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Qualitative analysis of some phytochemical constituents and antimicrobial tests of *Helotropium supinum* L. medicinal plant

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Abstract

Nowadays whole world blessed with medicinal plants for using as a major bio-source of modern synthetic drugs which are used widely in medicinal field. In this study Qualitative experiments of phytochemical and antibacterial tests were performed of ethanolic extracts of leaf and root parts of *Heliotropium supinum*. The Qualitative experiments revealed that the presence phytochemicals such as alkaloids, phenolic compounds, tannins, flavonoids, saponins, terpenoids, steroids, glycosides, and carbohydrates. In this experiment alkaloids, phenolic compounds, tannins, flavonoids, saponins, terpenoids, Carbohydrates and glycosides were observed in ethanolic extract leaves while alkaloids, flavonoids terpenoids were found in roots extract. For being presence of these essential properties *H. supinum* showed antibacterial activity. Maximum zone of inhibition was recorded in 500 µg concentration of leaves and root extracts where leaves extract showed highest inhibition zone (21 mm) against *S. aureus* while root extracts showed against E. coli (17 mm). Present study was designed to identify the sources and accurate information regarding the active components of this medicinal plant and to enhance awareness to public and private sector.

Keywords: Medicinal Plants; Phytochemicals; Qualitative; Antibacterial Activity; *Heliotropium Supinum*; Organic Solvent

1. Introduction

Heliotropium is a large genus of the family Boraginaceae, which consists of about 250-300 species worldwide. These species are widely distributed in temperate and tropical regions of both hemispheres. The name "heliotrope" derives from these plants turning their leaves to the sun [1]. In modern times, more than 80% world's population depends on the traditional system of medicines. And the Plants of the genus *Heliotropium* display a wide range of pharmacological activities. Especially the species *H. supinum* whole plant mixture of pulped plant with water can use to treat tumors [2]. The leaves, seed, and stem aqueous and alcoholic extracts have potential activities on pathogenic bacteria like *staph epidermidis*, *E. coli* and *proteus sp* [3]. Also, pancreatic islet cell tumors have been reported among rats treated with the open ester-alkaloids from *H. supinum* [4]. The hairy undershrub, *H. supinum* L., is also an epileptic child's enema in infusion and called nalufiaoli (pl. onamufiaoli) 'that which dies staying,' have the roots of either plant shredded into an infusion and drunk to relieve cramps [5].

Also, ethanolic extract of the whole plant of *H. indicum* another species from *Heliotropium* group revealed substantial antiproliferative activity against SKBR-3 human breast adenocarcinoma cell line using MTT assay. The crude extract of *H. strigosum* its resultant fractions possessed strong cytotoxic and phytotoxic activity. Its subsequent solvent fractions showed anti-inflammatory activity in carrageenan-induced edema and xylene-induced ear edema. [6] Furthermore, in the history of traditional drugs provide remedies for gout, inflammation, skin disorders, menstrual dysfunction,

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rheumatism, and poisonous bites. The active biochemical constituents extracted from the *Heliotropium* species comprise pyrrolizidine alkaloids, flavonoids, and terpenoids. Significant biological activities viz. antimicrobial, antiviral, antitumor, anti-inflammatory, cytotoxicity, phytotoxicity, and wound healing were revealed by many extracts and biochemically active constituents of various species of the *Heliotropium genus* [7]. On the other side some studies found *H. supinum* poisonous towards rats [8]. Also, PA poisoning of livestock caused by the accidental ingestion of feed contaminated with *Heliotropium* spp. is common worldwide [9, 10]. However, Medicinal plants are one of the most important sources of traditional medicines all over the world [11].

2. Material and methods

2.1. Collection and identification

Leaf and root parts of *Helotropium supinum* L. were collected in summer seasons from Rajshahi and Kurigram district, Bangladesh. Plants were authenticated by the Department of Botany, University of Rajshahi, Bangladesh.

2.2. Preparation of extracts

Ethanol extract: Leaf and root powder were made by grinder. Then 10g powder sample of *H supinum* leaf and root was taken in flax and added 100 ml of ethanol and kept on shaking bath at room temperature for 24 hours. Then filtered and concentrated using a rotary evaporator (at 40^{o}c). Then remaining solution was stored in refrigerator for further phytochemical screening [12].

2.3. Qualitative phytochemical analysis

Phytochemical analysis for ethanol extract of *H supinum* leaf and root was done to detect presence or absence of alkaloids, phenols, terpinoids, flavonoids, steroids, saponins, cardiac glycosides using standard procedures described by Goveas [13] and Dahanayake and co-workers [14] with some modifications.

