Investigating the Phytochemicals and Antimicrobial Activities of Bark Extract of Harpullia arborea (Blanco) Radlk against Food Isolates

Rajeswari R[1] and Dr. Murugesh S[2*]

[1]*Ph.D., Research scholar, Department of Botany, School of Life Sciences, Periyar University, Periyar Palkalai Nagar, Salem-636 011, Tamil Nadu, India.*

Professor & Head (Retd), Department of Botany, School of Life Sciences, Periyar University, Periyar Palkalai Nagar, Salem-636 011, Tamil Nadu, India.

Abstract:- *The aim of this study was to determine the phytochemical constituents and antimicrobial potential of Harpullia arborea (Blanco) Radlk. (Sapindaceae) bark against food isolates. The secondary metabolites present in Harpullia arborea were found to be carbohydrates, phenolics, saponins, tannins, steroids, alkaloids, flavonoids, quinones and terpenoids. The antimicrobial activity was determined in the bark extracts using agar disc diffusion method. Among the 2 solvents extracts, methanol extracts showed good inhibitory activity, than chloroform extract. Both bark extracts have demonstrated a substantial antibacterial activity towards Gram negative than Gram positive bacteria. 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, antibacterial activity, Agar well diffusion.*

*Corresponding author: Murugesh S, *Department of Botany, School of Life Sciences, Periyar University, Salem.*

INTRODUCTION

One of the main problems in developing countries is antimicrobial resistance. The increase in the prevalence of improper use of antibiotics is unreasonable. This is mainly due to the increase in the availability and abuse of drugs, which has led to the highest level of antibiotic resistance [1]. All over the world, India is one of the top people in the spread of infectious diseases. Recent reports show that the inappropriate and abnormal use of antibiotics for these diseases is the main reason for the increase in antimicrobial resistance. [2]. Another report showed that about 70,000 people failing to fight to antimicrobial resistance per year and around 10 million are expected to die from it by 2050 [3].

On a global scale, due to the increase in multiple drug-resistant pathogens, they have become more symbolic and considered huge trouble. New research recommended the seriousness of elucidating the antibiotic use to reduce antibiotic resistance in India [4]. Currently a number of scientists have identified the antibacterial activity of many plant extracts against antibiotic resistance isolates. Thus plant extracts can be explored for the treatment of microbial infections [5]. One such plant used in this research is *Harpullia arborea.*

Harpullia arborea is an evergreen shrub to a large tree with a dense, conical crown; it can grow up to 35 m tall but is usually smaller. The straight, cylindrical bole can be 60 -70 cm in diameter and is free of branches for most of its length; it is slightly fluted or with small buttresses up to 3 m high [6]. Till today various parts of *H. arborea* are used as traditional medicine among various tribal

communities of India such as Tamilnadu, Kerala and Maharastra as leech repellent, antihelmintic, appetizer and a cure to treat digestive disorders [7&8].

Phytochemicals *viz.*, glycosides, steroids, saponins, resins terpenoids, polyphenols, flavonoids and anthraquinones were detected in seeds leaves and stems of *H. arborea* exhibiting anti-oxidant properties. They also reported that seed leaves and stem extracts exhibiting antibacterial and antimalarial activity [9&10]. Poovapatthanachart and Thanakijcharoenpath, 2008 [11] reported a new norhopane from leaves of *H. arborea.*

Although the preparations of *H.arborea* have been cited in the literature and have a variety of medical properties since ancient times, relatively speaking, there is less research on it and little scientific information about its therapeutic use. Therefore, there is a huge necessity to scientifically explore and prove its medicinal value, thereby inspiring its therapeutic application and further clinical trials. Hence the aim of this study is to present novel results about the isolation and chemical characterization of phytochemicals from the bark extract of *H.arborea* and investigate the in vitro ability of methanol and chloroform extracts from the bark of *H.arborea* to potentates the activity against ESBL producing bacterial isolates.

Methodology

Sample collection

Fresh mature healthy barks of *Harpullia arborea* were collected during the month between April 2017 from Gedamalai, Namakkal district Southern eastern Ghats, India and It is lying on the in-between 11°54' 13" North latitude and 78° 26' 74" East longitude of Eastern Ghats.

