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Evaluation of phytochemical constituents of *Hemigraphis alternata* (Burm. F.) T. Anderson leaf extract

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ABSTRACT

Plants are exploited as medicinal source since ancient age. A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. Evaluation of the local flora exploited in traditional medicine for various biological activities is a necessary first step in the isolation and characterization of the active principle and further leading to drug development. In this aspect, *Hemigraphis alternata* (Burm.f.) T. Anderson was shown to possess many medicinal properties. The phytochemical constituents of *H. alternata* leaf extract was evaluated. The hot water leaf extract of *H. alternata* showed the presence of steroids, carbohydrates, tannins, phenol, proteins and amino acids. The cold water extract of *H. alternata* (leaf) showed the presence of carbohydrates and tannins. Numerous phytochemicals were found to be preserved by the hot water extract which could be recommended for curing human ailments.

Key words: *Hemigraphis alternata*, phytochemical activity, leaf extract.

INTRODUCTION

The traditional and folk medicinal system uses the plant products for the treatment of various infectious diseases. In recent times, plants are being extensively explored for harbouring medicinal properties. Studies by various researchers have proved that plants are one of the major sources for drug discovery and development [1]. The curative properties of medicinal plants are due to the presence of various complex chemical substances of different composition which occur as secondary metabolites. They are grouped as alkaloids, glycosides, cortico steroids, coumarin flavonoids, and essential oils. Over 50% of all modern clinical drugs are of natural origin and play an important role in development of drugs [2]. Many of the phytochemical bioactive compounds from medicinal plants have shown many pharmacological activities [3]. Screening of various bioactive compounds from plants has lead to the discovery of new medicinal drug which have efficient protection and treatment roles against various diseases [4].

H. alternata (Acanthaceae), an exotic plant adapted to India, is a versatile tropical low-creeping perennial herb that reaches a height of 15 to 30 cm. In Kerala, the plant is popular in the name 'murikootti' or 'murian pacha' because of its incredible potency to heal wounds. Literally, *Hemigraphis* means 'half writing' because the filament of the outer stamen bear brushes [5]. The plant is known by several names such as Aluminium plant, Cemetery plant, Metal leaf, Red flame Ivy, Waffle plant, Java Ivy etc. In Kerala, the plant is popular in the name 'murikootti' or 'murian pacha' because of its incredible potency to heal wounds. The leaf has metallic purple lustre on upper surface and a solid dark purple on ventral side. The leaves are opposite, ovate to cordate, serrate-crenate, about 2 to 8 cm

long and 4 to 6 cm wide, bearing well-defined veins. It blooms irregularly throughout the year in the tropics. Flowers are small (1 to 1.5 cm diameter), five lobed, bell shaped with imbricate bracts. These are white in colour with faint purple marks within and appear in terminal 2 to 10 cm long spikes. Capsules are small, slender, oval, linear and light green in colour. Seeds are small, flat and white in colour [6]. *H. alternata* is claimed in folk medicine that the plant has very good wound healing activity [7].

MATERIALS AND METHODS

Sample collection and processing

Freshly collected *H. alternata* leaves were washed in running tap water for 3 min. Then the plant parts were surface sterilized using 1% mercuric chloride solution under strict aseptic conditions. Finally they were rinsed with sterile distilled water thoroughly to remove mercuric chloride residues. Excess moisture was removed from the sterilized leaves and flower. Then they were subjected to hot water and cold water extraction.

Hot water extraction

About 10 g fresh leaves of *H. alternate* were boiled in 100 mL distilled water with constant stirring for 30 min. The solution was then allowed to cool to room temperature and then filtered using muslin cloth. The filtrate was centrifuged at 5000 rpm for 15 min. The supernatant was again filtered using Whattman's No. 1 filter paper under strict aseptic conditions. The filtrate was collected in fresh sterilized glass tubes and stored at 4 °C until use [8].

Cold Water Extraction

About 10 g fresh leaves of *H. alternate* was macerated in pestle and mortar with 100 ml distilled water at room temperature and then filtered using muslin cloth. The filtrate was centrifuged at 5000 rpm for 15 min. The supernatant was again filtered using Whattman's No. 1 filter paper under strict aseptic conditions and the filtrate was collected in fresh sterilized glass tubes and stored at 4 °C until use [8].

Phytochemical screening

The hot water as well as cold water leaf and flower extracts were tested for the presence of bioactive compounds by using standard [9,10].

Test for carbohydrates

The extracts (100 mg) were dissolved in 5 mL of sterile distilled water and filtered. The filtrate was then subjected to the following tests.

Fehling's test

One mL of the filtrate was boiled with 1 mL each of Fehling solutions A and B on water bath. Appearance of a red or brick precipitate indicates the presence of sugar.

Test for Fixed Oils

Spot test

A small quantity of each extract was pressed between two filter papers. Oil stain on the filter paper indicates that the test is positive.