2.4. Screening of phytochemical from medicinal plants

Different type of tests is present to identify the phytochemicals from medicinal plants are given bellow:

2.4.1. Alkaloids

Mayer's test

1 ml of extract treated with two of mayer's reagent. Formation of white precipitate indicates existance of alkaloids [15].

Wagner's test

1 ml of extract and two drops of wagner's reagent was added. Formation of redish-brown precipitate indicates the existance of alkaloid [16].

Hager's test

1 ml of extract and 1 ml of Hager's reagent were added. Formation of of yellow precipitate indicates the existance of alkaloids [17].

Dragendorff's test

1 ml extract and few drops of Dragendorff's reagent were added. Formation of yellow precipitate indicates the alkaloids present [18].

2.4.2. Phenolic compound and Tannins

Ferric chloride test

1 ml of extract was treated with few drops of Ferric chloride. Appearance of dark green colour indicates the existance of phenolic compounds [19].

Lead acetate test

1 ml extract and 1 ml 10% acetate was added. Bulky white precipitate indicates the presence of tannins [19].

2.4.3. Carbohydrates

Benedict's test

1 ml of extract and 4 ml of Benedict's reagent were dissolved then heated on a boiling water bath for few minutes orange red colour precipitate indicates the presence of carbohydrates.

Fehling's test

2 ml of extract was taken. 1 ml of Fehling's reagent A and 1 ml of Fehling's reagent B Solution were added and kept in boiling water bath for few minutes yellow, and brick red precipitate indicates the presence of reducing sugar [20].

2.4.4. Glycosides

Born Trager's test

2 ml of extract, 2 ml of chloroform were added, shaken vigorously chloroform layer separated equal volume of diluted ammonia was added. Pink colour indicates presence of glycosides [15].

Legal's Test

2 ml extract and 1 ml of pyridine and 1 ml of sodium nitroprusside and few ml of 10% NaOH were added. Apearance of pink to red colour indicates the presence of glycosides [21].

2.4.5. Flavonoids

Sodium hydroxide test

1 ml extract and 1 ml NaOH were added. A dirty yellowish-brown precipitate indicates the presence of Flavonoids [19].

Shinda test

Few ml of extracts and few fragments of magnesium turnings and then drops wise concentrated HCL was added. Pink colour was observed indicates the presence of flavonoids [19].

2.4.6. Saponins

Foam test

2 ml extracts and 4 ml distilled water was added. Vigorously shaken in test tube for few minutes. Formation of foam indicates the of saponins [22].

2.4.7. Terpenoids

Salkowski's test

2 mL of chloroform and concentrated sulphuric acid (3ml) were added carefully to 0.5 mL of each plant extract. Formation of red brown color at the interface indicated the presence of terpenoids.

2.4.8. Steroids

Liebermann Burchard test

2 ml of the organic extract was dissolved in 2 ml of chloroform and treated with concentrated sulphuric acid and acetic acid developed a greenish color, indicates the presence of steroids.

2.5. Susceptibility testing

Strains of microorganisms used for susceptibility tests were performed using five strains of microorganisms including gram positive and gram-negative bacteria. These microorganisms were collected from Microbiology laboratory with the help of Md Zahidul Hoque (Scientific officer, Microbiologist), Bangladesh.

2.6. Determination of Antibacterial activity

Sensitivity tests were performed by agar-well diffusion methods [23, 24, 25]. Hiton Agar medium were used for bacterial strains test. Different concentrations of leaves and root extract were poured in the wells. After applied the extracts all the plates holding at room temperature for an hour to allow diffusion of the extract into the ager, they were incubated for 24 hours at 37˚C. Micro aerophilic conditions were prepared for *Streptococcus sp*. After 24 hours, zone of inhibition was observed and recorded. The tests were performed three time for each sample for avoiding error and correct evaluation and the results were presented as arithmetic average by avoiding fractions. Here Ampicillin antibiotics was considered as standard.

2.7. Minimum Inhibitory Concentration (MIC)

The MICs were calculated by broth dilution technique. All the tubes were incubated at 37˚C for each type of bacterial cultures. Minimum Inhibitory Concentration (MIC), which was determined as the lowest concentration of plant extracts inhibiting the growth of the microbes, were determined.

