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Antimicrobial activity of *Plectranthus amboinicus* solvent extracts against Human Pathogenic Bacteria and Fungi

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ABSTRACT

Plectranthus amboinicus or locally known as bangun-bangun, is an indigenous vegetable which can be freshly eaten. However, the plant is unpopular among local people and being neglected. It has been reported to be traditionally used for medicine to cure common illnesses such as cough, stomachache, headache and skin infection. Based on the potential, a study was conducted to bioprospect the antimicrobial activity of the essential oil. *Plectranthus amboinicus* essential oil of methanol and chloroform extract was tested against nine bacteria and four fungi i.e., *Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Shigella flexneri, Klebsiella pneumoniae, Enterococcus faecal, Staphylococcus aureus, Salmonella typhi, Bacillus cereus, Candida albicans, Aspergillus niger, Aspergillus fumigatus* and *Aspergillus flavus.* The methanol extract of *Plectranthus amboinicus* showed the maximum antibacterial activity against the bacteria *Klebsiella pneumoniae* and fungi *Candida albicans.* The antimicrobial activity of *Plectranthus amboinicus* was more at 100 mg/ml concentration when compared to 50 mg/ml concentration. Comparatively, the Methanol extract of *Plectranthus amboinicus* exhibited maximum antimicrobial activity when compared to Chloroform extract. The *Plectranthus amboinicus* has showed good antimicrobial activity against most of the bacteria and only one fungal yeast *Candida albicans*. No zone of inhibition was recorded against other fungal isolates like *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and Negative DMSO control.

Keywords: *Plectranthus amboinicus*, Bacteria, Fungi, Antimicrobial activity and Well diffusion assay.

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1. INTRODUCTION

India has a rich heritage of knowledge on plant based drugs both for use in preventive and curative medicine. A country like India is very much suited for development of drugs from medicinal plant. Because of its vase and wide variations in soil and climate, the Indian sub – continent is suitable for cultivation of large number of medicinal and aromatic plant which can be used as raw materials for pharmaceutical, perfumery, cosmetics, flavour and food and agrochemical industries. Some of these plants produce valuable drugs which have high export potential [1].

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is founds in "Rig veda", which is said to have been written between 4500-1600 B.C. and is supposed to be the oldest repository of human knowledge. It is Ayurveda, the foundation of medicinal science of Hindu culture, in its eight division deals with specific properties of drugs and various aspects of science of life and the art of healing [2].

In the modern world multiple drug resistance has developed against many microbial infections 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 for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants [3].

Plectranthus is a large genus, with more than 300 species from the family of Lamiaceae. It has a rich diversity of ethnobotanical and medicinal uses. Several species of the genus possess interesting medicinal properties such as the

extract of *Plectranthus barbatus* is used for the treatment of stomachache and as a pugitive, nausea and gastritis and intestinal spasms in Brazil. *Plectranthus caninus, Plectranthus laxiflorus* and *Plectranthus barbatus* are used in the treatment of teeth and gum disorders. It is also reported that *Plectranthus amboinicus* and *Plectranthus barbatus* are used to treat a wide range of diseases such as for the treatment of digestive system, skin conditions and allergies, infections and fever, genito-urinary conditions, pain, respiratory conditions and muscular-skeletal conditions [4].

Plectranthus amboinicus or locally known as bangun-bangun, bebangun, sedingin or hati-hatihijau, is an indigenous vegetable which can be freshly eaten. The leaf of *Plectranthus amboinicus* has many medicinal uses, especially for the treatment of common illnesses such as of cough, stomachache, headache, skin infection, asthma and urinary conditions [5]. The plant extracts especially the volatile essential oils from the leaves have been reported to possess antioxidant, antibacterial, antimicrobial, anti-inflammatory and fungitoxic activities [6, 7] but due to the geographical region and variety, the activity and composition of essential oils may be vary [8]. Therefore, it is important to access the local *Plectranthus amboinicus* to screen the potential biological activity especially antimicrobial properties and volatiles components of the plant.

