

Phytochemical and antimicrobial study of the flowers of *Arenga wightii* Griff. - an endemic palm of Southern Western Ghats

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Abstract

Objective: To make scientific validation of the flowers of the endemic palm *Arenga wightii* Griff. in relation to phyto chemistry and antimicrobial activity by agar disc diffusion method.

Key Findings: Among the three extracts tested against twelve pathogenic bacteria, acetone flower extract was effective against *Bacillus cereus*, *Staphylococcus aureus* and *Streptococcus pyogenes*, while chloroform extract was effective against *B. cereus*, *S. aureus*, *Serratia marcescens* and *St. pyogenes*, while aqueous extract was effective against the gram negative strain *Enterobacter* sp. and *Acinetobacter baumannii*. The fungal strains showed no response to the flower extracts. Preliminary phytochemical screening showed the presence of phyto constituents viz. steroid, tannin, xantho proteins, phenols, flavonoid, coumarin, saponin and carbohydrates.

Conclusion: It may be concluded that the crude extracts obtained from the flowers of *A. wightii* may be used as drug to treat disease caused by the bacteria. Therefore, further investigations are needed to isolate and characterize their active compounds for pharmacology testing.

Keywords: Endemic palm; Phytochemical screening; Microbial Pathogens

Introduction

Nature has bestowed Western Ghats with a cradle of medicinal plants and an impressive number of modern drugs have been isolated from these plants. Many of these isolations were based on the uses of traditional medicine which continues to play an essential role in health care [1]. These compounds may derived by primary or rather secondary metabolism of living organisms. The secondary metabolite are diverse chemical compounds with obscure function and exhibits some kind of biological activities human therapy, veterinary, agriculture, scientific research and in countless other areas [2-5]. These biologically active ingredients include alkaloids, flavonoids, glycosides, steroids, terpenes and tannins [6-10].

Infectious diseases are the number one among all causes of death. About 50-75% of hospital deaths are reported due to infectious diseases caused by microorganisms [11]. Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. This situation forced scientists to search with a new eye for new antimicrobial substances and as an alternative source to existing drugs.

Antibiotics serve as the most important weapons in fighting bacterial infections and have greatly benefited the health of human life. However, over the past few decades these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not only because many of them produce toxic reactions but also due to emergence of drug resistant microorganism. To tolerate the drug resistant strains it is essential to develop newer drugs with lesser resistance. Systematic studies of pharmacological compounds revealed that the drug may have the possibility of possessing diverse functions and thus may be useful in different spheres of medicine.

Drugs derived from natural sources play a significant role in the prevention and treatment of human ailments. In many developing countries, traditional medicine is one of the primary healthcare systems [12]. Herbs are widely exploited in the traditional medicine and their curative potentials are well documented [13]. About 61% of new drugs developed between 1981 and 2002 were based on natural products and they have been very successful especially in the areas of life threatening diseases [14].

In the recent years, research on medicinal plants has attracted a lot of attentions globally [15,16]. Large body of evidence has accumulated to demonstrate the promising potential of Medicinal plants used in various traditional, complementary and alternate systems of treatment of human diseases [17-19]. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, and glycosides etc., which have been found *in vitro* to have antimicrobial properties [20].

Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases [7]. The harmful microorganisms can be controlled with drugs and these results in the emergence of multiple drug resistant bacteria and it has created alarming clinical situations in the treatment of infections [11,12]. The pharmacological industries

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have produced a number of new antibiotics; resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are utilized as therapeutic agents. Therefore, research work must be carried out to solve this problem, such as to minimize the use of antibiotics and develop new drugs to inhibit the growth of either synthetic or natural to control pathogenic microorganism. In an effort to expand the spectrum of antimicrobial agents from natural resources, many researches have been carried out. Most of the studies are concentrated on leaves than other parts [21-25]. Little information is available on other parts of plants especially the flowers [26-33].

Arenga wightii is an unbranched monocious palm found endemic to southern Western Ghats. Traditionally fresh toddy obtained from the young inflorescence is given internally for jaundice. The male flowers of *A. wightii* are left unutilized after pollination. However, to the best of our knowledge, there is no report available on the exploration of its phyto chemistry and antimicrobial activity. This study will provide base-line data for further detailed investigations of various biological activities of *A. wightii* palm and of its use as a functional food.

