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CONTENTS

Studies on the *In vitro* antioxidant, antimicrobial and phytochemical studies of *Helicteres isora* leaves

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ABSTRACT

The medicinal plant like *Helicteres isora* L. (Family: Sterculiaceae) is used for the treatment of various diseases like communicable and non-communicable diseases. The plant parts such as root, stem, leaves, fruit, and bark were used for the treatment of Diabetes, Orthopedic, Arthritis, Cardiovascular and communicable diseases like Dysentery, Asthma, and Antidot etc. These plant parts are also used for the treatment of the snake bite. The plant was collected from Thalakona, Chittoor district, Andhra Pradesh and were shade dried, powdered and extracted with ethanol and distilled water. The extracts were evaluated for the quantification of antioxidants and polyphenolics using *in vitro* assays. The effect of extracts determined for antioxidants, phenolics as well as antimicrobial activity. The DPPH scavenging effect revealed its radical reducing capacity and antimicrobial activity revealed its broad spectrum of antibiotic activity might be beneficial as stress relieving in addition to curing for infectious diseases.

The results of *H. isora* leaves of revealed that the total phenolics of ethyl alcohol and water extracts are 240 mg/ml and 275.5 mg/ml equivalents of gallic acid (standard) in the gram respectively. The present investigation revealed its antibacterial, antioxidant studies in addition to the quantification of phenolic substances might be useful in understanding the mechanism of curing ailments and justification for the traditional usages.

Keywords; *Helicteres isora*, Antioxidant, Polyphenolics and Antibacterial activity.

INTRODUCTION

Helicteres isora L. (Family: Sterculiaceae; common name: Indian screw tree) is a tree or shrub and its geographical distribution is Asian continent countries like Saudi Arabia, India, south china as well as Australia. The plant contains medicinal as well as nutrient values. The nutrients are proteins, calcium, iron, phosphorous, fiber, carbohydrates and lipids like vanillin, Gallic acid, p-Coumaric acid, caffeic acid. The phytochemicals are alkaloids, phytosterols, tannins, glycosides, flavonoids, phenols,

carotenoids. The plant roots consist of steroids are isocucurbitacin b, cucurbitacin b [1], other steroids are D-glucopyranosyl isorinic acid were isolated by Helistellarein, Helisorin, and Rosmarinic acid. It has been proved for the medicinal values, like antimicrobial, anticancer, antidiabetic and antioxidants [2].

Due metabolism escalation, the free radicals are released and should be reduced by the antioxidants, otherwise leads to several diseases. Its plant parts are bark, fruit, leaves, stem and root posses' high number of antioxidants, which has been proved to cure several diseases like cardiovascular, degenerative diseases. HIV type-1 potently inhibited by the fruit [3]. Its bark cures useful in, inflammatory, osteoarthritis, cardiovascular, kidney and gastrointestinal diseases [4]. The primary metabolites are lipids, proteins, nucleic acids, are oxidized by the free radicals, as a result it leads to several diseases. The antioxidants capture the free radicals and minimize the damage. In addition, the studies shown that microbes have become antibiotic resistance as well as side effects to the humans. The natural medicines were proved as nontoxic and safe [5]. Due to its antioxidant properties and presence of saponins and is used in cardiovascular and type-II diabetes [6, 7].

