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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF BARK EXTRACT OF HARPULLIA ARBOREA (BLANCO) RADLK AGAINST FOOD ISOLATES

Botany

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ABSTRACT

The aim of this study was to evaluate the antibacterial activity of Harpullia arborea extracts against Gram positive and Gram-negative bacterial species. The antimicrobial activity was determined in the extracts using agar disc diffusion method. Among the both solvents extracts, methanol extracts showed good inhibitory activity against gram positive and negative isolates, zone of ranged from 10 mm to 18mm. The phytochemical analyses of the plants were carried out. The microbial activity of the Harpullia arborea was due to the presence of various secondary metabolites. This plant extracts which proved to be potentially effective can be used as natural alternative preventives to control food isolates.

KEYWORDS

Harpullia arborea, methanol and chloroform extracts, phytochemicals, Antimicrobial activity.

Introduction

Antibiotic resistance is a global public health problem in world especially in developing countries. Even though production of new antibacterial compounds by the pharmaceutical companies had been increased in the last decades, the resistance of the microbial pathogens to these drugs was also increased [1]. This resistance is developed due to overuse and inappropriate use of drug and this phenomenon was greater threat in poorer nations than in richer ones.

According to Infectious Diseases Society of America report, MRSA, VRE, ESBL-EP, ESBL-KP and Pseudomonas aeruginosa as terrible pathogens and also not easily destroyed. These multidrug resistance bacterial strains is increasingly limiting the effectiveness of current drugs and significantly causing treatment failure of infections. In this situation, urgently, we need alternative medicine to eradicate the microbial pathogens [4].In accordance with World Health Organization (WHO), medicinal herbs could be an important resource for developing new antimicrobial drugs [6]. It has estimate that 60 to 90% of peoples are utilized the plants for primary health care in developing countries. Plants play an important role in developing modern medicines as they contain active phytochemical components. These components were proven to be a remedy for a number of human diseases [9].

Harpullia arborea is a tree belonging to the Sapindaceae that reaches a size of up to 15 meters high. It is found in Indomalasia and Australia and in the Western Ghats. The barks, fruits and seeds of *Harpullia arborea* are traditionally used by the ethnic communities of Cannore (Kerala) as leech repellent, hair wash and excellent source of an antirheumatic activity [2]. In previous study, methanol extract of *Harpullia arborea* showed inhibitory activity against clinical isolates of *Bacillus subtilis, Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli* and *Proteus vulgaris* [13]. According to previous study, few reports only showed the bark extract of *Harpullia arborea* against to clinical isolates.

Therefore, present study was designed to investigate the antimicrobial potential of bark extract of *Harpullia arborea* against MDR isolates.

Materials and methods

Collection of plant

The Bark of *Harpullia arborea* were collected from Gedamalai, Namakkal district, Tamil nadu and were shade dried, powdered and extracted in soxhlet apparatus successively with methanol and chloroform solvents. The extracts were stored at 4º C for phytochemicals screening and antimicrobial analysis.

Plant identification:

The study plant was identified with the help standard flora such as "Flora of presidency of Madras" (J.S. Gamble, 1915) [3]. The study plant was also identified with the help of "The flora of the Tamilnadu, Carnatic India"(K .M .Mathew, 1988) [10], Botanical Survey of India, Coimbatore (BSI).

Phytochemicals Screening of bark extract

The presence of various phytochemical compounds in the Bark of *Harpullia arborea* was confirmed by using the methods [14].

Fluorescence Analysis:

The fluorescence analysis of powdered crude drug may serves to evaluate the purity, when the powdered plant parts were treated with various acids and alkalis like Distilled water, Acetone, Ethanol, Chloroform, Diethyl acetate, Methanol, Petroleum ether, HCL, H^2SO_4, 5% $FeCl_3$, Nitric acid and 1N NaoH and observed under day light as well as in UV light and the characters exhibited were noted. The fluorescence analysis will serve to conform the given plant material even is powdered form [7].

Microorganisms

The Multidrug resistance isolates of *Escherichia coli, Staphylococcus aureus, Vibrio spp and Proteus vulgaris, Klebsiella pneumonia, Enterococcus spp and Pseudomonas aeruginosa were procured* from Microtech, Microbiology Laboratory, Coimbatore and used for the study.