Test for alkaloids

About 0.5 g of the extract was mixed with 5 mL of 1% aqueous hydrochloric acid on a water bath. The filtrate is carefully tested with different alkaloidal reagents for the presence of alkaloids.

Wagner's test

About 1 mL of HCl was added to 3 mL of extract in a test tube. The mixture was heated for 20 min, cooled and filtered. Two drops of Wagner's reagent was added to 1 mL of the filtrate and observed for reddish brown precipitate.

Test for flavonoids

Alkaline reagent test

Extracts (2 mL) was dissolved in 10% aqueous sodium hydroxide solution, it gives yellow color. A change of color from yellow to colourless on addition of dilute HCl indicates the presence of flavonoids.

Test for terpenoids**Horizon test**

To 1 mL of extract, 2 mL of trichloroacetic acid (TCA) was added. The formation of yellow to red precipitate shows the presence of terpenoids.

Test for steroids**Salkowski test**

A little quantity of each plant extracts was dissolved in 1 mL of chloroform and about 1 mL of concentrated sulphuric acid was added to it to form two phases. Formation of red colouration was taken as an indication for the presence of steroids.

Test for tannins**Ferric chloride test**

The extracts were boiled for 5 min in water bath and added 2 drops of 5% FeCl₃ to it. Production of greenish precipitate was an indication for the presence of tannins.

Test for saponins**Frothing test**

The extracts were shaken with a drop of sodium bicarbonate in test tubes. Formation of honey comb like froth which persisted for 15 min indicates the presence of saponins.

Test for cardiac glycosides

About 5 mL of the filtrate was added to 0.2 mL of fehling solution A and B until it turns alkaline and heated in a water bath for 2 min. A lightest blue coloration was observed (instead of brick precipitate) which indicates the presence of steroidal ring.

Test for proteins and aminoacids**Millon's test**

To the 2 mL of the filtrate, few drops of Millon's reagent were added. A white precipitate indicates the presence of proteins.

Test for phenols**Ferric chloride test**

The extract (50 mg) was dissolved in 5 mL of distilled water. To this few drops of neutral 5% ferric chloride solution was added. A dark green colour indicates the presence of phenolic compounds.

RESULTS AND DISCUSSION**Qualitative phytochemical analysis**

Qualitative analysis was carried out to screen the phytochemicals present in the hot water and cold water extracts of *H. alternata* leaves. The hot water extract of *H. alternata* leaf shows the positive result towards steroids, carbohydrates, tannins, phenol, proteins and amino acids and the negative results were obtained in alkaloids, tapernoids, saponins, flavonoids, cardiac glycosides. The cold water extract of *H. alternata* leaf shows positive result in carbohydrates, tannins and negative results in alkaloids, tapernoids, saponins, flavonoids, cardiac glycosides, steroids, phenols, proteins and amino acids (table 1).

Table 1 Qualitative phytochemical analysis of aqueous extracts of *H. alternata*

Name of the test	<i>H. alternata</i> leaf (coldwater)	<i>H.alternata</i> leaf (hot water)
Alkaloids (Wagners test)	-	-
Tapernoids (Horizon test)	-	-
Saponins	-	-
Flavonoids (Shinnoda test)	-	-
Cardiac glycosides	-	-
Steroids (Salkowski test)	-	+
Carbohydrates (Fehling test)	+	+
Proteins and amino acids (Millons test)	+	+
Tannins (Ferric chloride test)	+	+
Phenols (Ferric chloride test)	-	+

Natural products especially which are derived from plants have been used as a source for various therapeutic processes for long times from the human civilisation. The most common strategy of drug development from plants is

Careful observation of use of natural resources in folk medicine in different cultures by ethnopharmacology [11]. *H. alternata* (Blume) leaf paste on the wound promotes wound healing in mice but the oral administration was ineffective [12]. The phytochemical constituents on *H. alternata* were identified by examining the crude extracts of its leaves and stem using various solvents, screened the antibacterial activity against selected pathogens. The crude aqueous, acetone, benzene, chloroform, ethanol, and petroleum ether extracts of the plant had phenols, carbohydrates, steroids, saponins, coumarins, tannins, proteins, flavonoids, alkaloids. The hypoglycaemic and anti-diabetic properties of *H. alternata* were identified for the first time using wistar rats and Swiss albino mice by [13].

CONCLUSION

Many plants are known to have beneficial therapeutic effects as noted in the traditional Indian system of medicine, Ayurveda. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world. Hence the last decade witnessed and increased in the investigation of plants as a source of human disease management. Based on above ideas *H. alternata* possessed medicinal properties and so it can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address unmet therapeutic needs such as screening of natural organic compounds and identifying active agents is the need of the hour, because successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development. From the results of the study it is concluded that *H. alternata* possessed considerable level of bioactive compounds and therefore, these species can be used as a potential source of drugs.

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