yet to be discovered; though large numbers of plants are constantly being screened for their antimicrobial effects. It has been suggested that phytochemical extracts from plants hold promise to be used in allopathic medicine as they are potential sources of antiviral, antitumoral and antimicrobial agents (Nair et al, 2005).

For the experiment one gram positive bacteria viz-Staphylococcus aureus was collected from the department. It was seen that all the plants studied have significant inhibitory effects on S. aureus. Methanolic extracts of all the plants had antimicrobial activity. But distilled water extracts in most cases did not show significant results. It might be due to the fact that the active principles were distilled water insoluble.

The result of our experiments from the above table 2 showed that in vitro antimicrobial screening of extracts and pure compounds of *Murraya paniculata* indicated positive activity against *Staphylococcus aureus*. They showed acetone extracts of the plants showed in inhibition zone of 13mm, methanol extract showed in inhibition zone of 9mm and ethanol extract showed in inhibition zone of 7mm but the distilled water extract showed no inhibition zone.

We also performed work on selaginella and lycopodium but got no inhibition zone.

The differences in the inhibitory effect of various plant extracts may be due to qualitative and quantitative differences in the antibacterial principles or compounds present in them. In this investigation it becomes certain that most effective crude extract was methanol for which maximum zone of inhibition was recorded; followed by water that also inhibited the growth of bacteria. While acetone extract showed minimal antibiotic activity. This action may be synergistic and not due to the efficiency of one single substance. The above results revealed that the plant extracts could be effective antibiotic in controlling gram positive pathogen.

*Psidium guajava* is a potent ethno medicinally important plant used both in traditional medicine as an analgesic and for wood and its activity against S. aureus established its traditional uses too. Selaginella is also used traditionally in wounds, postpartum, menstrual disease against microbes Lycopodium is used in Rheumatism and disease of lungs and kidneys and our study also revealed the scientific base behind its uses. From the result it is clear that
three ethno botanically important plants can be used to control the various human pathogens. The above results also revealed that plant extracts could be effective antibiotics.

It has been shown in various studies that polarity of antibacterial compound is crucial for their activity. Therefore it is obvious that extracts prepared using organic solvents were more active against bacterial species. Similar observations have been reported by Thongson et al. Thus it can also be inferred that phytochemical screening should be done to discover the bioactive principles of the studied plants. More over similar types of work can be instigated to unreveal the wealth of traditional knowledge to usher a new dawn of drug discovery and economic pursuit of the region.

DISCUSSION

Our results support the findings of Rabe & Van Staden (1997)\textsuperscript{19}, where greater overall antimicrobial activity was seen with a methanol extract than with the essential oil and other extracts. While it is a common practice in traditional medicine to use plant extracts prepared with water, as is the case with infusions, decoctions and plasters, it seems unlikely that the active compounds present in water extracts are the same as those responsible for the action in the methanol extracts. The chemistry involved in chewing, boiling, soaking, rubbing and extracting plant medicines is complex. The results in Table 1 suggest that there are multiple and different anti-microbial agents present in each type of extract acting in different ways on different bacterial strains. Vieira et al. (2001)\textsuperscript{25} have also reported the antibacterial effect of guava leaves extracts and found that they inhibited the growth of the \textit{S. aureus}. GNAN & DEMELLO (1999)\textsuperscript{10} testing guava leaf extract found good antimicrobial activity against nine different strains of \textit{Staphylococcus aureus}. In contrast to the results reported here, Jaiarj et al. (1999)\textsuperscript{15}, testing for antibacterial action of aqueous, methanol and chloroform extracts of \textit{P. guajava}, on strains of \textit{S. aureus} isolated from clinical patients, obtained better results from aqueous extracts than from methanol extract. BERDY et al. (1982)\textsuperscript{4} and CACERES et al. (1993)\textsuperscript{5} described the antibiotic activity of the aqueous extract of dried leaves of \textit{Psidium guajava} to two compounds, namely guajaverin and psidiolic acid. Data on Table 1 both support and contradict previous findings concerning which type of plant extraction method results in greatest microbial inhibition. Such variability is perhaps explained in the light of multiple different active agents and different bacterial species. The secondary metabolism of plants is complex and results in an incredible variety of metabolic products. SANCHES et al. (2005)\textsuperscript{21} evaluated the antibacterial activities of \textit{Psidium guajava} against Gram positive and negative bacteria testing ethanol: water extract of \textit{P. guajava} leaves, stem bark and root, and aqueous extract against \textit{Staphylococcus aureus} were found to be more active by using ethanol:water extract than with just aqueous extract. High variability is not unexpected and serves to highlight gaps in our understanding of
plant medicines, and provides questions and direction for further research. When comparing these data with others, what really matters is the powerful inhibitory effect of guava leaves on many common diseases causing enteric pathogens.