Determination antibacterial activity of plant extract

This test was carried out according to the methods [5]. The plates were inoculated with freshly prepared over night inoculums which were swabbed over the entire surface of the medium, rotating the plate 60 degrees after each application by using a sterile cotton swab, to ensure the spread of the tested microbes on the surface of the plate completely. Inoculums were 10^8 CFU/ml of bacteria. The 6mm diameter of well was made with borer on the agar plates. Different concentration of plant extract was filled in well with the help of micropipette. Ampicillin (10µg/ml) was used as positive control and 100µl of DMSO was added in to well, which was negative control.

Results

The results of phytochemical screening of *Harpullia arborea* showed the presence of various phytochemicals (Table 1). Two solvents such as chloroform and methanol showed positive results for the presence of Alkaloids, Carbohydrate, Flavonoids, Tannins and Terpenoids. In addition, the methanol extracts showed the presence of Phenols and Saponins. The Sterols and proteins are absent in both of the extracts and methanol extract had highest number of phytochemicals.

The results of fluorescence analysis of powdered crude drug may serves to evaluate the purity, when the powdered plant parts were treated with various acids and alkalis like Distilled water, Acetone, Ethanol, Chloroform, Diethyl acetate, Methanol, Petroleum ether, HCL, H_2SO_4, 5% $FeCl_3$, Nitric acid and 1N NaoH and observed under day light as well as in UV light and the characters exhibited were

noted. The fluorescence analysis will serve to conform the given plant material. (Table.2).

Results of antibacterial activities of the chloroform and methanol extracts of *Harpullia arborea* were shown in Tables 3 and 4 respectively. The results showed that both the non-polar (chloroform) and polar (methanol) solvent extracts were active against 6 bacterial genera. In case of methanol extract, the highest zone of inhibitory were observed against *Proteus spp* and *Pseudomonas aeruginosa*, the zones of inhibition were ranging from 10 to 18 mm in diameter. Among the 6 types of genera, *Enterococcus spp* was resistance to methanol extract, 12.5mg of extract showed 10mm of zone of inhibition. (Figure.1 & 2)

In case of chloroform extract, the highest zone of inhibition observed against to *Vibrio spp*, *S.aureus* and *E.coli* and the zone of inhibition ranged from 8 to 14mm. The inhibitory activity of the methanol extract of *Harpullia arborea* showed highest antimicrobial activity than chloroform extract. In this extract both extracts are not able to inhibit the Enterococcus spp.

Discussion

Antimicrobial resistance has always been a global health concern challenging treatment of human infections caused by MDR-bacterial pathogens. Among them MRSA, VRE and ESBL's isolates are not easily eradicate [11]. Therefore, there is a need to look for substances from other sources with proven antimicrobial activity. Among the potential sources of new agents, plants have long been investigated. Because, they contain many bioactive compounds that can be interest in therapeutic. In this way our goal was inhibition of MDR isolates by two extracts of *Harpullia arborea*.

In the present study, a variety of MDR Gram-positive and Gram-negative bacteria were used in screening antimicrobial activity of methanol and chloroform extracts of *Harpullia arborea*. In this study, the 12.5mg/mL of extracts was the most effective on all pathogens and the methanol extract exhibited a stronger antibacterial activity against both Gram-negative than Gram-positive bacteria. These results are in agreement with previous findings from previous study [13]. They were also observed the highest antimicrobial activity from methanol extract. In this study, all isolates were suppressed by both extract except Enterococcus spp. [11]. In observed the antimicrobial activity of bark extract of *Harpullia arborea* against four bacterial genera. In their study, methanol extract not inhibited the *Enterococcus spp* and *S.aureus*. Successful determination of such biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure [12].

Plants contain a variety of important secondary metabolites as tannins, alkaloids and flavonoids, which possess *in vitro* antimicrobial properties [8]. It is well known that the antimicrobial property of *Harpullia arborea* extracts is attributed to the different phytochemical constituents [13]. Investigated the phytochemical constituents of *Harpullia arborea* extracts and revealed the presence of Glycosides, Steroids, saponin and Resins. In this present studies, Alkaloids, Carbohydrate, Flavonoids, Tannins and Terpenoids were observed from both extracts. These extracts consist of chemicals and are usually considered to play a role of antibacterial activity against pathogenic microorganisms.

According to previous literature, this is the first study investigating the antibacterial activity of *Harpullia arborea* (Bark) extracts against these MDR pathogens. These emerging pathogens become a significant health problem because of resistance to multiple antimicrobial classes and to survive in environments.

Our studies have suggested that the plant *Harpullia arborea* is found potential antimicrobial agent against gram positive and gram negative drug resistant isolates at 12.5 mg/ml concentration. Further studies are therefore suggested to ascertain their antimicrobial, anti ulcerative and anti helminthic activities. Furthermore, necessary to investigate and standardize the inhibitory effect of *Harpullia arborea* extracts against these emerging pathogens.

