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Original Article

ANTIRADICAL, ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF *HARPULLIA ARBOREA* (BLANCO) RADLK. (SAPINDACEAE)

RAGHAVENDRA H. L.[1], PRASHITH KEKUDA T. R.[2*], KARTHIK K. N.[2], ANKITH G. N.[2]

[1]Department of Biochemistry, School of Medicine, Wollega University, P. O. Box: 395, Nekemte, Ethiopia, [2]Department of Microbiology, S. R. N. M. N. College of Applied Sciences, N. E. S. Campus, Balraj Urs Road, Shivamogga 577201, Karnataka, India
Email: p.kekuda@gmail.com

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ABSTRACT

Objective: The present study was conducted to investigate antiradical and antimicrobial potential of extract of *Harpullia arborea* (Blanco) Radlk. (Sapindaceae) leaves obtained by maceration process.

Methods: Antiradical activity of leaf extract was performed by DPPH and ABTS radical scavenging assays. Antibacterial activity of leaf extract was carried out by Agar well diffusion assay. Antifungal activity of leaf extract was carried out by Poisoned food technique.

Results: Leaf extract displayed concentration dependent scavenging of radicals with potent scavenging activity against ABTS radicals (IC_{50} value 4.26µg/ml) when compared to DPPH radicals (IC_{50} value 27.26µg/ml). Extract exhibited inhibitory activity against all test bacteria. Marked and least activity was observed against *Staphylococcus epidermidis* and *Escherichia coli* respectively. Considerable reduction in the mycelial growth of test fungi was observed in poisoned plates. *Curvularia* sp. and *Alternaria* sp. were inhibited to highest and least extent respectively.

Conclusion: In suitable form, the plant can be used to treat oxidative damage, infectious diseases caused by pathogenic bacteria and to manage seed-borne fungi.

Keywords: *Harpullia arborea*, Maceration, Agar well diffusion, Poisoned food technique, DPPH, ABTS, IC_{50}

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INTRODUCTION

Harpullia arborea (Blanco) Radlk. Belonging to the family Sapindaceae is a medium sized evergreen tree native to Indo-Malayan. Leaves are compound, alternate, usually even, pinnate, up to 40 cm long. Flowers are greenish yellow, in axillary or subterminal panicles. Sepal 5, ovate and tomentose. Petals 5, ovate and clawed. Stamens 5-8, inserted on the disc. Ovary ovoid, 2-celled; ovules 2 in each cell, superposed. Fruit is a inflated, coriaceous, 2-lobed, 2-celled, loculicidally 2-valved dehiscent capsule, orange-red in color with 2 black seeds. In Karnataka, the plant is distributed in Chikamagalur, Shimoga, Hassan, Kodagu, Dakshina Kannada and Uttara Kannada [1, 2]. Various parts of *H. arborea* are used traditionally by the ethnic communities of Kerala, India as leech repellent, hair wash and to treat rheumatism [3]. In Tamil Nadu, the seeds of *H. arborea* were sold as an anthelmintic and a formulation Perali ennai containing the fruit of *H. arborea*, used as an appertizer and to cure digestive problems, was by herbal vendors [4]. In Maharashtra, the bark is traditionally used as leech repellent [5]. The seeds of *H. arborea* were shown to possess glycosides, steroids, saponins and resins [3]. Terpenoids, polyphenols, flavonoids and anthraquinones were detected in leaves while terpenoids and flavonoids were detected in stem [6]. A new norhopane triterpenoid, 3beta-eicosanoyl-6beta-hydroxy-21alphaH-24-norhopan-4(23), 22(29)-diene, was isolated from leaves of *H. arborea* [7]. Solvent extracts of seeds were shown to possess antibacterial activity against Gram positive and Gram negative bacteria [3]. Leaves and stem extracts were shown to exhibit antimalarial activity against *Plasmodium falciparum* HRP2 [6]. The present study was carried out to investigate antiradical and antimicrobial activity of leaf of *H. arborea*.

MATERIALS AND METHODS

Collection and identification of plant material

The plant was collected near Shiralakoppa, Shivamogga district, Karnataka during February 2017 and identified by Dr. Vinayaka K. S, Assistant Professor, Department of Botany, KFGC, Shikaripura.

Extraction

The leaves were separated, washed using clean water, dried under shade and powdered. 10g of powdered leaf material was subjected to maceration process using methanol (100 ml) in a stoppered container. The powder was left in methanol for 48 h during which the container was stirred occasionally. The content was filtered through 4-fold muslin cloth followed by Whatman filter paper (No. 1). The filtrate was evaporated to dryness. The crude leaf extract obtained was stored in refrigerator [8].