The natural drugs are safe and environmentally friendly, it has been proved that secondary metabolites and phytochemicals boost the immune system and it gives strength to fight against various diseases in humans. The phytochemicals like flavonoids, alkaloids, phenols, comarins, tannins and anthocyanin shown immunomodulating, anti-inflammatory, anti-tumor and antibacterial activities [8]. Secondary metabolites have been proved to cure urinary bladder stones, dysentery and diarrhea [9]. One of the triterpenes is diosgenin, an intermediate product in the anabolism of sex hormones is produced by the roots of the *H. isora* [10]. The parts like bark, roots and leaves have evidence as a treatment for skin infections like Scabies and eczema and also used for snake bite. The fruit has also been used for vermifuge, astringent, anti-dysenteric, gout, post-delivery health weakness, flatulence, to minimize pain in infants, stomachache, gastrointestinal infections and sores of ear. The seeds of the plant also have some values like dysentery. This plant has more secondary metabolites of different parts of the plant; due to this it has ethnomedicinal value. The fruit consist of chemicals such as p-coumaric acid, rosmarinic acid, caffeic acid. The plant bark consist of chemicals are 10 methyl dodeca-hydro-ethanophenanthrene, β-sitosterol. The root reported the phytochemicals such as gallic acid, cucurbitacin b, betulic acid, isorin, oleanolic acid. The leaves have p-coumaric acid, caffeic acid, gallic acid, vanillin [11].

The plant bark and roots used for the treatment of asthma, diabetes, emphysema, astringent, diarrhea, anti-galactagogue. The type-2 diabetes and hyperlipidemic, hypolipidimic activity. Insulin based disorders like cardiovascular, obesity, insulin resistance associated disorder (IRAD) and hypertension. It has been found evident that absorption of lipids and carbohydrates has been decreased by the intestine [12]. Based on the thorough review on the available literature, the leaves were used in the present investigation for the study of antioxidant, antimicrobial activities in addition to quantification of total phenols and antioxidants, which are not reported earlier.

MATERIALS AND METHODS

2.1. Plant material extract

The plant was collected from Thalakona, Chittoor district, Andhra Pradesh, India. The plant was identified and authorized by the Dr. L Md. Bhakshu, PVKN. Government College (A), Chittoor. The leaves were shade dried for two weeks and made into powder using a grinder. One gram of plant powder soaked in the 10 ml of ethyl alcohol and distilled water separately and heated using a water bath until the solvent come to 5 ml and the extracts were filtered and stored in refrigerator until further use.

2.2. Estimation of phenolic compounds:

The total phenolic substances were estimated using one ml of test samples which contains, 25 µl of alcohol/water extract mixed with 75 µl of ethanol/water, 200 µl of Folin-Ciocaltue reagent and kept in the dark place for 15-20min. After that 500 µl of distilled water mixed with 200 µl of 20% sodium bicarbonate solution. The absorbance was read at 700 nm using colorimeter using blanks or control. Development of blue color indicates the positive indication of phenolic substances. Gallic acid used as standard and the concentration of phenolics in the extracts were calculated using standard curve [13].

2.3. Estimation of total antioxidants:

The extracts were mixed with Ammonium molybdate reagent (100ml of distilled water mixed with Ammonium molybdate (4mM), sodium

phosphate (28mM) and 0.6 mM of sulphuric acid). 100 µl of extract mixed with ammonium molybdate reagent and incubated and the reaction mixture at 90ºC for 90 min. The blue color (indication of positive reaction for presence of antioxidants) with absorbance at 695 nm and neared the optical density values with help of colorimeter compared with the Ascorbic acid standard. The total antioxidants were measured using calibration curve [14].

2.4. DPPH scavenging activity:

The leaf extracts were 5-50 µl were mixed with one ml methanolic solution of 2,2, diphenyl 1 piccrihydrazyl (0.003 gr/L) and made up to 1ml with ethanol and incubated in dark for 15 minutes. The absorbance in terms of optical density has measured using the calorimeter at 540nm. The percentage of inhibition was calculated using the following formula and the inhibition concentration at 50% (IC_{50}) calculated [15].