2. MATERIALS AND METHODS

2.1. Collection of Plant materials

Healthy leaves of *Plectranthus amboinicus* were collected from Tirupattur, Vellore district, Tamil Nadu, India. The fresh leaves were cut into small pieces and dried under sun shade for 2 week and make as a fine powder form. The *Plectranthus amboinicus* leave powdered samples were hermetically sealed in separate polythene bags until the time of the extraction. This was used as the raw material for the extraction of antimicrobial compounds against the microbes used by using Soxhlet apparatus for 24 hrs. The purified extracted was stored at 4 °C until further use.

2.2. Test microorganisms used

Nine test bacterial strains (*Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Shigella flexneri, Klebsiella pneumoniae, Enterococcus faecalis, Staphylococcus aureus, Salmonella typhi* and *Bacillus cereus*) and three fungal strains (*Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger* and *Candida albicans*) were obtained from Department of Microbiology, Sacred Heart College (Autonomous), Tirupattur, Vellore District, Tamil Nadu, India.

2.3. Preparation of plant extract

Forty grams of powdered leaves of *Plectranthus amboinicus* was extracted successively with 200 ml of Methanol and Chloroform in Soxhlet extractor until the extract was clear. The extracts were evaporated to dryness and the resulting pasty form extracts were stored in a refrigerator at 4 °C for future use [9].

2.4. Determination of Antimicrobial activity of *Plectranthus amboinicus*

2.4.1. Inoculum preparation

Bacterial inoculum was prepared by inoculating a loopful of test organisms in 5 ml of Nutrient broth and incubated at 37 °C for 24 hours. Fungal inoculum was prepared by inoculating a loopful of test organisms in 5 ml of Sabouraud's dextrose broth and incubated at room temperature for 3 days.

2.4.2. Determination of Antimicrobial activity by Agar well Diffusion Method

Muller Hinton agar plates were inoculated with test organisms by spreading the fungal inoculum on the surface of the media. Wells (8 mm in diameter) were punched in the agar. Methanol and Chloroform extracts of *Plectranthus amboinicus* with different concentrations (50 mg/ml and 100 mg/ml) were mixed with 1 ml of Dimethyl sulfoxide (DMSO) and added into the well. Well containing DMSO alone act as a Negative control. For bacteria, the plates were incubated at 37 °C for 24 hours. For fungi, the plates were incubated at room temperature for 3 days. The antimicrobial activity was assessed by measuring the diameter of the zone of inhibition (in mm) [10].

3. RESULT AND DISCUSSION

The antimicrobial activity of methanol, chloroform leaf extract of *Plectranthus amboinicus* were studied in different concentrations (50 mg/ml and 100 mg/ml). Antibacterial potential of leaf extract was assessed in terms of zone of inhibition of bacterial and fungal growth.

The antibacterial activity of methanol extract of *Plectranthus amboinicus* was analyzed in the present study and the results were furnished in Table – 1. Among the two concentrations tested (50 and 100 mg/ml), maximum inhibitory zone was observed at 100 mg/ml when compared to 50 mg/ml. Maximum antibacterial activity was observed in the methanol extract of *Plectranthus amboinicus* against the bacteria *Klebsiella pneumoniae* (35 mm). Least antibacterial activity was observed in *Shigella flexneri* and it does not showed any zone of inhibition. No zone of inhibition was observed in the Negative DMSO control.

The antibacterial activity of Chloroform extract of *Plectranthus amboinicus* was analyzed in the present study and the results were furnished in Table – 2. Among the two concentrations (50 and 100 mg/ml) tested, maximum inhibitory zone was observed at 100 mg/ml than 50 mg/ml. Maximum antibacterial activity was observed in the bacteria *Enterococcus faecalis* and *Bacillus cereus* (17 mm). No zone of inhibition was observed against *Shigella flexneri, Pseudomonas aeruginosa* and Negative DMSO control.