Table. 1 Priliminary Phytochemical Screening of Chloroform and Methanol Extracts of *Harpullia arborea* (Bark Extract).

S.No	Phytochemicals test	Chloroform Extract	Methanol Extract
1.	Alkaloids	+	+
2.	Carbohydrate	+	+
3.	Flavoniods	+	+
4.	Phenols	-	+
5.	Saponins	-	+
6.	Tannin	+	+
7.	Terpenoids	+	+
8.	Quinon	+	-
9.	Sterols	-	-
10.	Proteins	-	-

"+"= Positive
"-"= Negative

Table. 2 Fluorescence analysis of *Harpulliaarborea*.(Bark Powder)

S.No	Solvents	Visible	UV light
1.	Distilled water	Light brown	Light brown
2.	Acetone	Cream colour	Cream colour
3.	Ethanol	Light yellow	Brown
4.	Chloroform	Cream clour	Light yellow
5.	Diethylacetate	Light brown	Brown
6.	Methanol	Light brown	Brown
7.	Petroleum ether	Brown	Dark brown
8.	HCL	Cream clour	Dark brown
9.	H2SO4	Dark red	Dark red
10.	5% FeCl3	Cream	Brown
11.	Nitric acid	Orange	Orange
12.	1N NaoH	Brown	Brown

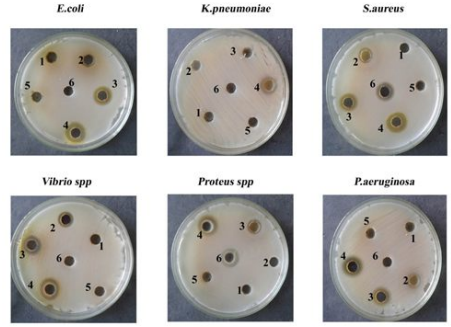
Table.3 Antimicrobial Activity of Chloroform extract of *Harpullia arborea* (Bark Extract)

S.No	Isolates	Con. Of plant extract (mg) Zone of inhibition (mm)					
		5	7.5	10	12.5	Control	Ampi
1.	*E.coli*	-	8	11	14	-	-
2.	*K.pneumoniae*	-	-	-	10	-	-
3.	*S.aureus*	-	8	11	14	-	11
4.	*Vibrio spp*	-	9	11	14	-	-
5.	*Proteus spp*	-	-	8	12	-	12
6.	*P.aeruginosa*	-	8	11	13	-	-

Table.4 Antimicrobial activity of Methanol extract of *Harpullia arborea* (Bark Extract)

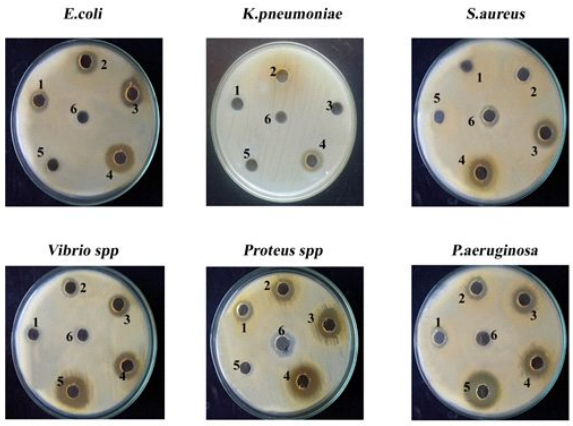
S.No	Isolates	Con. Of plant extract (mg) Zone of inhibition (mm)					
		5	**7.5**	**10**	**12.5**	**Control**	**Ampi**
1.	*E.coli*	8	10	13	16	-	-
2.	*K.pneumoniae*	-	-	-	10	-	-
3.	*S.aureus*	-	-	10	14	-	11
4.	*Vibrio spp*	8	11	14	17	-	-
5.	*Proteus spp*	10	12	15	18	-	12
6.	*P.aeruginosa*	10	12	15	18	-	-

Figure 1 Antimicrobial Activity of Chloroform extract of *Harpullia arborea*



1. 5mg, 2.7.5mg, 3.10mg, 4.12.5mg, 5. DMSO, 6. Ampicilin (10µg)

Figure 2 Antimicrobial activity of Methanol extract of *Harpullia arborea*



1. 5mg, 2.7.5mg, 3.10mg, 4.12.5mg, 5. DMSO, 6. Ampicilin (10µg)

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