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Extraction of phytochemicals and study of its antimicrobial and antioxidant activity of *Helicteres isora L.*

S. P. Mahire[1*] and S. N. Patel[2]

Abstract

Background: Herbal medicines are used for the treatment of many infectious diseases throughout the history of mankind. The increasing antibiotic resistance exhibited by microorganisms has led to the phytochemical screening of medicinal plants for antimicrobial activity. Many infections can be treated by phytochemicals possessing potent antibacterial efficiency. The pharmacological activities of any plant is because of the presence of primary metabolites, secondary metabolites and its secretary products, comprising the phenolic compounds, alkaloids, tannins, saponins, carbohydrates, glycosides, flavanoids, steroids, etc. *Helicteres isora* L. is a medicinal plant used in traditional medicine. The root and bark are expectorant, demulcent, astringent to the bowels, antigalactagogue, lessen gripping; a cure for scabies when applied topically.

Method: Soxhlet extraction or hot continuous extraction: In this method, finely ground sample was placed in thimble chamber of the Soxhlet apparatus. Extraction solvents was heated in the bottom flask, vaporizes into the sample thimble, condenses in the condenser and drip back. When the liquid content reaches the siphon arm, the liquid contents emptied into the bottom flask again and the process is continued. Gram positive bacteria *Staphylococcus aureus* NCIM 2079, Gram negative bacteria *Escherichia coli* NCIM 2109, Fungi (Yeast) *Candida albicans* NCIM *3471*, Fungi (Mould) *Aspergillus niger* NCIM *545* are used as reference Strain for antimicrobial activity. Antioxidant activity was tested by DPPH method.

Result: Chloroform Extracts of leaves, Stem and fruits of *Helicteres isora* L. exhibited potent antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus, niger*. The stem extract showed highest antimicrobial activity against *Escherichia coli* with zone of inhibition of 19.24 mm and leaves lowest activity against *Candida albicans* with zone of inhibition of 11.26 mm. Antioxidant activity was tested by DPPH method.

Conclusion: The use of herbal crude drugs, in tracts and their remedies have significantly increased throughout the world. Efforts must be made to ensure safe, effective and affordable treatments for wide range of diseases by traditional methods which use locally available medicinal plants. The scientific and authentic researches on these aspects are to be done in order to exploit traditional knowledge of medicinal plants.

Keywords: Phytochemicals, *Helicteres isora* L., Hot extraction, Soxhlet, Chloroform extract, Antimicrobial, Antioxidant activity, Etc.

* Correspondence: sarojchem31@rediffmail.com
[1]P G and Research Department of Chemistry, Shirpur, MS, India
Full list of author information is available at the end of the article

Background

Although there is great advancement in medical science, plants are considered as important contributors in health care [1]. According to World Health Organizationabout 80% of population relies on traditional medicines for their primary health needs [2]. Plants have ability to synthesize secondary metabolites. Around 1200 secondary metabolites have been isolated. These synthesized metabolites are aromatic substances, used by plants as defensive molecules against predation by microorganism, insects and other herbivores [3]. However, these defensive molecules give plants their medicinal value which is appreciated by human being.

In 1985 Farnsworth et al. identified 119 secondary plant metabolites which were used as drugs. According to WHO 11% of the basic and essential drugs are obtained from plant and number of synthetic drugs are also obtained from natural precursors. Phytochemicals possess antioxidants [4], antifungal [5], and antibacterial [6]. A huge number of plants are used as remedy against several ailments by tribal communities. Besides, the origin of most of the drugs available now a days, are from medicinal plants. Research on medicinal plants is increasing day by day due to high cost and possible side effects associated with the use of modern drugs. Treatment of ailments using medicinal plants is often cheaper and in almost all cases it lacks side effects [7]. It is very important to search for effective but of low cost and reliable traditional therapeutic agents, hence also the abuse of drugs for ailment is in high increase which motivated drug resistant organisms. This work is aimed at studying *Helicteres isora* L.

Helicteres isora L., an ayurvedic herb, is distributed widely in forest throughout India. Almost every part of the plant is used traditionally. Parts of plant are useful in ailments such as gastric problems diarrhoea, dysentery, wounds, ulcers, hemorrhages snake bite, worm infections, and diabetes [7, 8]. *Helicteres isora* L. is a large genus of tropical trees and shrubs (family Sterculiaceae). *Helicteres isora* L. is medicinally important and is used in the treatment of several diseases [9]. It is distributed in dry forests throughout Central and Western India, from Bihar to Jammu and Western Peninsula. It is sub-deciduous small tree or large shrub with long, greenish-brown, beaked and cylindrical fruits. It is commonly known as East India Screw tree [7]. The plant is reported to exhibit various bioactivities such as antioxidant, hypolipidemic, antimicrobial, antiviral, analgesic, hepatoprotective, anticancer, antidiarrhoeal and wormicidal activity [7, 8].

