Research Paper Biotechnology

Phytochemicals Analysis and Antimicrobial Activity of Murryakoenigii

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ABSTRACT

Murrya koenigiiwas assessed for the phytochemical analysis and antimicrobial activity. Evaluated the phytochemicals e.g. carbohydrade, 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 aeruginosausing the agar cup method.The elemental analysis of M. koenigiiwas carried out by Atomic Absorption Spectrometry (AAS) and has 55.57 of calcium.

KEYWORDS Murrya koenigii, phytochemicals, Atomic Absorption Spectrometry, antimicrobial activity

Introduction

Diet is one which helps to improve and maintain health, keeping away frommany 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; Selitrennikoff2001). 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 advantageof using plant extract or their essential oils instead syntheticdrugs in feed: synthetic drugs residues inanimal meat and eggs can cause health problemsin people who consume them, especially due to increasing resistance of pathogenspresent in the human body as a result ofprolonged 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 works differently (Argal and pathak 2006). Nees (*A. paniculata*, *Chuanxinlian*), native to Taiwan, Mainland China and India, is a medicinal herb with an extremely bitter taste used to treat liver disorders, bowel complaints of children, colic pain, common cold and upper respiratory tract infection. *A. paniculata*is have a number of bioactivities such as anti- inflammation, anti- cancer, immunomodulation, anti- infection, anti- hepatotoxicity, antiatherosclerosis, anti- diabetes and anti- oxidation (Negi et. al. 2008).

Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils (Jansen et. al. 1987), as well as in tannin (Saxena et. al. 1994). The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency (Almagboul et. al. 1985; Sousa et. al.1991; Artizzu et. al. 1995; Gislene et. al. 2000). The antimicrobial efficacy attributed to some plants in treating diseases has been beyond belief. It is estimated that local communities have used about 10% of all flowering plants on earth to treat various infections, although only 1% have gained recognition by modern scientists (Lewis. et. al. 2006). According to the WHO, medicinal plants would be the best source for obtaining variety of drugs (Santos et. al. 1995). These evidences contribute to support and quantify the importance of screening natural products.

The scientific name of the curry plant is *Murraya Koenigii Sprengand* it belongs to the *Rutaceae* family. The plant is native to India and is usually found in tropical and subtropical regions. It is cultivated in various other countries such as China, Australia, Nigeria and Ceylon. The most useful parts of this plant are the leaves. *Murrayakoenigii* L. (curry leaves) It is reported to have antioxidant, antidiabetic, anticarcinogenic, antidysenteric, stimulant, hypoglycaemic and antimicrobial activities (Khanum et. al. 2000;Yadav et. al. 2002; Ningappa et. al. 2010).The aim of the present study was to investigate the phytochemical and elemental analysisof leaves extracts of *Murraya koenigii* L. (curry leaves) and antimicrobial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*

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 chloride5.0 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 leavesextract

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 study.The *M. koenigii*leaves were crushed with sterile distilled water (1:1.5) and then filter it with sterileWattman filter paper and filtrate was used for phytochemical analysis.

Phytochemical Analysis

The carbohydrate, protein and amino acid estimation was carried by using DNSA method, lowery and ninhydrin method respectively.The testsof flavonoid, free anthraquinones, Tannins,

phlobatannins, anthraquinones and terpenoidswere carried out on the aqueous extract and powdered of *M. koenigii*by using standard procedure to identify the constituents as described by Ajayiet. al.(2011)

Estimation of phenol
The leaves extract prepared in 80% ethanol used for the estimation of phenol content. Extract dissolve in distilled water and add folin phenol reagent and wait for 3 min. After that sodium carbonate was added and kept in boiling water bath for 1 min and measured optical density at 650 nm.

Test for flavonoid
*M. koenigii*leaves powder was 1:10 (w/v) diluted withof distilled water and boiled for 5 minutes and filtered. The few drops of 20 % sodium hydroxide solution were added to 1 ml of filtrate. A change to yellow color which on addition of acid changed to colorless solution showed the presence of flavonoids

Test for Tannins
1 g of sample was separately boiled with 20 ml distilled water for five minutes in a water bath and was filtered while hot. 1 ml of cool filtrate was distilled to 5 ml with distilled water and a few drops (2-3) of 10 % ferric chloride were observed for any formation of precipitates and any color change. A bluish-black or brownish-green precipitate indicated the presence of tannins.

Test for phlobatannins
An aqueous extract was boiled with 1 % aqueous hydrochloric acid and deposition of a red precipitation indicated the presence of the phlobatannins.