Materials and Methods

Collection and authentication of plant material

To study the phyto chemistry and antimicrobial activity, disease free male flowers of *Arenga wightii* were collected from Western Ghats region. The plants were identified using regional floras and voucher specimens were deposited in the Herbarium, Department of Botany, Scott Christian College, Nagercoil, Tamil Nadu, and India.

Preparation of flower extracts

Fresh flowers of *A. wightii* were obtained from Western Ghats and washed thoroughly under tap water and dried under shade and powdered. The solvents used for the extraction procedure in the present study were acetone, chloroform and water. About 25 g of dried flower powder was extracted using 250 ml of the extraction solvents with continuous shaking on a rotary shaker at 150-180 rev/min for 48 h. The filtrates were concentrated using a rota-vapour at 45°C and stored at 4°C in air tight containers for future use.

Preliminary phytochemical screening

Preliminary phytochemical screening of the fresh flowers of *A. wightii* was performed using the methods proposed by Harborne [34].

Microbial pathogens

Twelve different clinically isolated pathogenic bacteria viz., *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Acinetobacter baumannii*, *Enterobacter* sp., *Enterococcus faecalis*, *Streptococcus pyogenes* and *Bacillus cereus* were used and four different fungi viz., *Candida albicans*, *C. cruzi*, *C. parapsolisis* and *C. tropicalis* were used in the present study.

The bacterial and fungal stock cultures were incubated for 24 h at 37°C on Nutrient Agar and Potato Dextrose Agar medium (Microcare lab., Surat, India) respectively following refrigeration storage at 4°C.

The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C, (The bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C) whereas the yeasts and molds were grown in sabouraud dextrose agar (SDA) and potato

dextrose agar (PDA) media, respectively, at 28°C. The stock cultures were maintained at 4°C.

Antimicrobial activity Study

Agar disc diffusion procedure (Kirby-Bauer method) was used to study the antimicrobial activity [35]. Filter paper discs (Whatman No.41) of uniform size (5 mm diameter), impregnated with specified concentrations of the different extracts (100 µg/disc) and control were placed on the surface of the agar plate, that has been pre-seeded with the bacterial pathogens on Muller Hinton Agar (Himedia, India) plates with a pH of 7.4. The plates were incubated at 37°C for 17 hours. The antimicrobial activity was determined by measuring the diameter of the zone of inhibition in millimeter.

The antibiotic Amikacin was used as positive control for bacterial strains whereas; Flucanazole was used for fungal stains. All the experiments were done in triplicates. The average of triplicate values was calculated.

Results

Preliminary phytochemical screening

Preliminary phytochemical screening of acetone extracts of *A. wightii* showed the presence of tannins, xanthoprotein, phenols, coumarins and carbohydrates whereas the chloroform extract of the palm showed the availability of steroids, tannins, phenols, saponin and carbohydrates. Biomolecules like alkaloids, proteins, quinones, xanthoprotein, carboxylic acids, flavonoids and coumarins were absent in chloroform extract. Tannins, xanthoprotein, phenol, flavonoid, saponin and carbohydrate were found in the aqueous extract of *A. wightii*. All the other tested secondary metabolites were found to be absent in aqueous extract.

Antimicrobial activity

The antimicrobial activity of the flower extracts of *A. wightii* are presented in Table 1. Of the twelve bacterial pathogens studied, seven were susceptible which includes four gram positive (*Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus pyogenes* and *Bacillus cereus*) and three gram negative bacteria (*Serratia marcescens*, *Acinetobacter baumannii* and *Enterobacter* sp.). The remaining five organisms showed resistance towards the flower extracts.

The acetone flower extracts showed maximum zone of inhibition against the gram positive bacterium *Bacillus cereus* (10 mm). *S. aureus* and *St. Pyogenes* had 7 mm zone each. The chloroform flower extracts of the palm showed maximum inhibition against the gram positive

Biomolecules	Extracts		
	Acetone	Chloroform	Aqueous
Alkaloid	-	-	-
Protein	-	-	-
Quinones	-	-	-
Steroids	-	+	-
Tannin	+++	+++	+++
Xanthoprotein	+++	-	+++
Carboxylic acid	-	-	-
Phenol	+++	+	+++
Flavonoid	-	-	++
Coumarin	+++	-	-
Saponin	-	+	+
Carbohydrate	+	+++	++

Table 1: Phytochemical study of the flowers of *Arenga wightii* Griff.

pathogen *Bacillus cereus* (12 mm), followed by *S. aureus* (9 mm), *Serratia marcescens* (8 mm) and *St. pyogenes* (7 mm). Aqueous extract also showed considerable inhibitory activity against the gram negative strain *Enterobacter* sp. (14 mm) followed by *A. baumannii* (12 mm). The gram positive strain *S. aureus* showed 9 mm inhibitory zone followed by *St. pyogenes* (8 mm).