Material and method

Sample species collection

The sample Species were collected from Satpura ranges in Nandurbar and Dhule Disrict. The collected sample species were observed and identified by popular taxonomist, as *Helicteres isora* L. Healthy plants were washed to remove clay, plant parts are separated as leaves, stem and fruit, then separated dried plant parts were crushed [10]. This crushed sample was used for next extraction.

Extraction

The extraction procedure for the isolation of crude products from plant has been practiced since long time. The precise mode of extraction depends on texture and water content of plant material being extracted and type of substance which is to be isolated. Normally crude extract is taken from Soxhlet apparatus using Chloroform as a solvent [11]. Chloroform shows good solubility for maximum organic compounds, and due to low boiling point it can be easily recovered.

Soxhlet extraction or hot continuous extraction

In this method, crushed sample was placed in thimble chamber of the Soxhlet apparatus. The extraction solvent Chloroform was heated in the bottom flask, vaporizes into the sample thimble, condenses in the condenser and drip back. When the liquid content reached the siphon arm, the liquid contents emptied into the bottom flask again and the process was continued [12].

About 100 g of dried sample powder was weighed and extraction process is carried out by using 250 ml of Chloroform in Soxhlet apparatus for 48 h. The extract was concentrated by evaporation at 70 °C for 8 h and then dried. The concentrated extract was made in Gel form and stored at room temperature prior to phytochemical screening [11, 13].

Phytochemical screening

Preliminary screening of secondary metabolites

1. **Saponin:** 0.5 g Extract was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3

Table 1 Phytochemical Screening of chloroform extracts of Fruit, Stem, and Leaves of *Helicteres isora* L. [3]

Test	Fruit extract	Stem extract	Leaves extract
Alkaloid	+	+	+
Steroid	+	–	–
Terpenoid	–	+	–
Flavanoids	–	–	–
Polyphenols	–	–	–
Tannins	–	–	–
Cardiac glycosides	–	+	–
Saponins	–	–	–

+ = Presence, – = absence

Table 2 An antimicrobial activity (diameter in mm) of Chloroform extract of parts of *Helicteres isora* L. [12]

Extract	Bacteria		Fungi	
	E. coli	*S. aureus*	*C. albicans*	*A. niger*
Fruit	–	12.98	–	15.83
Stem	19.24	13.11	–	13.88
Leaves	18.57	12.20	11.16	15.37
C	30.32	29.82	–	–
A	–	–	18.55	18.13

E. coli Escherichia coli, *S. aureus Staphylococcus aureus*, *C. albicans Candida albicans*, *A. niger* Aspergillus niger, *C* Chloramphenicol (Standard antibacterial), *A* Amphotericin B (Standard antifungal)

drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

2. **Steroids:** 2 ml of acetic acid added to 0.5 g extract with the addition chloroform, heated, with the addition of 2 ml H_2SO_4. The colour change to orange indicates presence of steroids [14].
3. **Flavanoids:** 0.5 g Extract was shaken with 1 ml of dilute ammonia solution. A yellow observation indicates the presence of Flavanoids [15].
4. **Tannins:** 0.5 g Exctract was boiled in 20 ml of water in a test tube and then filtered. Few drops of 0.1% ferric chloride was added and observed for brownish green or blue black colour [16].
5. **Cardiac glycosides (Keller-Killam test):** 0.5 g Extracts was treated with 2 ml of glacial acetic acid containing a drop of ferric chloride solution. Then 1 ml of concentrated H_2SO_4 was added. A brown ring of the interface indicates a deoxy-sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer, indicates the presence of cardiac glycosides [17].
6. **Free alkaloids:** 0.5 g extract was dissolved in 1.5 ml of 2% HCL and treated with two drops of Mayer's reagent. Turbidity and formation of creamy white precipitate was regarded as evidence for the presence of free alkaloids in the extract [15].
7. **Alkaloids salts:** The 0.5 g aqueous extract of each organs of the plant (25 ml) was stirred with 15 ml of 10% HCl on a steam bath for 30 min. The mixture was extracted three times with dimethyl ether. 1 ml of the aqueous layer was treated with two drops of Wagner's reagent. Formation of brownish precipitate was regarded as evidence for the presence of salts alkaloids in the extract [18].
8. **Terpenoides:** The presence of Terpenoides was determined as described for steroids except that red, pink or violet colour indicates the presence of Terpenoides.
9. **Salkowskitest:** 0.5 g of extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of Terpenoides.
10. **Anthracenosides:** To 0.5 g extract was added 15 ml of 10% HCl. The mixture was refluxed for 30 min. After cooling, the mixture is extracted three with 15 ml of diethyl extract. After evaporation of 8 ml of etheric layer, the residue was treated with 2 ml of hot water and some drops of 10% NH_4OH. Appearance of red orange colour revealed the presence of anthracenosides [18].

