Phytochemicals Analysis and Antimicrobial Activity of Murraya koenigii

Pratibha S. Patil
Department of Biotechnology, Shivaji University, Kolhapur-416 004, India

ABSTRACT

Murraya koenigii was assessed for the phytochemical analysis and antimicrobial activity. Evaluated the phytochemicals e.g. carbohydrate, protein, phenol, amino acids, etc. by using leaves extract of the M. koenigii. The antimicrobial activity of the extracts was assayed against Staphylococcus aureus and Pseudomonas aeruginosa using the agar cup method. The elemental analysis of M. koenigii was carried out by Atomic Absorption Spectrometry (AAS) and has 55.57% of calcium.

KEYWORDS

Murraya koenigii, phytochemicals, Atomic Absorption Spectrometry, antimicrobial activity

Introduction

Diet is one method helps to improve and maintain health, keeping away from many chronic health risks such as obesity, heart disease, diabetes and cancer. Numerous antimicrobial molecules, such as antimicrobial peptides, proteins and small molecular weight organic substances are present in plants acting as host defense mechanisms (Broekaert et al. 1995; Seilertnikoff 2001). As well as many compounds show broad spectrum of inhibitory activity against pathogenic bacteria and fungi. The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives.

In recent years, there has been gradual revival of interest in the use of medicinal plants in developed as well as developing countries, because plant divided drug have been reported to be safe and without side effects. There is also, one other important advantage of using plant extract or their essential oils instead of synthetic drugs in feed: synthetic drugs residues in animal meat and eggs can cause health problems in people who consume them, especially due to increasing resistance of pathogens present in the human body as a result of prolonged use of synthetic drugs (Barbour et al. 2010).

Human beings have been utilizing plants for basic preventive and curative health care since time immemorial. The plant extracts or bioactive herbal compounds have been reported scientifically for their biological activities. Plant produces chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. There are many phytochemicals in fruits and herbs and each of them work differently (Argal and pathak 2006). Nees (A. paniculata, Chuanxinlan), native to Taiwan, Mainland China and India, is a medicinal herb with an extremely bitter taste used to treat many diseases such as host defense mechanisms (Broekaert et al. 1995; Seilertnifikoff 2001). As well as many compounds show broad spectrum of inhibitory activity against many chronic health risks such as obesity, heart disease, diabetes and cancer. Numerous antimicrobial molecules, such as antimicrobial peptides, proteins and small molecular weight organic substances are present in plants acting as host defense mechanisms (Broekaert et al. 1995; Seilertnikoff 2001). As well as many compounds show broad spectrum of inhibitory activity against pathogenic bacteria and fungi. The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives.

In recent years, there has been gradual revival of interest in the use of medicinal plants in developed as well as developing countries, because plant divided drug have been reported to be safe and without side effects. There is also, one other important advantage of using plant extract or their essential oils instead of synthetic drugs in feed: synthetic drugs residues in animal meat and eggs can cause health problems in people who consume them, especially due to increasing resistance of pathogens present in the human body as a result of prolonged use of synthetic drugs (Barbour et al. 2010).

Human beings have been utilizing plants for basic preventive and curative health care since time immemorial. The plant extracts or bioactive herbal compounds have been reported scientifically for their biological activities. Plant produces chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. There are many phytochemicals in fruits and herbs and each of them work differently (Argal and pathak 2006). Nees (A. paniculata, Chuanxinlan), native to Taiwan, Mainland China and India, is a medicinal herb with an extremely bitter taste used to treat many diseases such as host defense mechanisms (Broekaert et al. 1995; Seilertnikoff 2001). As well as many compounds show broad spectrum of inhibitory activity against pathogenic bacteria and fungi. The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives.

Human beings have been utilizing plants for basic preventive and curative health care since time immemorial. The plant extracts or bioactive herbal compounds have been reported scientifically for their biological activities. Plant produces chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. There are many phytochemicals in fruits and herbs and each of them work differently (Argal and pathak 2006). Nees (A. paniculata, Chuanxinlan), native to Taiwan, Mainland China and India, is a medicinal herb with an extremely bitter taste used to treat many diseases such as host defense mechanisms (Broekaert et al. 1995; Seilertnikoff 2001). As well as many compounds show broad spectrum of inhibitory activity against pathogenic bacteria and fungi. The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives.