Saranraj *et al*. [11] evaluated the antibacterial potentiality of ethanol and ethyl acetate solvent extracts of mature leaves of *Acalypha indica* against nine pathogenic bacterial isolates *viz*., *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Escherichia coli, Salmonella typhi, Shigella flexneri, Klebsiella pneumoniae, Vibrio cholerae* and *Pseudomonas aeruginosa*. The turbidity of the bacterial inoculums was compared with 0.5 Mc Farland standards and the antibacterial potential of *Acalypha indica* ethanol extract was tested by using Agar well diffusion method. The ethanol extract of *Acalypha indica* (100 mg/ml) showed maximum zone of inhibition (30 mm) against *Pseudomonas aeruginosa, Escherichia coli* and *Bacillus subtilis*. *Staphylococcus aureus* showed less zone of inhibition (12 mm). The ethyl acetate extract of *Acalypha indica* (100 mg/ml) showed maximum zone of inhibition (23 mm) against *Escherichia coli*.

Sivasakthi *et al*. [12] evaluated the antibacterial potentiality of ethanol and ethyl acetate solvent extracts of mature leaves of *Datura metel* against nine pathogenic bacterial isolates *viz*., *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Escherichia coli, Salmonella typhi, Shigella flexneri, Klebsiella pneumoniae, Vibrio cholerae* and *Pseudomonas aeruginosa*. The turbidity of the bacterial inoculums was compared with 0.5 Mc Farland standards and the antibacterial potential of *Datura metel* ethanol extract was tested by using Agar well

diffusion method. The ethanol extract of *Datura metel* (100 mg/ml) showed maximum zone of inhibition (26 mm) against *Pseudomonas aeruginosa, Escherichia coli* and *Bacillus subtilis*. *Staphylococcus aureus* showed less zone of inhibition (8 mm). The ethyl acetate extract of *Datura metel* (100 mg/ml) showed maximum zone of inhibition (19 mm) against *Escherichia coli*. There was no zone of inhibition against *Pseudomonas aeruginosa*.

Saranraj and Sivasakthivelan [13] tested the antibacterial activity of *Phyllanthus amarus* was tested against Urinary tract infection causing bacterial isolates *viz*., *Staphylococcus aureus*, *Serratia marcescens*, *Escherichia coli*, *Enterobacter* sp., *Streptococcus faecalis*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. The *Phyllanthus amarus* was shade dried and the antimicrobial principles were extracted with methanol, acetone, chloroform, petroleum ether and hexane. The antibacterial activity of *Phyllanthus amarus* was determined by Agar Well Diffusion Method. It was found that methanol extract of *Phyllanthus amarus* showed more inhibitory activity against UTI causing bacterial pathogens when compared to other solvent extracts.

Table - 1: Antibacterial activity of Methanol extract of *Plectranthus amboinicus*

S. N.	Bacterial Strains	Concentration of the Methanolic extract and Zone of inhibition (mm in dm)	
		50 mg/ml	100 mg/ml
1	*Escherichia coli*	25 mm	30 mm
2	*Proteus vulgaris*	17 mm	23 mm
3	*Pseudomonas aeruginosa*	25 mm	30 mm
4	*Shigella flexneri*	NZ	NZ
5	*Klebsiella pneumoniae*	31 mm	35 mm
6	*Enterococcus faecalis*	14 mm	29 mm
7	*Staphylococcus aureus*	23 mm	30 mm
8	*Salmonella typhi*	20 mm	23 mm
9	*Bacillus cereus*	20 mm	21 mm

*NZ – No zone of inhibition

Table - 2: Antibacterial activity of Chloroform extract of *Plectranthus amboinicus*

S. N.	Bacterial Strains	Concentration of the Chloroform extract and Zone of inhibition (mm in dm)	
		50 mg/ml	100 mg/ml
1	*Escherichia coli*	13 mm	15 mm
2	*Proteus vulgaris*	13 mm	15 mm
3	*Pseudomonas aeruginosa*	NZ	NZ
4	*Shigella flexneri*	NZ	NZ
5	*Klebsiella pneumoniae*	11 mm	12 mm
6	*Enterococcus faecalis*	15 mm	17 mm
7	*Staphylococcus aureus*	12 mm	14 mm
8	*Salmonella typhi*	12 mm	14 mm
9	*Bacillus cereus*	15 mm	17 mm

*NZ – No zone of inhibition

The antifungal activity of Methanol extract of *Plectranthus amboinicus* was analyzed in the present study and the results were furnished in Table – 3. Among the two concentrations (50 and 100 mg/ml) tested, maximum inhibitory zone was observed at 100 mg/ml when compared to 50 mg/ml. Maximum antifungal activity was observed against the fungal yeast *Candida albicans* (40 mm in dm) at 100 mg/ml concentration and no zone of inhibition was recorded against other fungal isolates like *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and Negative DMSO control.