Material and method for antimicrobial study

Gram positive bacteria *Staphylococcus aureus* NCIM 2079, Gram negative bacteria *Escherichia coli* NCIM 2109, Fungi (Yeast) *Candida albicans* NCIM *3471*, Fungi (Mould) *Aspergillus niger* NCIM *545* are used as reference Strain.

Concentration used for anti microbial tests

Stock solution [1000 microgram per ml] of each compound was prepared in Dimethylsulfoxide (100%

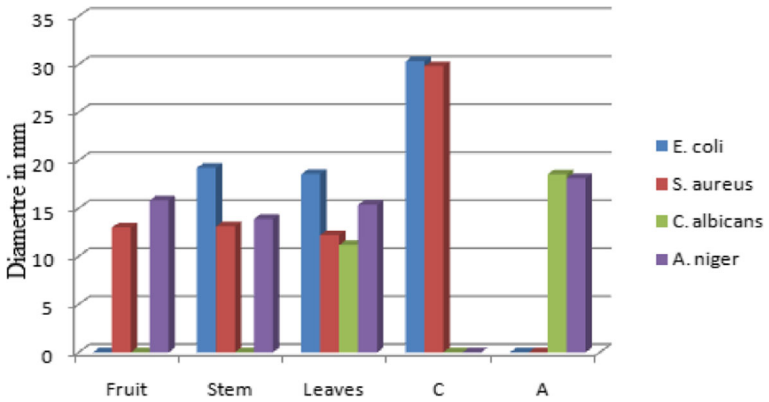

Fig. 1 An antimicrobial activity (diameter in mm) of Chloroform extract of fruit, stems, and leaves of *Helicteres isora* L. [12]

Table 3 %Antioxidant activity of chloroform extracts by DPPH method [20, 21]

Extract	Concentration		
	200 μ g/ml	400 μg/ml	600 μg/ml
Fruit	57.28%	59.22%	57.97%
Stem	30.02%	52.01%	65.60%
Leaves	30.09%	36.61%	36.19%
Ascorbic acid	81.27%	83.49%	96.67%
Control	00	00	00

DMSO). Assay carried out by taking concentration 100 microgram per disk. Hi-media antibiotics disk: Chloramphenicol (10 microgram/disk, Amphotericin-B (100 units/disk) moistened with DMSO are used as standard [12, 19].

Media used

Microbiological media used for bacteria (*Staphylococcus aureus, and Escherichia coli)* is Nutrient agar (Himedia).

Composition (gL-1)

Sodium chloride, 5.0; Beef extract 10.0; Peptone 10.0 (pH 7.2).

Microbiological media for fungi (*Aspergillusniger*) is Potato dextrose agar (Hi-media).

Composition (gL-1)

Potatoes infusion, 200.0; Dextrose 20.0 (pH 5.2).

Microbiological media for yeast (*Candida albicans*) is MGYP (all ingredients of Hi media).

Composition (gL-1)

Malt extract, 3.0; Glucose, 10.0; Yeast extract, 3.0; Peptone, 5.0 (pH 6.4) [12, 19].

Antioxidant activity

DPPH radical scavenging assay

DPPH radical scavenging activity was done using the reaction mixture containing 1 ml of DPPH solution (2.0 mm /L, in 95% methanol v/v) with 3 ml extract was shaken and incubated for 30 min at room temperature in dark. After the incubation absorbance was read at 517 nm against a blank. The radical scavenging activity was measured as a decrease in the absorbance of DPPH in triplicates and calculated using the following equation [20, 21]:

$$Effect\ of\ scavanging(\%) = \left[\frac{1\text{–}A\ sample}{A\ control}\right] \times 100$$

$$\text{Effect of scavenging } (\%) = [(1\text{–A sample})/\text{A control}] \times 100$$