Methods and materials

Test microorganisms

The test bacteria Pseudomonas aeruginosa NCIM 5032 and Staphylococcus aureus NCIM 2654 were used for the antimicrobial activity. All bacterial strains were maintained routinely on the nutrient slants containing (g l−1): NaCl 5.0, bacteriological peptone 10.0, yeast extract (2.0), sodium chloride 5.0 and agar 15.0. All the cultures were stored at 4°C.

Plant material

Healthy, disease-free, mature and fresh leaves of M. koenigii were collected from Sindhudurg district, Maharashtra, India. The fresh M. koenigii were cleaned with sterile water and used for the study.

Preparation of leaves extract

The clean M. koenigii leaves were crushed and successively extracted with methanol (1:1.5). The methanol layer was dried by evaporation at room temperature and used to assess the antimicrobial activity. The M. koenigii leaves were crushed with sterile distilled water (1:1.5) and then filter it with sterile Watten filter paper and filtrate was used for phytochemical analysis.

Phytochemical Analysis

The carbohdrate, protein and amino acid estimation was carried out by using DNSA method, lowery and ninhydrin method respectively. The testsof flavonoid, free anthraquinones, Tannins, and curative health care since time immemorial. The plant extracts or bioactive herbal compounds have been reported scientifically for their biological activities. Plant produces chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. There are many phytochemicals in fruits and herbs and each of them work differently (Argal and pathak 2006). Nees (A. paniculata, Chuanxinlan), native to Taiwan, Mainland China and India, is a medicinal herb with an extremely bitter taste used to treat many diseases such as host defense mechanisms (Broekaert et al. 1995; Seilertnikoff 2001). As well as many compounds show broad spectrum of inhibitory activity against pathogenic bacteria and fungi. The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives.

Human beings have been utilizing plants for basic preventive and curative health care since time immemorial. The plant extracts or bioactive herbal compounds have been reported scientifically for their biological activities. Plant produces chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. There are many phytochemicals in fruits and herbs and each of them work differently (Argal and pathak 2006). Nees (A. paniculata, Chuanxinlan), native to Taiwan, Mainland China and India, is a medicinal herb with an extremely bitter taste used to treat many diseases such as host defense mechanisms (Broekaert et al. 1995; Seilertnikoff 2001). As well as many compounds show broad spectrum of inhibitory activity against pathogenic bacteria and fungi. The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives.

Human beings have been utilizing plants for basic preventive and curative health care since time immemorial. The plant extracts or bioactive herbal compounds have been reported scientifically for their biological activities. Plant produces chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. There are many phytochemicals in fruits and herbs and each of them work differently (Argal and pathak 2006). Nees (A. paniculata, Chuanxinlan), native to Taiwan, Mainland China and India, is a medicinal herb with an extremely bitter taste used to treat many diseases such as host defense mechanisms (Broekaert et al. 1995; Seilertnikoff 2001). As well as many compounds show broad spectrum of inhibitory activity against pathogenic bacteria and fungi. The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives.

Human beings have been utilizing plants for basic preventive and curative health care since time immemorial. The plant extracts or bioactive herbal compounds have been reported scientifically for their biological activities. Plant produces chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. There are many phytochemicals in fruits and herbs and each of them work differently (Argal and pathak 2006). Nees (A. paniculata, Chuanxinlan), native to Taiwan, Mainland China and India, is a medicinal herb with an extremely bitter taste used to treat many diseases such as host defense mechanisms (Broekaert et al. 1995; Seilertnikoff 2001). As well as many compounds show broad spectrum of inhibitory activity against pathogenic bacteria and fungi. The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives.
The phytochemical screening showed the presence of M. koenigii leaves against a wide array of microorganisms (Marjorie et al., 2011). The plates were incubated for 24 h at 37°C. The nitrogen results were shown in Table 2. The largest zone of growth inhibition zone was tested for antibacterial activity against human pathogens bacteria as Pseudomonas aeruginosa, Staphylococcus aureus and Proteus mirabilis. In our study, when the methanolic extract of M. koenigii leaves was tested for antibacterial activity against human pathogenic bacteria as Staphylococcus aureus and Pseudomonas aeruginosa, it showed growth inhibition zone. The specific concentration of methanol extracts of M. koenigii leaves showed various growth inhibitory zones against pathogens and zone diameter shown in Table 2. The largest zone of growth inhibition was observed from methanolic extracts 1200 and 1000 μg ml⁻¹ against Staphylococcus aureus and Pseudomonas aeruginosa respectively. Phenol and flavonoids present in the methanol extract may be responsible for the antibacterial activity. Phenol is produced by the plants for the defense against the infection. Two reasons accounting for the higher antibacterial activity of the methanolic extracts may be: First reason is the nature of biological active components (alkaloids, flavonoids, essential oil, terpenoids, phenols, etc.), which may be enhanced in the presence of different metals. And second is the stronger extraction capacity of methanol may have produced a greater number of active constituents responsible for antibacterial activity. Use of the crude methanol extract of M. koenigii as an agent to microbial pathogens needs further extensive research for their better economic and therapeutic utilization.