The standard drug Amikacin showed maximum zone of inhibition against *Pseudomonas aeruginosa* (21 mm) and *Serratia marcescens* (21 mm) and least activity against *Proteus mirabilis* (9 mm). Moderate activity was noticed against *E. coli* (15 mm), *K. pneumoniae* (14 mm), *Salmonella typhi* (10 mm), *A. baumannii* (14 mm), *Enterobacter* sp. (18 mm), *Enterococcus faecalis* (13 mm) and *B. cereus* (18 mm). The fungal strains showed no response to the tested flower extracts. For fungal strains, Flucanazole was used as a positive control. *C. albicans* was resistant to Flucanazole. *C. tropicalis*, *C. cruzi* and *C. parapsolisis* showed 36 mm, 33 mm and 32 mm zones respectively.

Discussion

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world [3-5]. The world health organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the words population. In the present work, the extracts obtained from *A. wightii* flowers showed strong activity against most of the tested bacterial strains. The results were compared with standard antibiotic drugs. In this screening work, no extracts of the flowers showed activity against the fungal strains.

This study also shows the presence of different phytochemicals with biological activity that can be of valuable therapeutic index. The result of phytochemicals in the present investigation showed that the plant contain more or less same components like saponin, triterpenoids, steroids, glycosides, anthraquinone, flavonoids, Proteins and amino acids. Plant rich in tannin and phenolic compounds have been shown to possess antimicrobial activities against a number of microorganisms [28,36]. Generally, the antimicrobial activities of plant extracts dependent on various factors; the environmental and climate conditions under which the plant grew, the solvent that used for the extraction, the choice of extraction method, test concentration, the method of determination of antimicrobial activity and the test microorganisms [37,38] (Table 2).

Arenga wightii flower extracts expressed tremendous activity against four gram positive (*Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus pyogenes* and *Bacillus cereus*) and three gram negative bacteria (*Serratia marcescens*, *Acinetobacter baumannii* and *Enterobacter* sp.). The bioactivity of plant extracts depends on the type and quantity of phytochemicals present in the extract. The phytochemicals show various modes of antibacterial activities [39].

Conclusion

The present study justifies the claimed uses of flowers in the traditional system of medicine to treat various infectious disease caused by the microbes. Therefore, it may be concluded from the above results, that the crude extracts obtained from the flowers of *A. wightii* may be used enough as drug to treat disease caused by the bacteria, but before use in human being isolation of pure compound, toxicological study, and clinical trial in animal model should be carried out thereafter. Therefore, further investigations are needed as far as the chemical constitutes of this plant extracts concerned. Additional deep research is necessary to isolate and characterize their active compounds

Microorganisms	Solvent extracts			
	Acetone	Chloroform	Aqueous	Amikacin/ Flucanazole
<i>Escherichia coli</i>	-	-	-	15 mm
<i>Klebsiella</i>	-	-	-	14 mm
<i>Pseudomonas</i>	-	-	-	21 mm
<i>Proteus mirabilis</i>	-	-	-	9 mm
<i>Serratia marcescens</i>	-	8 mm	-	21 mm
<i>Salmonella typhi</i>	-	-	-	10 mm
<i>Acinetobacter</i>	-	-	12 mm	14 mm
<i>Enterobacter</i>	-	-	14 mm	18 mm
<i>Staphylococcus aureus</i>	7 mm	9 mm	9 mm	R
<i>Enterococcus</i>	-	-	7 mm	13 mm
<i>Streptococcus pyogenes</i>	7 mm	7 mm	8 mm	R
<i>Bacillus cereus</i>	10 mm	12 mm	-	18 mm
<i>Candida albicans</i>	-	-	-	R
<i>C. cruzi</i>	-	-	-	40 mm
<i>C. parapsolisis</i>	-	-	-	38 mm
<i>C. tropicalis</i>	-	-	-	37 mm

Table 2: Antimicrobial activity of the flower extracts of *Arenga wightii* Griff.

for pharmacology testing.

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