2.5. Antimicrobial activity:

The antimicrobial activity was performed using disc diffusion assay and the cultures were grown and maintained in the nutrient agar. The microbial pure cultures were collected from Institute of Microbial Technology (IMTECH), Chandigarh, India. The bacterial pure cultures used as experimental microbes were *Escherichia coli* MTCC 1668, *Pseudomonas aeruginosa* MTCC 7296, *Klebsiella pneumoniae* MTCC 7028, *Salmonella typhimurium* MTCC 98, (Gram negative), *Staphylococcus aureus* MTCC 7443 (Gram positive). The different concentrations of the extracts were prepared (10-50 mg/ml) and Whatmann No.1 filter paper disc (6 mm) were dipped in the extracts and were left for drying. The dried impregnated discs were used for the antibacterial activity. The 12hour old culture of bacteria were mixed with liquified sterile nutrient agar medium and mixed well for uniform distribution of bacterial cells [16]. This medium poured into sterile petri dishes and allowed to cool to the room temperature. The discs were kept carefully on the surface of the solidified medium and incubated at 35ºC for twenty-four hours, under aseptic conditions. The positive control (Amoxillin) and negative control with solvent. The plates were observed for the growth and antibacterial activity around the discs [17]. The antibacterial effect has been determined by the observation of clear zone of inhibition around the discs and zones were measured using the metric scale.

RESULTS:

3.1. Total phenolics

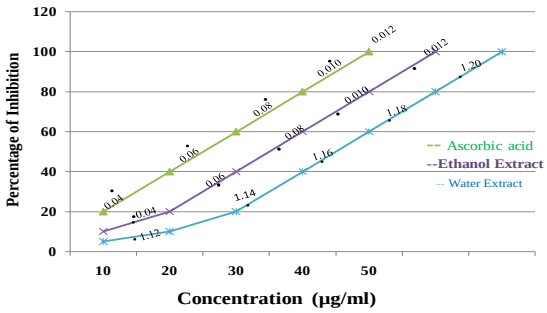
The present research work on *H. isora* leaves has been focused on the scavenging effect extracts on the DPPH radical, quantification of phenolics and antioxidants in addition to antibacterial activity. The phenolics were examined by ethyl alcohol extract and water extracts is 240 mg/ml, and 275.5 mg/ml per gram dry leaf respectively.

3.2. Antioxidant activity: The antioxidants quantified by using reduction of ammonium molybdate and formation blue color in the reaction taken as a positive result. The total antioxidants were estimated in the extracts of *H. isora* ethanolic and aqueous extracts are 31.5 mg/gram and 65.4 mg/gram dry leaf wight in equivalents to the standard (Ascorbic acid).

The DPPH scavenging studies revealed that the extracts were significantly decolorized the blue tinge of the control solution in dose dependent manner. The minimum concentration to reduce at 50% is 35.7 µg/ml and 65.5 µg/ml for the ethanolic and water extracts, respectively. The free radicals are unpaired electrons. They release in the body, due to metabolism. The free radicals are neutralized by the antioxidant. If free radicals are more in the body, they cause diseases like arthritis, cardiovascular disease, cancer and diabetes [18].

3.3. DPPH Scavenging activity:

Fig. 1 DPPH Scavenging activity of *H. isora* leaf extracts



This graph has concentration and percentage of inhibition, the ascorbic acid has 0.04 value concentration at 10 µg/ml of 20% of inhibition. 0.06 value concentration at 25 µg/ml at 55% of inhibition.

0.08 value 35 µg/ml at 79% of inhibition. 0.010 value 45 µg/ml at 98% of inhibition. 0.012 value 50 µg/ml at 100% of inhibition. The ethanol has consisted of 0.04 values at 15 µg/ml at 20% of inhibition. 0.06 value at 30 µg/ml of 35% of inhibition. 0.08 value at 40 µg/ml of 50% of inhibition. 0.010 value at 50 µg/ml of 70% of inhibition. 0.012 value at 60 µg/ml of 100% of inhibition. The water extract has concentration of 1.12 values at 10 µg/ml of 5% inhibition. 1.14 values at 30 µg/ml of 23% of inhibition. 1.16 values at 45 µg/ml of 43% of inhibition. 1.18 values at 60 µg/ml of 63% of inhibition. 1.20 values at 70 µg/ml of 85% of inhibition. The ethanol has more percentage of inhibition than water extract. Ascorbic acid is a template of percentage of inhibition (Fig. 1).

