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Phytochemical screening and isolation of bioactive ingredients of *Achyranthe aspera*

C. Elavarasi*, G. Nithya, V. Collins Arun Prakash, A. Arokia Nepolean raj

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Abstract

The use of plants and herbs as medicines has been practiced from ancient times and Indian tradition medicine has got its own place and reputation throughout the world. In particular, south Indian states are well known for practicing traditional medicine and a number of plant varieties are used for curing various ailments. *Achyranthes aspera* is a plant found on the sides of roads, boundaries of fields as a weed throughout India up to an altitude of 3000 feet and can be found in South Andaman Island. The plant is also common in Baluchistan, Ceylon, Tropical Asia, Africa, Australia and America [1,2]. This plant was known to be used for complaints like pain, ulcer, inflammation etc. Hence the present study was aimed to investigate active phytochemical ingredients present in the leaves extract of *Achyranthes aspera* using n-hexane as solvent and subsequent isolation of compounds using chromatographic analysis. The phytochemicals flavonoid and saponins were successfully isolated and characterized using qualitative chemical test and FTIR spectroscopy. The obtained results have shown that the leaf extract contain flavonoids and alkaloids which could be isolated further for getting individual compounds.

Keywords: *Achyranthes aspera,* Extracts, chromatography and phytochemicals.

1 Introduction

Plants are the precious gift of nature which are important part of biodiversity owing to unique medicinal values of every plant. Plants are used as both conventional and non-conventional medicines for the treatment of various diseases like malaria, diabetes, antiseptics, etc. The crude extract obtained from the plant is directly used in treating the many diseases since the prehistoric time. The world health organization (WHO) has recognized more than 20,000 species all over the world which are used as medicines [3,4]. The medicinal value is due to the presence of biologically active compounds present in the plants. Effort are being made by the scientists to discover novel drugs by identifying and isolating the phytochemicals present in the plants and these isolated compounds are directly used as drugs or synthetically modified to produce a more effective drug. Achyranthes aspera Linn. belongs to family Amaranthaceae, commonly known Rough chaff flower in English, is an annual herb that grows throughout India, consists of 160 genera and approximately 2400 species of shrubs, herbs, climbers [5]. *Achyranthes aspera* is a well-known plant in Ayurvedic, unnai, siddha, allopathic, homeopathic, naturopathic and home remedies [5]. It is also used to treat cough, renal dropsy, skin rash, nasal, fever, malaria, impotent, asthma, piles and snake bites. The flowering spikes or seeds, ground and made into a paste with water, are used as external application for bites of poisonous snakes and reptiles, used in night blindness and cutaneous diseases. For snake bites the ground root is given with water until the patient vomits and regains consciousness. It is used in indigenous system of medicine as emenagouge, antiarithitic, antifertility, antihelminthic, aphrodisiac, antiviral, antiplasmodic, antihypertensive, anticoagulant, diuretic and antitumour properties [6]. Though it has been used in traditional systems of medicines, seeds, roots and shoots are the most important parts which are used medicinally. A recent review reveals that wide numbers of phytochemical constituents have been isolated from this plant which possesses activities like antiperiodic, diuretic, purgative, laxative, antiasthmatic. For example, the dried leaf powder (2-5gms) is taken with honey for diarrhoea. Leaf juice is useful remedy for skin diseases like prutitis and scabies. Leaf paste is applied externally for toxic bites. Whole plant ash is a good remedy for bleeding piles and abdominal problems. Root is used as tooth brush to clean the mouth and to cure halitosis. Infusion of the twig is also used as a wash for toothache. Root extract is used as an eye drop at bed time for night blindness [7,8].

A number of research efforts were already made with a similar type of the genus and the reports reveal that the plant could be potentially used for treatment of cough, asthma, malarial fever, hypertension, diabetes, etc. The species chosen in the present study is a special variety that blooms in the vicinity of the study area and therefore it is chosen and methods were employed to successfully extract the components from the plant leaf extract.