Test for terpenoids
5 ml of extract was mixed in 2 ml of chloroform and 3 ml of conc. H_2SO_4 was then added to form a layer. A reddish-brown precipitate coloration at the interface indicated the presence of terpenoids

Test for free anthraquinones
5 ml of sample was mixed in 5 ml of chloroform. The resulting mixture was shaken for 5 min and filtrate it. The filtrate was then shaken with equal volume of 10 % ammonia solution. The presence of a bright pink color in the aqueous layer indicated the presence of free anthraquinones.

Estimation of calcium and iron
The Aqueous fraction of leaves was used for estimation of calcium and iron content was determined by atomic absorption spectroscopy (AAS) methods.

Antimicrobial assay
Antimicrobial activity was evaluated by the agar cup method on nutrient agar medium. For agar cup method wells (6 mm diameter) were punched into the agar medium and filled with 100 µl (150 mg ml^{-1}) of leaves extract and solvent blanks as control. The plates were incubated for 24 h at 37°C.

Result and discussion
Phytochemical Analysis
Phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, saponins and other several otheraromatic compounds are secondary metabolites of plants that serve a defense mechanism against prediction bymany microorganisms, insects and other herbivores (Bonjar et. al. 2004). These bioactive compounds are known to act by different mechanism and exert antimicrobial action.Flavonoidsarehydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and itshould not be surprising that they have been found *in vitro* to be effective antimicrobial substances against awide array of microorganisms (Marjorie 1999).

The Phytochemical screening of *M. koenigii* showed the presence of carbohydrates, protein, amino acids, phenol and flavonoids, free anthraquinones, Tannins, phlobatannins, anthraquinones and terpenoids (Table1). The carbohydrate and protein contents of the *M. koenigii* leaves are relatively low and comparatively same in concentration. The *M. koenigii* showed least 270.0 and 225.0 $\mu g g^{-1}$ carbohydrate and protein content respectively. The content of the *M. koenigii* was µg g^{-1}. The amino acid and phenolic content of the *M. koenigii* 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 ppmrespectively which. This result indicated the *M. koenigii* leaves use as calcium source in routine diet

Antibacterial activity
This initial observation *M. koenigii* leaves had high total phenolic contents, that prompted us to evaluate antimicrobial activity. *Murrayakoenigii*was a common plant with medicinal properties.In our study, when the methanolic extract of *M. koenigii* was testedfor antibacterial activity against human pathogenic bacteria as *Staphylococcus aures* and*Pseudomonas aeruginosa,* itshowed 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 zone was observed from methanolic extracts 1200 and 1000 µg ml^{-1} against *Staphylococcus aures* and *Pseudomonas aeruginosa* respectively.

Phenol and flavonoids present in the methanol extract may be responsible for the antibacterial activity. Flavonoids are produced by the plants for the defense against the infection. Two reasons accounting for the higher antibacterial activity of methanolic extracts may be: First reason is the nature of biological active components (alkaloids, flavonoids, essential oil, tarpenoids, phenols, etc.), which may be enhanced in the presence of methanol; and second is the stronger extraction capacity of methanol may have produced a greater number of active constituents responsible for antibacterial activity. So, 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 areuginosa and staphylococcus aures*had put forth essential aspects related to drug resistance for several antibiotics available to cure the diseases caused by these pathogens. The *M. koenigii* leaves were show high antimicrobial activity against *Staphylococcus aures*in plate assay. Thus it is concluded that *M. koenigii*leaves are useful in various preparations of medicines which are showing resistance against s*taphylococcus aures.*To conclude the present study, we have found that most of the biologically active phytochemicals like phenols, proteins, carbohydrates, aminoacids and flavonoids were present in the *M. koenigii* leaves extract. The presence of phytochemicals had greater influence in showing antimicrobial activity, as phenols and flavonoids also play an immunological role in plant system. Also *M. koenigii* leaves are useful in curing the disorders caused due to deficiency of calcium and iron because of presence of higher concentration of calcium and iron and they also indirectly leads with an antimicrobial activity. This preliminary screening is more useful to human beings as *M. koenigii*leaves used the daily component of diet. Also concluded the higher amounts of *M. koenigii*leaves may use as to preserve food in case of staphylococcal food poisoning

case.

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Table 1 Observation of phytochemical test

Sample	Curry leaves
Carbohydrate	+
Protien	+
Aminoacids	+
Phenol	+
Flavonoids	+
Tannins	+
Freeanthraquinone	-
Phlobatanin	+
Terpenoids	-

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