Extraction of plant (Khandelwal, 2002) [12]

The dry powdered plant bark samples of *Harpullia arborea* were extracted individually with methanol and chloroform for the determination of extractive values. The extraction was carryout with maceration method. The 5 gm of coarsely powdered plant material was weighed and transferred to a dried 250 ml conical flask containing 100 ml of the solvents separately. The flasks were corked and incubated for 24 hrs at room temperature with frequent agitation for the first 6 hrs and left undisturbed for the next 18 hrs. The extracts were filtered through Whatman No. 1 filter paper and 25 ml of the filtrate was evaporated to dryness in a tarred flat bottom shallow dish at 105°C to a constant weight and weighed. The extractive value in percentage was calculated as follows:

$$\text{Extractive value} = \frac{\text{Wt. of the dried extract}}{\text{Wt. of the plant material}} \times 100$$

Sample extraction for phytochemicals (Sreelatha and Padma, 2009)

The 20 gm of finely powdered sample was filled in the thimble of the extraction apparatus and dropped into the soxhlet tube and extracted for 8 hrs overheat. Extraction was carried out individually with 250ml of methanol and chloroform. The extract (condensed vapour) obtained was subsequently concentrated and dried using Rotary vacuum evaporator (Equitron, India) at 60°C under reduced pressure. The solvent was distilled off and then the dried crude residues were aseptically weighed and redissolved in DMSO and stored at -20°C in a sterile, labeled, airtight container until further analysis [13].

Qualitative analysis of phytochemicals

The bark extract of *H.arborea* were tested for the presence alkaloids, carbohydrates, flavonoids, phenolics, saponins, tannins, quinones, steroids and proteins as per the method described by Harborne (1998) [14]and Kokate (2005) [15].

Bacterial pathogens

Sample collection and isolation of bacteria

A total of 5 samples comprising of goat meat and chicken meat were collected from the plastic container and transported to the laboratory for identification of bacterial isolates. One gram of samples was transferred to a conical flask containing peptone water and incubated for 30 min at 80 rpm at room temperature in a rotator. After incubation, samples from peptone water were streaked into selective media and chromogenic agar media. All the plates were incubated aerobically at 37 °C for 24 h. All isolates were identified by following standard microbiological techniques which include studies of colony morphology and staining reactions. Pure isolates were identified by performing the standard biochemical tests (IMVIC and sugar fermentation test) [16]. All bacterial isolates were maintained on nutrient agar slants at temperature of 4 ºC.

Determination of antibiotic susceptibility test

Antibiotic susceptibility screening was done for the 14 bacterial isolates as per the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS, 1997). Kirby- Bauer's disc diffusion technique was adapted for an antibiogram. All food isolates were selected and subjected to the antibiogram test. After the incubation period the zone of inhibition size was observed and compared with the standard chart [17].

Result and discussion

The extractive value or extraction yield was used as an indicator to demonstrate how effective the extraction was and based on the findings we can further fine-tune the extraction conditions like temperature, time, and solvents [18]. The current study evaluated two different solvents *viz.*, methanol, and chloroform and extraction yield have relatively increased with respect to the polarity of solvents. The maximum yield was noticed in methanol (84.2 mg/5gm) than in chloroform (62.8 mg/5gm). Possibly, this can be attributed due to the increased polarity of methanol than chloroform and at elevated extraction temperature; methanol has more dielectric constants than that of the organic solvent, chloroform [19].

The results of the qualitative analysis of phytochemicals of all solvents bark extract of *Harpullia arborea* are given in Table 1. The results revealed that methanol bark extract of *Harpullia arborea* demonstrated positive reactions for carbohydrates, phenolics, saponins, tannins, steroids, and terpenoids and revealed negative reactions for alkaloids, flavonoids, quinones and proteins. Whereas the chloroform bark extract demonstrated positive reaction for alkaloids, carbohydrates, flavonoids, tannins, quinines, and terpenoids and negative reactions for phenolics, saponins, steroids, and proteins.