While the overall inhibitory effect of the essential oils in this experiment was less than for methanol, its individual effect on *S. aureus* was greater. According to Robbers *et al.* (1997)\textsuperscript{20}, a higher concentration of active chemical compounds in essential oils explain their stronger inhibitory action (Jaiarj *et al.* (1999)\textsuperscript{15}; Andrade-Neto *et al.* (1994)\textsuperscript{1}). Within the genus *Psidium*, essential oil constituents are principally comprised of monoterpenes, 1.8-cineol, r-cimen and acetate of a-terpenil. The complex composition of essential oils offers a variety of pharmacological resources and great potential for the development of novel drugs.

Investigating the antimicrobial effect of guava leaves involved a comparison of their effect (*Table 1*) with commercially developed antibiotics (*Table 2*) by comparing the inhibition haloes in those tables. We note that the commercial antibiotics had a larger inhibitory effect than the guava leaf extractions. This is not surprising and reinforces the position that commercially perfected and tested antibiotics should be used in treatments whenever available. *Table 2* also provides data which support the findings of Silva & Hofer (1995)\textsuperscript{22} about *E. coli* isolated from marine fish, where multiresistance to commercial antibiotic was reported. Multidrug resistance is defined as resistance to at least two different classes of antibiotics\textsuperscript{9}. An increase in the occurrence of bacterial strains resistant to more than one commercial antibiotic isolated from natural environments is cause for concern. Increased resistance has typically been ascribed to the indiscriminate use of drugs with antibiotic properties on human beings, livestock protection and food production, resulting in the development of resistant strains\textsuperscript{23}. Our data demonstrate that multiple resistance is frequently found in strains from environmental samples where this type of exposure seems unlikely. In developing countries, especially in rural, coastal and estuarine environments, where child diarrhea is common and life-threatening, special attention is required when preparing fish for consumption. In conclusion, guava leaf extracts and essential oil are very active against *S. aureus*, thus making up important potential sources of new antimicrobial compounds.

**CONCLUSIONS**

The present work demonstrates the antimicrobial potential of *Psidium guajava* leaves extract by using various solvents. The results indicate that ethanol and methanol are better than n-hexane and water for the extraction of the antibacterial properties of guava. The results also indicate that the plant extracts have no antibacterial effect on the Gram-negative bacteria, showing that they do not contain active ingredients against the organisms. The observed inhibition of Gram-positive bacteria, Bacillus cereus and *Staphylococcus aureus*, suggests that guava possesses compounds containing antibacterial properties that can effectively suppress the growth when
extracted using methanol or ethanol as the solvent. Comparisons with related data from the literature indicate that according to the different methodologies of studies on antibacterial activity, the most diverse outcomes can be obtained. This study provides scientific insight to further determine the antimicrobial principles and investigate other pharmacological properties of guava. On the basis of the present finding, P. guajava leaves possess the capabilities of being a good candidate in the search for a natural antimicrobial agent against infections and/or diseases caused by B. cereus and S. aureus

Extensive literature survey revealed that guava, acclaimed as 'poor man's apple of the tropics', has a long history of traditional use for a wide range of diseases. The fruit as well as its juice is freely consumed for its great taste and nutritional benefits. Much of the traditional uses have been validated by scientific research. Toxicity studies in mice and other animal models as well as controlled human studies show both leaf and fruit are safe without any side effects. A number of chemicals isolated from plants like quercetin, guaijaverin, flavonoids and galactose-specific lecithins have shown promising activity in many human trials. The plant has been extensively studied in terms of pharmacological activity of its major components, and the results indicate potent anti-diarrheal, antihypertensive, hepatoprotective, antioxidant, antimicrobial, hypoglycemic and antimutagenic activities. In recent years, emphasis of research has been on utilizing traditional medicines that have a long and proven history of treating various ailments. In this regard, further studies need to be carried out to explore P. guajava L for its potential in preventing and treating diseases

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ANTIMICROBIAL ACTIVITY OF SOME IRANIAN MEDICINAL PLANTS

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