*Corresponding author: e-mail collunsarunprakash@shctpt.edu,
[1]Department of Chemistry,Sacred Heart College (Autonomous)
[2]Department of Chemistry, Sacred Heart College (Autonomous)
[3]Department of Chemistry, Sacred Heart College (Autonomous),
Tirupattur – 635 601, Tamil Nadu, India

2 Experimental

2.1 Plant material

The leaves of *Achyranthes aspera* were collected from a prominent hill station Yelagiri Hills, situated at 12.5796° N, 78.6399° E, which has abundant source of medicinal plants for researchers in India [9]. The leaves of *Achyranthes aspera* plant is washed with water followed by ethanol and dried carefully in the absence of sun light to remove all water molecules present in the plant material. The dried leaves of plants are made into fine powders using domestic blender machine. The fine powder of leaves is properly stored in airtight containers for further analysis.

2.2 Preparation of Extract

About 40g of the fine powder of the plant leaves are taken in a thimble and placed in an apparatus specially designed for this purpose for the extraction of phytochemicals present in the plant leaves. The extraction is carried out successively using solvents such as n-hexane, ethanol, ethyl acetate and water in the order of increasing polarity [10]. The extracts obtained in each step are collected separately, the solvents are evaporated using vacuum distillation and vacuum dried. The dry samples are stored in an airtight container for further analysis.

2.3 Phytochemical Analysis:

The extract obtained from n-hexane was subjected to the qualitative analysis for the presence of phytochemicals. Phytochemical tests were carried out by adopting standard procedure[11,12]. Tests were performed for the presence of alkaloids, tannins, flavonoids, terpenoids, steroids and saponins and the results are recorded.

Alkaloids

2ml of extract was acidified with few drops of dilute hydrochloric acid. Then 1ml of Dragendorff's reagent was added. The appearance of orange to red precipitate indicates the presence of alkaloids.

Tannins

To 2ml of extract few drops of 10% lead acetate were added. The appearance of white precipitate indicates the presence of tannins.

Flavonoids

4ml of extract solution was treated with 1.5ml of methanol solution. The solution was warmed and metal magnesium was added to this solution 5-6 drops of concentrated hydrochloric acid were added and colour was observed for flavonoids and orange colour or flavones.

Terpenoids

To 1ml of extract add 0.5ml of chloroform followed by a few drops of concentrated sulphuric acid, formation of reddish brown precipitate indicates the presence of terpenoids.

Steroid

A small amount of sample is treated with 2ml of acetic anhydride followed by the addition of 3ml of H_2SO_4 Solution. Color changes from violet to green or blue indicates the presence of steroids.

Saponins

5ml of extract is diluted with 20ml of distilled water and agitated for 10 minutes. Foam is formed which indicates the presence of saponins.

2.4 Separation technique:

Chromatography was selected on the basis of separation achieved by TLC. TLC Plates were prepared using silica gel as the stationary phase. The plates were coated after thorough prior cleaning and rinsing with distilled water. The plates were kept in sunlight for some time and then activated in oven for 60 minutes at 120^{o}C.

The obtained plant extract is spotted on TLC plate and eluted using different combinations of eluting solvents like n-hexane, ethyl acetate and ethanol in the ratio 6:2:2 which was found to be good and efficient for separation and therefore used in column chromatography for the isolation of the compounds. Column is prepared using silica gel as a stationary phase.

2.5 FTIR Spectroscopic Analysis

Fourier transform infrared spectroscopy (FTIR) spectrum was recorded using Perkin-Elmer spectrophotometer in which the isolated compounds from the column chromatography are made into pellets using KBr.

3 Results and Discussion

3.1 Qualitative analysis

The crude extract of *Achyranthes aspera* was screened for the presence of phytochemicals and the result shows that the hexane extract of the plant leaves shows the presence of alkaloids, flavonoids and saponins.

Table 1: Phytochemical analysis of leaves of *Achyranthes Aspera*

S. No	Phytochemicals	Hexane Extract
1	Alkaloids	+
2	Flavonoids	+
3	Terpenoids	-
4	Tannins	-
5	Steroids	-
6	Saponins	+

+ = Present; - = Absent

3.2 Isolation of the compounds

The number of compounds present in the extract are identified in TLC using different solvents by trial and error method and found that solvent n-hexane, ethyl acetate and ethanol in the ratio 6:2:2 was found good for the separa-

tion of compounds. The hexane extract showed two spots in the TLC. These compounds are isolated in column chromatography successfully. The isolated compounds were tested for the presence of phytochemicals once again and it was found that the compound 1 is tested positive for flavonoid and the compound 2 is saponin which is in good agreement with the following IR spectrum.