Table 1.Preliminary phytochemicals screening on bark extracts of *Harpullia arborea*

S.no	Phytochemicals	Test Name	Observation	Methanol	Chloroform
1	Alkaloids	Wagner's	Reddish brown colour	-	+
2	Carbohydrates	Molisch's	Purple ring at the junction	+	+
3	Flavonoids	With NaOH	Yellow colour	-	+
4	Phenolic compounds	Ferric chloride	Deep chloride	+	-
5	Saponins	Foam test	Froathing	+	-
6	Tannins	Braymer's test	Greenish colour	+	+
7	Quinones	With HCl	Yellow precipitate	-	+
8	Steroids	Salkowski test	Bluish red	+	-
9	Terpenoids	Salkowski test	Reddish brown	+	+
10	Proteins	Millon's test	Red color	-	-

Tannins present in both the plant extracts are considered to be cardio-protective, anti-inflammatory, anti-carcinogenic and anti-mutagenic, among others. Tannins enhance glucose uptake and inhibit adipogenesis, thus being potential drugs for the treatment of noninsulin-dependent diabetes mellitus (NIDD) [20]. In recently 2019, Sharmila *et al* [21] also observed the tannins and terpenoids from fruiting of *Harpullia arborea.*

A total of 14 bacteria were isolated and identified from the 10 samples of fresh meat collected from the local market. The isolates were identified up to species level and all these 14 isolates were grouped under 6 genera. The gram-negative isolates were identified as *Escherichia coli* accounting to 21.43% and *Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Proteus mirabilis* each accounting to 14.29%. The gram-positive isolates were identified as *Staphylococcus aureus* and *Enterococcus faecalis* and accounting for 21.43 and 14.29% respectively.

All isolates were subjected to an antibacterial susceptibility tests against 12 antibiotics. Among the 6 genera, the highest antibiotic resistance was observed on *P. aeruginosa* (54.1%), followed by *S.aureus* (50%) and the lowest resistance was observed in *K. pneumonia* (33.5%). In the case of antibiotic-wise, kanamycin (78.5%) showed highest resistance against all isolates, and ceftazidime, cefotaxime, ceftriaxone and norfloxacin. Presently, 28.5% of isolates were resistant to more than 50% of antibiotics. This states the role of raw food as a reservoir of antibiotic resistant bacteria that can be transferred to humans thereby causing gastrointestinal disorders and food-borne illness which can be life-threatening.

In this study, most of the isolates had higher resistance and these isolates were not to easily eradicate, therefore, urgently we need to discover a new way to reduce the problem and develop the research for new drugs natural. Among the potential sources of new agents, plants have long been

investigated. Because, plants contain many bioactive compounds, that can be interested in the therapeutic activity. In this way, our goal was inhibition of food isolates by the bark extract of *Harpullia arborea*.

In the present study, *Harpullia arborea* bark extracts were individually studied against those 6 bacterial isolates that have demonstrated greater resistivity activity. The chloroform bark extract of *H. arborea* exhibited antibacterial activity against all the test pathogens. Plant extract at 5 mg concentration was active against only *P. mirabilis*, whereas 12.5 mg concentration demonstrated antibacterial activity for all the 6 isolates (Fig.1).

The highest zone of inhibition was recorded against *S. aureus* and *P. mirabilis* (17 and 16 mm respectively) with maximum antibacterial activity, whereas the least activity was demonstrated against *K. pneumoniae* (10 mm) at 12.5 mg concentration and for *E. coli* (14mm) at 12.5 mg concentration. Thus chloroform bark extract has demonstrated substantial antibacterial activity towards Gram-negative than Gram-positive bacteria. Of the 6 isolates tested 3 isolates *viz.*, *E. coli*, *P. aeruginosa* and *E.faecalis* demonstrated a MIC of 7.5mg, *P. mirabils* 2.5 mg, and *K. pneumonia* recorded MIC of 12.5 mg. The lowest MIC was recorded against *S.aurues* (5mg).

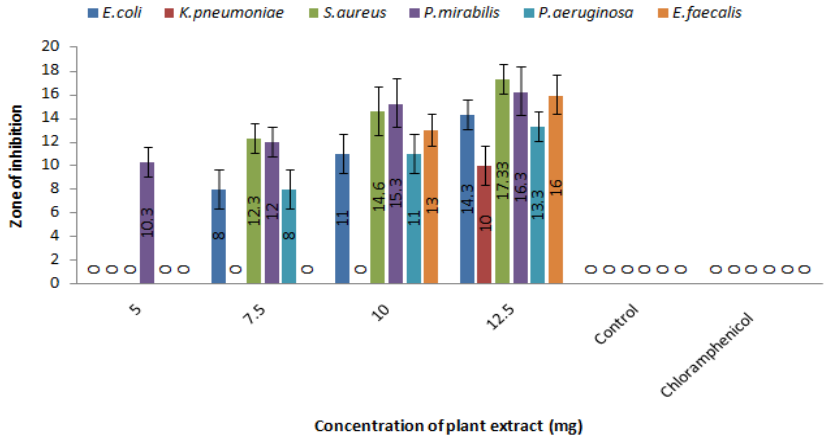

Fig.1 Antibacterial activity of chloroform extract of *H. arborea* against food isolates