Antibacterial activity of *H. arborea*

Agar well diffusion method was employed to investigate antibacterial activity of leaf extract against 7 bacteria (Gram positive bacteria-*Staphylococcus aureus* NCIM 5345, *Staphylococcus epidermidis* NCIM 2493, *Bacillus subtilis* NCIM 2063 and *Bacillus cereus* NCIM 2016; Gram negative bacteria-*Escherichia coli* NCIM 2065, *Pseudomonas aeruginosa* NCIM 2200 and *Salmonella typhimurium* NCIM 2501). The 24 h old Nutrient broth cultures of test bacteria were swab inoculated on sterile Nutrient agar plates and wells of 8 mm were created in the plates with the help of sterile cork-borer. The wells were labeled and filled with leaf extract (20 mg/ml of Dimethyl sulfoxide [DMSO]), reference antibiotic (Chloramphenicol, 1 mg/ml of sterile distilled water) and DMSO. The plates were incubated in upright position at 37 °C for 24 h and the zones of inhibition were measured [8, 9].

Antifungal activity of *H. arborea*

In order to evaluate the antifungal potential of leaf extract against three seed-borne fungi namely *Alternaria* sp., *Fusarium* sp. and *Curvularia* sp., we performed Poisoned food technique. Control (without extract) and poisoned Potato dextrose agar (1 mg extract/ml of medium) plates were inoculated with the well sporulated cultures of test fungi followed by incubating the plates in upright position for 5 d at room temperature. The diameter of fungal colonies was measured and extent of mycelial growth inhibition by extract was calculated using the formula:

Mycelial growth inhibition (%) = (C–T/C) x 100, where 'C' refers to colony diameter of test fungi on control plates and 'T' refers to colony diameter of test fungi in poisoned plates [8, 9].

DPPH radical scavenging activity of *H. arborea*

Various concentrations of leaf extract and ascorbic acid in 1 ml of methanol were mixed with 3 ml of DPPH radical solution (0.004% in methanol) in clean and dry tubes. The tubes were incubated for 30 min at room temperature in dark. The absorbance was read at 520 nm in a spectrophotometer. Radical scavenging potential of each concentration of leaf extract and ascorbic acid was determined using the formula:

Scavenging of DPPH radicals (%) = (Ac–At/Ac) x 100, where Ac and At represents the absorbance of DPPH control (1 ml methanol+3 ml DPPH radical solution) and absorbance of DPPH in presence of extract/ascorbic acid [8, 10].

ABTS radical scavenging activity of *H. arborea*

Various concentrations of leaf extract and ascorbic acid in 1 ml of methanol were mixed with 3 ml of ABTS radical solution (generated previously by mixing 7 mmol ABTS stock and 2.45 mmol potassium persulfate) in clean and dry tubes. The tubes were incubated for 30 min at room temperature in dark. The absorbance was read at 730 nm in a spectrophotometer. Radical scavenging potential of each concentration of leaf extract and ascorbic acid was determined using the formula:

Scavenging of ABTS radicals (%) = (Ac–At/Ac) x 100, where Ac and At represents the absorbance of ABTS control (1 ml methanol+3 ml DPPH radical solution) and absorbance of ABTS in presence of extract/ascorbic acid [8, 10].

RESULTS AND DISCUSSION

Antibacterial activity of *H. arborea*

Since discovery, antibiotics are considered as life-saving drugs as the use of antibiotics has saved millions of death due to infectious agents such as bacteria and fungi. However, the overuse and abuse of antibiotics results in the development of resistance in pathogenic microorganisms against the antibiotics. The resistant pathogens are of serious concern in both community and hospital settings as they are not susceptible to antibiotics and thereby cause marked morbidity and mortality. Plants have been considered as an excellent source of antimicrobials. Worldwide, many plants are used in the therapy of several diseases. Extracts and purified compounds from plants have shown to inhibit a wide range of pathogenic bacteria including antibiotic resistant strains [8, 9, 11-14]. Table 1 shows the result of antibacterial potential of leaf extract of *H. arborea*. All bacteria were susceptible to leaf extract but to a varied extent. Gram positive bacteria displayed high susceptibility to leaf extract when compared to Gram negative bacteria. *S. epidermidis* and *B. cereus* were susceptible to highest and least extent respectively among Gram positive bacteria. Among Gram negative bacteria, *P. aeruginosa* and *E. coli* were inhibited to highest and least extent respectively. Reference antibiotic caused high inhibition of test bacteria when compared to leaf extract. There was no inhibition caused by DMSO. In a study, the seed extract of *H. arborea* was shown to exhibit antibacterial activity in disk diffusion method [3]. In the study of Chung *et al.* [15], only *S. aureus* was sensitive to bark extract while other bacteria and *C. albicans* were not inhibited by bark as well as leaf extract of *H. arborea*. Khan *et al.* [16] reported antibacterial effect of various parts of *H. ramiflora*. Ethyl acetate fraction of flower exhibited highest activity. Solvent fractions such as dichloromethane, ethyl acetate and butanol of methanol extract of *H. petiolaris* leaves, stem and root barks and heartwoods exhibited antibacterial activity [17]. Two new benzeneacetic acid derivatives viz. Harpulliaside A and B, isolated from *H. pendula*, were shown to exhibit antibacterial activity with prominent activity against Gram positive bacteria [18].