3.4. ANTIMICROBIAL ACTIVITY

Table -1 Antimicrobial activity of phenol *H. isora* of leaf extract Zone of inhibition (mm)

Microorganisms	2mg/ml	4mg/ml	6mg/ml	8mg/ml	10mg/ml	Reference antibiotic
E. coli	-	-	15	17	24	20
S. typhi	-	-	16	19	26	18
K. pneumoniae	-	-	-	16	18	19
P. aeruginosa	-	-	14	17	23	20
S. aureus	15	19	21	23	29	18

The *H. isora* leaves were tested against genetically modified bacteria. The media was poured in to the petriplate and the culture was poured on the media, after that the culture was spread on the media by the sterile glass rod. The discs were dipped in 5 concentrations i.e., 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml, 10 mg/ml. The concentration of 2 mg/ml had only *E. coli* which had only antibiotic activity. The other pathogens never show the antimicrobial activity. The concentration of 4 mg/ml E. *coli* had 18mm of antimicrobial activity, *S. typhi* had shown 18mm of antimicrobial activity. The other pathogens never show the antimicrobial activity (Table 1).

The concentration of 6 mg/ml *S. typhi* had 21 mm of antimicrobial activity. *E. coli* had 20mm of antimicrobial activity. *K. pneumoniae* had 18mm of antimicrobial activity. *S. aureus* had 15mm of antimicrobial activity. *P. aeruginosa* never shows the antimicrobial activity. The concentration of 8mg/ml *S. typhi* had 24mm of antimicrobial activity *E. coli* had 24 mm of antimicrobial activity. *K. pneumoniae* had 18mm of antimicrobial activity. *S. aureus* had 17 mm of antimicrobial activity. *P. aeruginosa* had never shows the antimicrobial activity. When the concentration increases antimicrobial activity increases. The concentration of 10 mg/ml *S. typhi* had 28 mm of antimicrobial activity, followed by *E. coli* 26 mm of antimicrobial activity, *K. pneumoniae* 23 mm of antimicrobial activity, *S. aureus* 21 mm of antimicrobial activity and *P. aeruginosa* 17 mm of antimicrobial activity (Table 2).

Table 2. Antimicrobial activity of ethyl alcohol of *H. isora* of leaf extract.

Zone of inhibition (mm)

Microorganisms	2mg/ml	4mg/ml	6mg/ml	8mg/ml	10mg/ml	Reference Antibiotic
E. coli	16	18	20	24	26	17
Sal. Typhi	-	18	21	24	28	19
Kl. pneumoniae	-	-	18	18	23	20
Ps. aeruginosa	-	-	-	-	17	19
St. aureus	-	-	15	17	21	23

The 5 concentrations were 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml, and 10 mg/ml. The 2 mg/ml concentration the *S. aureus* had only the antimicrobial activity I,e 15 mm. The other pathogens never show any antimicrobial activity. The 4 mg/ml concentration only *S. aureus* has only showed the antimicrobial activity i.e., 19 mm. The other pathogens never indicate any antimicrobial activity. The 6mg/ml concentration *S. aureus* had higher concentration, followed by *S. typhi, E. coli, P. aeruginosa*. The *K. pneumoniae* does not show any antimicrobial activity. While concentration increases the antimicrobial activity increases. The 8mg/ml the *S. aureus* shows higher concentration i.e., 29 mm followed by *Sal. typhi* 19mm, *E. coli* 17 mm, pseudomonas 17 mm, *K. pneumoniae* 16 mm. The 10 mg/ml concentration *S. aureus* has 29 mm had highest antimicrobial activity, followed by *S. typhi* 26mm, *E. coli* 24 mm, *P. aeruginosa* 23 mm, *K. pneumonia* 18mm (Table 3).