Results

Discussion

In our study phytochemical screening of different parts of *Helicteres isora* L. using chloroform as a solvent is carried out. Results obtained as shown in Table 1. Secondary metabolites of plant are mainly responsible for different pharmaco-logical properties and their therapeutic benefits [22]. Extracts of parts of plant were tested against the pathogenic bacteria and fungi. Antioxidants have inhibitory capacity to oxidative stress induce cellu-lar damage and their main mechanism underlying this property is to scavenge free radicals due to their redox capacity [23]. In present work, we observed that the investigated plant extracts possess good DPPH free radical scavenging potential. Fruit extract shows presence of alkaloids and steroids. Observed results of microbial activity as inhibitory zone formation are shown in Table 2 and also clarified by Fig. 1. The results obtained in antioxidant activity as % scavenging activity as shown in Table 3 and clarified in Fig. 2. Alkaloids and steroid present in fruit extract shows activity against

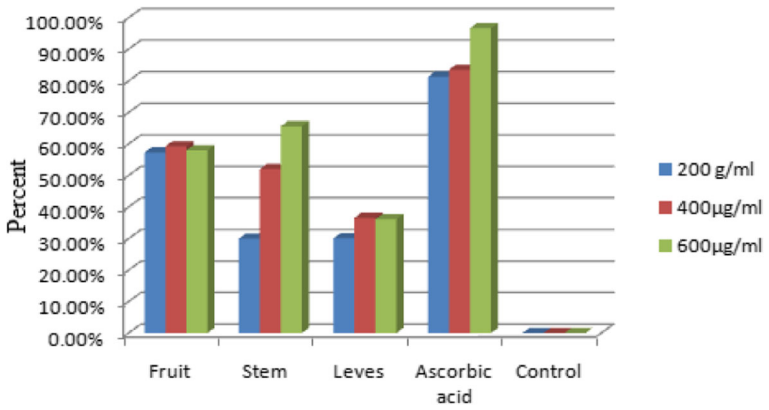

Fig. 2 %Antioxidant activity of chloroform extracts by DPPH method [21]

bacteria *Escherichia coli* with forming diameter of 12.98 mm and *Staphylococcus aureus* with forming diameter of 15.83mm. Alkaloids and steroid present in fruit extract shows activity against bacteria *Escherichia coli* with forming diameter of 12.98 mm and *Staphylococcus aureus* with forming diameter of 15.83 mm. Alkaloids and steroid present in fruit extract shows 57.28%, 59.22%, and 57.97% scavenging activity at 200 μg/ml, 400 μg/ml and 600 μg/ml. Stem extract shows presence of alkaloids, terpenoides and cardiac glycosides, shows activity against bacteria *Escherichia coli* with forming diameter of 19.24 mm, *Staphylococcus aureus* with forming diameter of 13.11 mm and fungi *Aspergillus niger* with forming diameter of 13.838 mm. These secondary metabolites show 30.02%, 52.01%, and 65.60% scavenging activity at200 μg/ml, 400 μg/ml and 600 μg/ml respectively. Leaves extract shows presence of alkaloids. Alkaloids present in leaves extract shows activity against bacteria *Escherichia coli* with forming diameter of 18.57 mm, *Staphylococcus aureus* with forming diameter of 12.20 mm and fungi *Candida albicans* with forming diameter of 11.16 mm and *Aspergillus niger* with forming diameter of 15.37 mm. These alkaloids show 30.09%, 36.61%, and 36.19% scavenging activity. The chloroform extract of different parts can be considered as good source of anti bacterial, antifungal and anti oxidant agent due to presence of alkaloids, terpenoides, steroids and cardiac glycoside. However further studies are suggested to isolate the individual components using chromatographic technique and to understand underlying mechanism of observed activities.

Conclusion

The use of herbal crude drugs, in tracts and their remedies have significantly increased throughout the world. Chloroform extract of fruit, stem, and leaves shows activity against bacteria and fungi *Escherichia coli*, *Staphylococcus aureus* and fungi *Candida albicans* and *Aspergillus niger*, it also shows scavenging activity at three concentrations 200 μg/ml, 400 μg/ml and 600 μg/ml. The scientific and authentic researches on these aspects are to be done in order to exploit traditional knowledge of medicinal plants.

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Authors' contributions

SP Mahire carried out the collection of plant, extraction process, Preliminary Screening of secondary metabolites and wrote the manuscript. Dr. S. N. Patel supervised research work and improved the quality of final manuscript. The author(s) read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Author details

[1]P G and Research Department of Chemistry, Shirpur, MS, India. [2]S. P. D. M. ARTS, S. B. B. & S. H. D. Commerce and S M A Science College, Shirpur, MS, India.

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