Antifungal activity of *H. arborea*

Seed is an important input for production of majority of food crops. The use of good quality and disease free seeds results in desired germination and emergence of plants. Seeds often act as passive carriers of several fungi such as species of *Aspergillus, Fusarium, Helminthosporium, Curvularia, Alternaria, Epicoccum, Mucor, Rhizopus, Cercospora, Pyricularia* and *Rhizoctonia* and these fungi reduces seed viability, nutrients and cause diseases in seedlings and other stages of growth and result in yield losses. Management of seed-borne fungi is usually done with the use of synthetic chemicals. Interest in plants with antifungal activity against fungi from plant origin is intensified due to several drawbacks, such as high cost, environmental pollution and toxic effects on humans and other organisms, that are associated with the use of synthetic fungicides. Plant based agents are biodegradable, non-toxic and cheaper.

Many studies have shown the potential of botanicals to inhibit a range of seed-borne fungi [19-26]. In the present study, we evaluated the antifungal potential of leaf extract of *H. arborea* by Poisoned food technique. The result of antifungal potential of *H. arborea* is shown in table 2. All fungi were susceptible to extract as considerable reduction in the mycelial growth was observed in plates poisoned with leaf extract. The extract was found to inhibit the mycelial growth of all test fungi to >40%. Inhibition of *Curvularia* sp. was highest (55.5%) followed by *Fusarium* sp. (53.5%) and *Alternaria* sp. (42.3%). In an earlier study, the seed extract of *H. cupanioides* exhibited strong antifungal activity against phytopathogenic fungi viz. *Rhizoctonia solani*, *Curvularia lunata*, *Colletotrichum musae* and *Alternaria alternata* [27].

Table 1: Antibacterial activity of leaf extract of *H. arborea*

Test bacteria	Zone of inhibition in cm		
	Leaf extract	***Antibiotic***	**DMSO**
S. aureus	1.7	3.2	00.00
S. epidermidis	2.0	3.4	00.00
B. subtilis	1.8	3.5	00.00
B. cereus	1.6	3.4	00.00
P. aeruginosa	1.4	2.9	00.00
E. coli	1.1	2.4	00.00
S. typhimurium	1.3	2.8	00.00

Table 2: Antifungal activity of leaf extract of *H. arborea*

Test fungi	Colony diameter in cm	
	Control	***H. arborea***
Curvularia sp.	4.5	2.0
Alternaria sp.	5.2	3.0
Fusarium sp.	4.3	2.0

Antiradical activity of *H. arborea*

A free radical is any molecular species which contains an unpaired electron in an atomic orbital and the presence of this unpaired electron is responsible for sharing certain common properties by most radicals. These radicals are generated during normal metabolism and exposure to environmental factors such as pollution and radiation. The most important oxygen-containing free radicals that are implicated in many disease states (such as cancer, ageing, cardiovascular diseases and neurodegenerative diseases) are hydroxyl radical, superoxide anion radical and nitric oxide radical and peroxy nitrite radical. The free radicals are unstable, highly reactive and are known to damage biomolecules such as proteins, lipids and nucleic acids. Antioxidants are substances which can neutralize and decrease the deleterious effects caused by free radicals and other non-radical species. Interest in botanicals with antioxidant activity has been intensified due to suspected negative effects that are associated with the use of synthetic antioxidants. Plants are shown to be potent sources of natural antioxidants and many of the plant metabolites especially polyphenolic compounds are known to act as potent antioxidants [28-34].