3.3 Fourier Transform Infrared Spectroscopy

The IR spectrum of compound 1 shows characteristic peaks for flavonoid. The peaks and their corresponding vibrations are shown in the following **Table 2**.

Table 2. FTIR Peak values of compound– 1 (Flavonoid)

S. No	Peak value	Functional group
1	1272 cm^{-1}	C-O stretching
2	1472 cm^{-1}	C=C bending
3	1716 cm^{-1}	C=O stretching
4	2845-2930 cm^{-1}	C-H stretching
5	3447 cm^{-1}	OH stretching

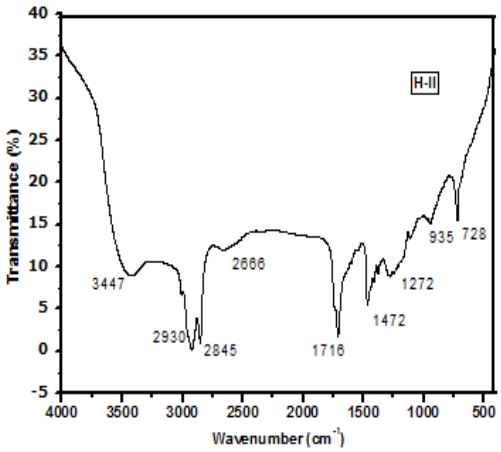

Figure 2. FTIR spectrum for compound 1 (Flavanoid)

The IR spectrum of compound 2 shows characteristic peaks for saponin. The peaks and their corresponding vibrations are shown in the following Table 3. The phytochemicals isolated using liquid column chromatography using n-hexane, ethyl acetate and ethanol in the ratio 6:2:2 as eluting solvent. Two compounds were isolated in the column, the first component was yellow in color which is found to be flavonoid and the second was green in color which is found to be saponin. The same has been endorsed by the characteristic peaks in FT IR spectra. The FT IR spectra shown in figure2 reveals the presence of carbonyl group in the range 1716cm^{-1} and OH Stretching of alcohol at 3447 which are essential functional groups in flavonoids and the other supporting stretching frequencies and their corresponding group in the molecule are listed in the table. Similarly, the FT IR shown in figure 3 is a supportive evidence for saponin in which a less intense carbonyl stretching at 1716cm^{-1} and at 1745cm^{-1} and a week alcoholic OH stretching at 3429cm^{-1}. As IR spectra does not give complete structural information about the molecule yet it supports the qualitative test by showing the expected stretching frequency of the functional group present in the molecule.

Table 3. FTIR Peak values of compoun– 2 (Saponin)

S. No	Peak value	Functional group
1	718 cm^{-1}	Methylene rocking
2	1170 cm^{-1}	C-O stretching
3	1453 cm^{-1}	Bending and fundamental Vibrations
4	1716-1745 cm^{-1}	C=O stretching
5	2856-2930 cm^{-1}	C-H stretching
6	3426 cm^{-1}	OH stretching

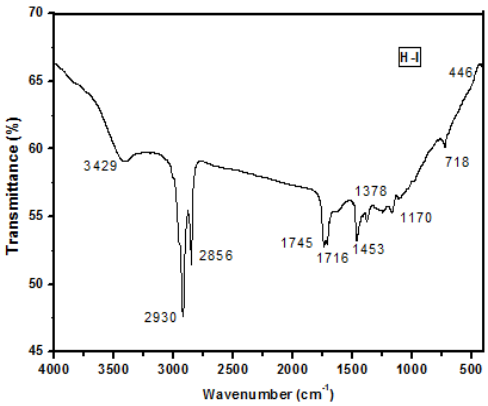

Figure 3. FTIR spectrum for compound 2 (Saponin)

4 Conclusions

The plant *Achyranthes aspera* was collected and the phytochemicals present in the plant leaves are extracted using n-hexane and the crude extract is analyzed for the presence of phytochemicals. The obtained results through solvent extraction preliminarily indicate the presence of flavonoids, alkaloids and saponins. The phytochemicals flavonoid and saponins were successfully isolated using thin layer and column chromatographic techniques. The components of the extract were characterized using qualitative chemical test which confirms the presence of flavonoid and saponin. The FTIR spectroscopic results also confirm their presence which is evident from the characteristic peaks present in the spectrum. Further separation and identification of the individual components are underway and soon to be published.

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