The methanol bark extract of *H. arborea*, likewise chloroform exhibited antibacterial activity against all the test pathogens. Plant extract at 5 and 7.5 mg concentration showed no antibacterial activity for *K. pneumonia*, *P.mirabilis* and *S. aureus*. Among the various concentrations, 12.5 mg showed activity against all the 6 isolates. The highest zone of inhibition was recorded against *P. aeruginosa* (18 mm) with maximum antibacterial activity, whereas least activity was demonstrated against *K.pneumoniae* (14mm) at 12.5 mg concentration. Thus similar to chloroform, methanol bark extract has demonstrated substantial antibacterial activity towards Gram negative than Gram positive bacteria (Fig.2). Of the 6 isolates tested 3 isolates *viz.*, *P. aeruginosa* and *E.faecalis* sp demonstrated a MIC of 5 mg, *S. aureus* recorded MIC of 7.5mg and 2.5mg for *E.coli.*

Several studies have demonstrated the antimicrobial activity of *Harpullia* sp. Khan *et al.* [22] reported the antibacterial effect of *H. ramiflora* and their research study concluded that the ethyl acetate fraction of the flower exhibited the highest activity. A study by Chung *et al.* [23] demonstrated that among the various microbes examined only *S. aureus* was sensitive to bark extract while other bacteria were not inhibited by bark as well as leaf extract of *H. arborea*. According to literature, this is the first investigating the antibacterial activity of *H.arborea* (Bark) extracts against these drug resistance isolates.

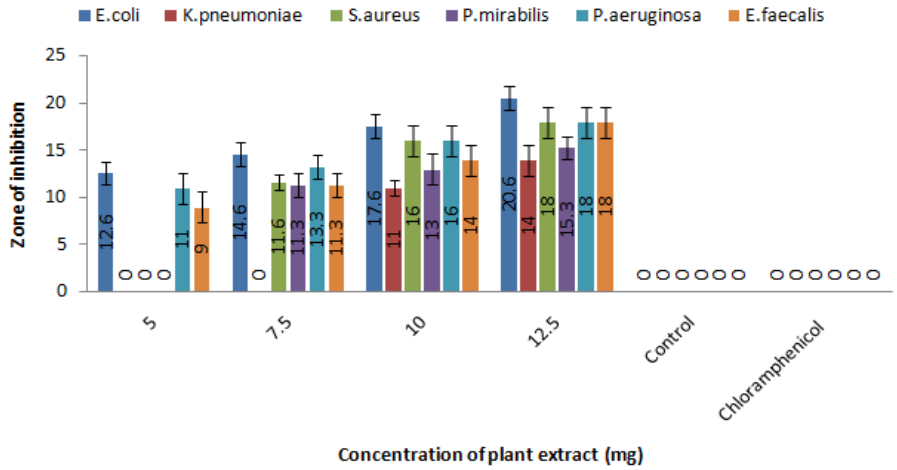

Fig.2 Antibacterial activity of methanol extract of *H. arborea* against food isolates

Among the 2 solvents, methanol showed the highest inhibitory activity than chloroform extract. Because methanol solvent is a high polar compound and shows very little difference in their extractive abilities. A similar line of results was observed by an earlier study by Gowri and Vasantha [9].

With the present findings it can be concluded that the bark extract of *Harpullia arborea* has antimicrobial activities against food isolates. The presence of various bioactive phytoconstituents identified in this study and its synergistic activity would be of greater therapeutic benefits in the management of diseases. However further studies shall be conducted in isolating and purifying the crude phytochemicals obtained in the study into an active ingredient and perform a clinical study in the human population thereby further developing into an active pharmaceutical formulation.

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