3. Results and discussion

Table 1 Screening of some Phytochemicals from of *H. supinum*

Scientific name of plants	***H. supinum* L.**	
Tests	**Ethanolic Extract of leaves**	**Ethanolic Extract of roots**
Alkaloids		
Mayer's Test	+	+
Wagner's Test	+	+
Dragendorff's Test	+	+
Hager's Test	+	+
Phenolic compounds and Tannins		
Ferric Chlorides Test	+	-
Lead Acetate Test	+	-
Flavonoids		
Sodium hydroxide Test	+	+
Shinda Test	+	+
Saponins		
Foam Test	+	-
Terpenoids		
Salkowski's test	+	+
Steroids		
Liebermann Burchard test	-	-
Carbohydrates		
Benedict's Test	+	-
Fehling Test	+	-
Glycosides		
Borntrager's test	+	+
Legal's Test	+	+

Note. + = indicates presence and - = indicates absence

H. supinum L contained with the pyrrolizidine alkaloids supinine, heliosupine, echinatine and 7-angelylheliotridine (and its trachelanthic and viridifloric ester ((-)-erythro-2, 3-dihydroxy-4-methylpentane-3-carboxylic acid)); were reported by Crowley and Culvenor [26] and Mattocks [27]. Extraction and screening of phytochemical from selected medicinal plants, as mentioned above, have been analyzed. The identified components which can play important role against various types of diseases such as antibacterial, antidiarrheal activity and help in electrolyte reabsorption prevent specific pathogen and dwell in intestinal motility [28].

In this present study the screening of phytochemicals using Ethanol from leaves and roots of the selected medicinal plants results showed the presence and absence of various types of alkaloids, phenolic compounds, tannins, flavonoids, saponins, terpenoids, steroids, glycosides and carbohydrates were depicted in Table-1. Here, alkaloids, phenolic compounds, tannins, flavonoids, saponins, terpenoids, Carbohydrates and glycosides were found in leaves extract and Alkaloids, flavonoids terpenoids were found in roots extract. Among of these the alkaloids is the major compound and the prominent one. The results are shown in table 1.

Table 2 Antibacterial activity of leaf and root extracts of *H. supinum*

Name of Microorganism	**Leaves extract**		**Root extract**		**Ampicillin**	
	Con. (µg)	**ZI (mm)**	**Con. (µg)**	**ZI (mm)**	**Con. (µg)**	**ZI (mm)**
E. coli	100	4	100	2	300	7
	200	6	200	5		
	300	9	300	8		
	400	14	400	12		
	500	18	500	17		
Bacillus subtilis	100	3	100	0	300	11
	200	5	200	2		
	300	7	300	4		
	400	11	400	6		
	500	16	500	11		
Streptococccus sp	100	2	100	2	300	9
	200	5	200	4		
	300	8	300	9		
	400	11	400	13		
	500	17	500	16		
Staphylococcus aureus	100	2	100	2	300	10
	200	7	200	6		
	300	13	300	9		
	400	18	400	10		
	500	21	500	15		
Shigella dysenteriae .	100	3	100	2	300	12
	200	7	200	4		
	300	9	300	10		
	400	13	400	12		
	500	16	500	16		

Con. = Concentration, ZI = Zone of Inhibition, µg = microgram & mm = millimetre

Al-Saimary and Baker, mentioned that among the leaf, stem, and seed extracts of *H. supinum* the aqueous extract of leaves showed highest inhibition zone diameter (25mm) for *E. coli* while lowest for aqueous alcoholic extract of stems (5mm) where minimal inhibitory concentrations ranged between 100 to 750 mg/ml. In this study, the extracts shown different degree of inhibitory effect. The inhibitory effect of extracts was directly proportional to increasing concentration of field grown leaf and root extracts [3].

The leaves and root extracts of ethanol inhibits the growth of pathogenic microorganisms. Here ethanolic extract of leaves showed more inhibitor than root extract. Maximum zone of inhibition was recorded in 500 µg concentration of leaves and root extracts where leaves extract showed highest inhibition zone (21mm) against *S. aureus* while root extracts showed against *E. coli* (17mm). In case of all the minimum zone of inhibition observed in 200µg concentration of leaf and root extract. The MIC of 100µg/ml was found against all the tested bacteria, but its' all concentrations did not show any inhibitory effect on *B. subtilis*. In some cases, extracts showed more inhibition zone than antibiotics (selected standard) the antibacterial activity tests results are shown in table 2.

4. Conclusion

This research work shows a primary information of *H. supinum* L. about phytochemical constituents and minimal inhibition zone against *E. coli*, *B. subtilis*, *Streptococcus* sp, *S. aureus* and *S. dysenteriae* as an antibacterial activity. It will be helpful for doing the farther research work in this topic.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared that no competing interests exist.

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