Conclusion The methanolic extract of M. koenigii leaves has been proved to be highly effective antimicrobial agent against pathogens Pseudomonas aeruginosa and Staphylococcus aureus. The results show the phytochemical potential of M. koenigii leaves. The phytochemical screening of M. koenigii showed the presence of carbohydrates, protein, amino acids, phenol and flavonoids, free anthraquinones, Tannins, phlobatannins, anthraquinones and terpenoids (Table 1). The carbohydrate and protein contents of the M. koenigii leaves are relatively low and comparatively same in concentration. The M. koenigii leaves showed least 270.0 and 225.0 μg g⁻¹ carbohydrate and protein respectively. The content of the M. koenigii leaves was μg g⁻¹. The amino acid and phenolic content of the M. koenigii leaves was showed 5.5 and 26.5 μg g⁻¹ respectively. The M. koenigii leaves are generally very low in carbohydrates, fats and proteins, and therefore contribute very little to the energy values of a meal but they are highly aromatic. So, the phenols and flavonoids in M. koenigii may responsible for the antimicrobial property.

Elemental analysis
There is a presence of elements such as Ca,Mg,Fe,P etc. in M. koenigii leaves. The detected elements which are present in higher amounts are Fe and Ca. The Fe and Ca content of M. koenigii leaves were analyzed by Atomic Absorption Spectroscopy method (AAS) and showed 0.056and 55.57 ppm respectively. This result indicated the M. koenigii leaves use as calcium source in routine diet.

Antibacterial activity
This initial observation M. koenigii leaves have high total phenolic contents, that prompted us to evaluate antimicrobial activity. Murraya koenigii is a common plant with medicinal properties. In our study, when the methanolic extract of M. koenigii was tested for antibacterial activity against human pathogenic bacteria as Staphylococcus aureus and Pseudomonas aeruginosa, it showed growth inhibition zone. The specific concentration of methanol extracts of M. koenigii leaves showed various growth inhibitory zones against pathogens and zone diameter shown in Table 2. The largest zone of growth inhibition was observed from methanolic extracts 1200 and 1000 μg ml⁻¹ against Staphylococcus aureus and Pseudomonas aeruginosa respectively.

Phenol and flavonoids present in the methanol extract may be responsible for the antibacterial activity. Phenol is produced by the plants for the defense against the infection. Two reasons accounting for the higher antibacterial activity of the methanolic extracts may be: First reason is the nature of biological active components (alkaloids, flavonoids, essential oil, terpenoids, phenols, etc.), which may be enhanced in the presence of different metals. And second is the stronger extraction capacity of methanol may have produced a greater number of active constituents responsible for antibacterial activity. Use of the crude methanol extract of M. koenigii as an agent to microbial pathogens needs further extensive research for their better economic and therapeutic utilization.

Conclusion
The methanolic extract of M. koenigii leaves has been proved to be highly effective antimicrobial agent against pathogens Pseudomonas aeruginosa and Staphylococcus aureus. The results show the phytochemical potential of M. koenigii leaves. The phytochemical screening of M. koenigii showed the presence of carbohydrates, protein, amino acids, phenol and flavonoids, free anthraquinones, Tannins, phlobatannins, anthraquinones and terpenoids (Table 1). The carbohydrate and protein contents of the M. koenigii leaves are relatively low and comparatively same in concentration. The M. koenigii leaves showed least 270.0 and 225.0 μg g⁻¹ carbohydrate and protein respectively. The content of the M. koenigii leaves was μg g⁻¹. The amino acid and phenolic content of the M. koenigii leaves was showed 5.5 and 26.5 μg g⁻¹ respectively. The M. koenigii leaves are generally very low in carbohydrates, fats and proteins, and therefore contribute very little to the energy values of a meal but they are highly aromatic. So, the phenols and flavonoids in M. koenigii may responsible for the antimicrobial property.