The method of scavenging of DPPH radical (stable, organic, nitrogen centered free radical) is extensively used for determining the radical scavenging potential of various kinds of samples including plant extracts. The method is popular in being simple, rapid, and cheaper and the results obtained are reproducible. In this method, the substances (antioxidants) having the potential to donate proton causes decolorization of purple colored DPPH radical to yellow colored non-radical DPPHH [8, 9, 30, 35-38]. Fig. 1 shows the result of scavenging potential of leaf extract of *H. arborea* against DPPH radicals. The leaf extract was found to scavenge radical's dose dependently with an IC_{50} value of 27.26µg/ml. A scavenging of>50% and higher was observed at extract concentration 50µg/ml and higher. At 100µg/ml, a scavenging activity of 66.66% of radicals was observed. Ascorbic acid scavenged radicals more efficiently with an IC_{50} value 3.06µg/ml when compared to leaf extract. In an earlier study, Moustafa *et al.* [39] observed DPPH radical scavenging potential of two *Harpullia* species viz. *H. cupanioides* and *H. pendula*. Although, leaf extract scavenged DPPH radicals to lesser extent when compared to ascorbic acid, it is evident that the leaf extract of *H. arborea* possess hydrogen donating potential and thereby it can act as a free radical scavenger.

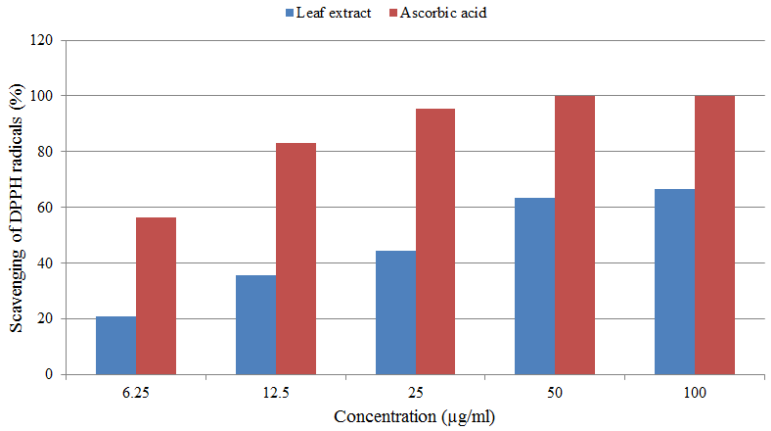

Fig. 1: Scavenging of DPPH radicals by leaf extract of *H. arborea* and ascorbic acid

Like DPPH assay, the assay involving scavenging of ABTS radicals is another simple, rapid and popular *in vitro* antiradical assay. In this assay, ABTS salt is oxidized to its radical cation which is intensely colored and the antiradical activity is measured as the ability of substances to decrease the color. In this assay, substances having electron donating potential converts blue-green colored ABTS radicals to colorless neutral form which is evidenced by suppression of its characteristic long wave absorption spectrum. ABTS radicals scavenging assay is widely used to evaluate antiradical activity of plants [8, 10, 40-43]. The result of scavenging potential of leaf extract against ABTS radicals is shown in fig. 2. The leaf extract was effective in scavenging ABTS radical's dose dependently with an IC_{50} value of 4.26µg/ml. A scavenging of>50% and higher was observed at all extract concentrations. Ascorbic acid was found to scavenge ABTS radicals more efficiently with an IC_{50} value 2.48µg/ml when compared to leaf extract. It is clear from the result that the leaf extract possess electron donating potential and hence, it can act as a free radical scavenger.

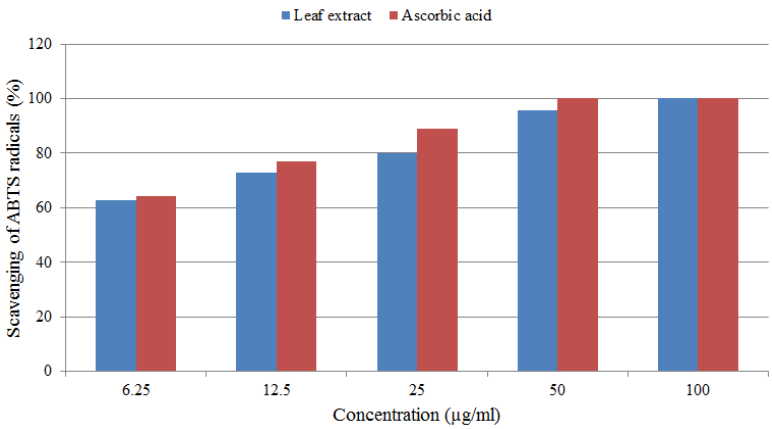

Fig. 2: Scavenging of ABTS radicals by leaf extract of *H. arborea* and ascorbic acid

CONCLUSION

The leaf extract of *H. arborea* was shown to possess marked antimicrobial and antiradical potential. In suitable form, the plant can be used to treat infectious diseases and oxidative damage and manage seed-borne fungal diseases. Further studies on purification of active principles from the extract and determination of their potent biological roles are to be carried out.

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CONFLICT OF INTERESTS

Declare none

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