Table 3. Antimicrobial activity of distilled water of *H. isora* of leaf extract Zone of inhibition (mm)

Microorganisms	2 mg/ml	4 mg/ml	6 mg/ml	8 mg/ml	10 mg/ml	Reference antibiotic
E. coli	-	-	19	22	28	21
S. typhi	-	-	15	20	25	22
K. pneumonae	-	14	17	22	25	16
P. aeruginosa	-	-	-	17	20	22
S. aureus	-	-	-	-	27	16

The *H. isora* leaves were tested against pathogens. The agar media was poured in to the petriplates and culture was poured on the media and spread by the sterile glass rod. The paper was in to circular shape 5 pieces was prepared. The concentration was 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml, and 10 mg/ml. The concentration of 2 mg/ml there was no antimicrobial activity. The concentration of 4 mg/ml *K. pneumoniae* had 14 mm of antimicrobial activity. The other pathogens never had any antimicrobial activity. The concentration of 6mg/ml *E. coli* had 19 mm of antimicrobial activity followed by *K.*

pneumoniae had 17mm of antimicrobial activity, *S. typhi* had 15 mm of antimicrobial activity. The other two pathogens never show antimicrobial activity. The concentration of 8 mg/ml *E. coli* and *K. pneumoniae* both of them had 22 mm, 22 mm of antimicrobial activity. The *S. typhi* had 20 mm of antimicrobial activity. *P. aeruginosa* had 17 mm of antimicrobial activity. The *S. aureus* never shows antimicrobial activity. The concentration of 10 mg/ml. *E. coli* had 28 mm of antimicrobial activity; it shows highest antimicrobial activity and *S. aureus* had 27 mm of antimicrobial activity. Followed by *S. typhi* 25 mm of antimicrobial activity, *K. pneumoniae* 25 mm of antimicrobial activity and *P. aeruginosa* 20 mm of antimicrobial activity (Table 3).

DISCUSSION:

The phenolic compounds are important group of phytoconstituents and its functions has the redox properties and as reducing agent. The phenol contains food are honeybush, mate, black tea and green tea proved as medicinal constituents [19].

The antioxidants and phenols are naturally found in the plants, these biomoleculesare called secondary metabolites stored in the different parts of the plant body and were obtained from the leaves are isocucurbitacin b, cucurbitacin b (steroids), the phenols are gallic acid, 3,4dihydroxy benzoic acid and benzoic acid propyl ester. Due to the presence of these biomolecules, the oxygen species and nitrogen species were being neutralized might be responsible for prevention of diseases in human beings which developed during the stress and age-related disorders [20].

The antimicrobial activity was tested on pathogens; they were *E. coli, S. typhi, S. aureus, K. pneumoniae, P. aeruginosa* and *E. coli* is an opportunistic pathogen; its origin is normal flora of intestine. Which cause an intestinal disease; symptoms are fever, diarrhea and abdominal pain. The genetically modified bacteria were tested against *H. isora* leaves. It shows antimicrobial activity. So, this plant leaves are used as a drug [21].

The *S. typhi* is a pathogen, it causes the typhoid. The genetically modified bacteria were tested against *H. isora* leaves. It shows the antimicrobial activity, so it is used for the drug. The *K. pneumoniae* is a human pathogen; it causes the Blood stream infections. This bacterium was tested against the *H. isora* leaves. It has been showed antimicrobial activity, so leaves can be use as a drug [22]. *P. aeruginosa* is a human pathogen, it causes pulmonary disease. This bacterium was tested against the *H. isora* leaves. It had shown antimicrobial activity. By this invention the drugs may be produced [23]. *S. aureus* is a human pathogen; it causes pleuropulmonary, skin, soft tissue, osteoarticular and endocarditis. The broad spectrum of antibacterial activity of *H. isora* leaves may be helpful in the development of novel antibiotic natural compounds.

CONCLUSION

The present on the *H. isora* leaf extracts revealed the potential antioxidant and antimicrobial activity which supports the medicinal claims for its usage. Further work required to upgrade the extracts after thorough *in vivo* studies.

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