Elemental analysis
There is a presence of elements such as Ca,Mg,Fe,P etc. in M. koenigii leaves. The detected elements which are present in higher amounts are Fe and Ca. The Fe and Ca content of M. koenigii leaves were analyzed by Atomic Absorption Spectroscopy method (AAS) and showed 0.056 and 55.57 ppm respectively. This result indicated the M. koenigii leaves use as calcium source in routine diet.

Antibacterial activity
This initial observation M. koenigii leaves have high total phenolic contents, that prompted us to evaluate antimicrobial activity. Murraya koenigii is a common plant with medicinal properties. In our study, when the methanolic extract of M. koenigii was tested for antibacterial activity against human pathogenic bacteria as Staphylococcus aureus and Pseudomonas aeruginosa, it showed growth inhibition zone. The specific concentration of methanol extracts of M. koenigii leaves showed various growth inhibitory zones against pathogens and zone diameter shown in Table 2. The largest zone of growth inhibition was observed from methanolic extracts 1200 and 1000 μg ml⁻¹ against Staphylococcus aureus and Pseudomonas aeruginosa respectively.

Phenol and flavonoids present in the methanol extract may be responsible for the antibacterial activity. Phenol is produced by the plants for the defense against the infection. Two reasons accounting for the higher antibacterial activity of the methanolic extracts may be: First reason is the nature of biological active components (alkaloids, flavonoids, essential oil, terpenoids, phenols, etc.), which may be enhanced in the presence of different metals. And second is the stronger extraction capacity of methanol may have produced a greater number of active constituents responsible for antibacterial activity. Use of the crude methanol extract of M. koenigii as an agent to microbial pathogens needs further extensive research for their better economic and therapeutic utilization.

Conclusion
The methanolic extract of M. koenigii leaves has been proved to be highly effective antimicrobial agent against pathogens Pseudomonas aeruginosa and Staphylococcus aureus. The results show the phytochemical potential of M. koenigii leaves. The phytochemical screening of M. koenigii showed the presence of carbohydrates, protein, amino acids, phenol and flavonoids, free anthraquinones, Tannins, phlobatannins, anthraquinones and terpenoids (Table 1). The carbohydrate and protein contents of the M. koenigii leaves are relatively low and comparatively same in concentration. The M. koenigii leaves showed least 270.0 and 225.0 μg g⁻¹ carbohydrate and protein respectively. The content of the M. koenigii leaves was μg g⁻¹. The amino acid and phenolic content of the M. koenigii leaves was showed 5.5 and 26.5 μg g⁻¹ respectively. The M. koenigii leaves are generally very low in carbohydrates, fats and proteins, and therefore contribute very little to the energy values of a meal but they are highly aromatic. So, the phenols and flavonoids in M. koenigii may responsible for the antimicrobial property.

Elemental analysis
There is a presence of elements such as Ca,Mg,Fe,P etc. in M. koenigii leaves. The detected elements which are present in higher amounts are Fe and Ca. The Fe and Ca content of M. koenigii leaves were analyzed by Atomic Absorption Spectroscopy method (AAS) and showed 0.056 and 55.57 ppm respectively. This result indicated the M. koenigii leaves use as calcium source in routine diet.

Antibacterial activity
This initial observation M. koenigii leaves have high total phenolic contents, that prompted us to evaluate antimicrobial activity. Murraya koenigii is a common plant with medicinal properties. In our study, when the methanolic extract of M. koenigii was tested for antibacterial activity against human pathogenic bacteria as Staphylococcus aureus and Pseudomonas aeruginosa, it showed growth inhibition zone. The specific concentration of methanol extracts of M. koenigii leaves showed various growth inhibitory zones against pathogens and zone diameter shown in Table 2. The largest zone of growth inhibition was observed from methanolic extracts 1200 and 1000 μg ml⁻¹ against Staphylococcus aureus and Pseudomonas aeruginosa respectively.
Acknowledgments
Author expresses gratitude to DST-SERB (Science and Engineering Research Board), New Delhi, India, for Fast Track Young Scientists Fellowship.

Table 1 Observation of phytochemical test

<table>
<thead>
<tr>
<th>Sample</th>
<th>Curry leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>+</td>
</tr>
<tr>
<td>Protein</td>
<td>+</td>
</tr>
<tr>
<td>Aminoacids</td>
<td>+</td>
</tr>
<tr>
<td>Phenol</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Freeanthraquinone</td>
<td>-</td>
</tr>
<tr>
<td>Phlobatanin</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
</tr>
</tbody>
</table>

REFERENCES