The antifungal activity of Chloroform extract of *Plectranthus amboinicus* was analyzed in the present study and the results were furnished in Table – 4. Among the two concentrations (50 and 100 mg/ml) used, maximum inhibitory zone was observed at 100 mg/ml than 50 mg/ml. Maximum inhibitory activity was observed in the fungal yeast *Candida albicans* (16 mm) at 100 mg/ml concentration. No zone of inhibition was recorded against other fungal isolates like *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and Negative DMSO control.

Sakthi *et al*. [14] screened the pharmacological activity of the ethanol and ethyl acetate extract of *Datura metel* and *Acalypha indica* for its antifungal activity against pathogenic fungi. Six different fungal isolates *viz*., *Candida albicans*, *Candida glabrata*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger* and *Penicillium chrysogenum* were tested for its antifungal activity. The collected leaf samples were powdered and the bioactive compounds were extracted by using ethanol and ethyl acetate in a Soxhlet extractor. The antifungal activity was determined by using Well diffusion method. Ethanol and ethyl acetate extracts with different concentrations (100 mg/ml, 200 mg/ml and 300 mg/ml) were mixed with 1 ml of Dimethyl sulfoxide (DMSO) and added into the well. The inhibitory effect of ethanol extract was relatively high when compared to ethyl acetate extract. The extract of *Datura metel* showed maximum zone of inhibition against fungal pathogens when compared to *Acalypha indica*.

Sekar *et al*. [15] screened the pharmacological activity of the ethanol and acetone extract of *Phyllanthus amarus, Acalypha* and *indica Datura metel* for its antimicrobial activity against selected pathogen. The antimicrobial activity was determined by using Disc diffusion method. Ethanol and acetone extracts with different concentrations (100 mg/ml,

200 mg/ml and 300 mg/ml) were mixed with 1 ml of Dimethyl sulfoxide (DMSO). The inhibitory effect of ethanol extract was relatively high when compared to acetone extract. The study of antimicrobial activity of herbal plant extract of *Datura metel*, *Acalypha indica* and *Phyllanthus amarus* showed that the ethanol extract shows promising antimicrobial activity against bacterial and fungal human pathogens when compared to acetone extract.

Table 3: Antifungal activity of Methanol extract of *Plectranthus amboinicus*

Fungal strains	Concentration of the Methanolic extract and Zone of inhibition (mm in dm)	
	50 mg/ml	100 mg/ml
Candida albicans	35 mm	40 mm
Aspergillus niger	NZ	NZ
Aspergillus fumigatus	NZ	NZ
Aspergillus flavus	NZ	NZ

*NZ – No zone of inhibition

Table 4: Antifungal activity of Chloroform extract of *Plectranthus amboinicus*

Fungal strains	Concentration of the Chloroform extract and Zone of inhibition (mm in dm)	
	50	100
Candida albicans	14	16
Aspergillus niger	NZ	NZ
Aspergillus fumigatus	NZ	NZ
Aspergillus flavus	NZ	NZ

*NZ – No zone of inhibition

4. CONCLUSION

The present research concluded that the methanol extract of *Plectranthus amboinicus* showed the maximum antibacterial activity against the bacteria *Klebsiella pneumoniae* and fungi *Candida albicans.* The antimicrobial activity of *Plectranthus amboinicus* was more at 100 mg/ml concentration when compared to 50 mg/ml concentration. Comparatively, the Methanol extract of *Plectranthus amboinicus* exhibited maximum antimicrobial activity when compared to Chloroform extract. The *Plectranthus amboinicus* has showed good antimicrobial activity against most of the bacteria and only one fungal yeast *Candida albicans*. No zone of inhibition was recorded against other fungal isolates like *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and Negative DMSO control.

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