

Phytochemical Investigational Analysis of *Achyranthes Aspera* Linn Leaf Extract

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Abstract: - *Achyranthes aspera* is also known as *chirchita* in hindi, *apamarga* in Sanskrit, *Aghedi* in Gujarati, *apang* in Bengali, *nayurivi* in Tamil in our country. One of the many plants used is *Achyranthes aspera*. *A. aspera* Linn. belongs to the family *Amaranthaceae*, locally known as *Nayurivi* is an annual, stiff erect or procumbent, annual or perennial herb, growing up to 1-2m in height, often with a woody base, commonly found as a weed of waysides, on road sides grows throughout the world in tropical and warmer regions. *Achyranthes aspera* Linn is a well-known plant drug in Ayurveda, Unani- Tibbi, Siddha, Allopathic, Homeopathic, and Naturopathic & Home Remedies. It is an annual shrub found distributed throughout the tropical and subtropical regions. It possesses valuable medicinal properties and it is used in the treatment of cough, bronchitis, rheumatism, malarial fever, dysentery, asthma, hypertension and diabetes in Indian folklore. Present study was designed to evaluate phytochemical investigation of an aqueous extract, alcoholic extract and organic solvent extract of *Achyranthes aspera* in IR spectroscopy.

Key Words: *Nayurivi*, *perennial herb*, *tropical and subtropical regions*.

I. INTRODUCTION

Nature is a beauty source of medicinal agents for thousands of years and have a variety of number of modern drugs have been isolated from the natural source [1]. Indian systems of medicine (AYUSH- Ayurveda, Yoga, Unani, Siddha, and Homeopathy) have developed over a long period of time. Ayurveda is one of the traditional systems of medicine [2]. These medication systems usually use plant or plant products for the treatment of several disease. A number of Indian medicinal plants have been used extensively in the oldest medicinal system for the treatment of numerous disease [3]. Traditional medicine which includes herbal drug therapies has maintained its popularity in all regions of the developing world and its use is rapidly spreading in industrialized countries [4].

Knowledge of herbs in the herbal medicine have a strong traditional or conceptual base and the potential to be useful as drugs in terms of safety and effectiveness leads for treating several diseases. World health organization has made an attempt to identify all medicinal plants used globally and listed more than 20,000 species [5]. According to the WHO, more than 80% of the world's population relies on traditional herbal medicine for their primary health care [6]. Plants have an extraordinary ability to synthesize aromatic substance which are usually phenols or their oxygen substituted derivatives. The medicinally active plant compounds are usually their secondary metabolites like terpenoids, quinones, flavonoids, tannins and etc that are responsible for protecting the plants from microorganisms, insects and other natural pests [7]. One of the many plants used is *Achyranthes aspera*. *A. aspera* Linn. belongs to the family *Amaranthaceae*, locally known as *Nayurivi* is an annual, stiff erect or procumbent, annual or perennial herb, growing up to 1-2m in height, often with a woody base, commonly found as a weed of waysides, on road sides grows throughout the world in tropical and warmer regions [8]. *Achyranthes aspera* Linn is a well-known plant drug in Ayurveda, Unani- Tibbi, Siddha, Allopathic, Homeopathic, and Naturopathic & Home Remedies [9]. It is an annual shrub found

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distributed throughout the tropical and subtropical regions. It is commonly found in India, Baluchistan, Sri Lanka, tropical Asia, Africa, Australia, and America [10].



Fig.1. Leaf of Nayuruvi

This wild tropical plant is known by different names such as Chirchita (Hindi), Apamarga (Sanskrit), Aghedi (Gujarati), Apang (Bengali), Nayurivi (Tamil), Kalalat (Malayalm), and Agadha (Marathi), in India. In Unani and Ayurvedic system of medicine, leaves and fruits used as remedy for piles, renal dropsy, pneumonia, cough, kidney stones, skin eruption, snake bites, gonorrhoea and dysentery. The plant *Achyranthes aspera* has antibacterial, anti-tumor, anti-inflammatory, anti-fertility, abortifacient activity [11]. *A. aspera* is used in Indian traditional system of medicine for the treatment of ophthalmic and other eye infections.



Fig.2. Medicinal plant *Achyranthes aspera*

Various studies have been reported for its hypoglycemic effect, anti-cancer, anti-fungal, and potential to increase the thyroid hormone levels [12]. Leaf extracts were also reported to possess thyroid stimulating and anti-per oxidative properties [13]. The plant *A. aspera* is used in indigenous system of medicine also as emenagogue, anti-arthritis, anti-plasmodic, diuretic, anticoagulant, [14]. antihypertensive, antiviral, aphrodisiac, anthelmintic, ecobolic and laxative. It is useful to treat cough, renal dropsy, fistula, scrofula, skin rash, nasal infection, chronic malaria, importance, fever, asthma, piles and snake bites [15]. This plant is astringent, digestive, laxative, purgative and stomachic. The juice of the plant is used in the treatment of boils, dysentery, hemorrhoids, rheumatic pains, itches and skin eruptions [16]. It is reported to contain alkaloids, flavonoids, saponins, steroids and terpenoids. Flavonoids have shown to prevent or slows the development of some cancers and mostly act as an anti-oxidant and anti-inflammatory [17].

1.1 Taxonomical Classification:

Kingdom	: Plantae
Subkingdom	: Tracheobionota
Super division	: Spermatophyta
Division	: Mangoliophyta
Class	: Mangoliopsida
Subclass	: Caryophyllidae
Order	: Caryophyllales
Family	: Amaranthaceae
Genus	: <i>Achyranthes</i>
Species	: <i>Aspera</i>

1.2 Geographical Source:

The plant is widespread in the world as a weed, in Baluchistan, Ceylon, Tropical Asia, Africa, Australia and America. It is found on roadsides, field boundaries and waste places as a weed throughout India up to an altitude of 2100 m in South Andaman Islands [18]. In the northern part of India it is known as a medicinal plant in different system of folk medicine.

1.3 Pharmacognosy:

Nayuruvi is an erect herb, 0.3-1m high stiff branches terete or absolutely quadrangular, striate, pubescent, leaves few, usually thick, elliptic-obovate, petiolate, acute and entire. Flowers are greenish white, numerous in small dense auxiliary heads or spikes, bracts and bracteoles persisting ending in a spine.



Fig.3. Plant of achyranthes aspera

Main root is long cylindrical thick; secondary and tertiary roots present slightly ribbed, yellowish brown in color; odor is slight; taste is slightly sweet and mucilaginous; stem is yellow brownish, erect branched, cylindrical hairy about 60cm high. Seeds are sub cylindrical, truncates at apex, rounded at base, black and shining. The plant is distributed throughout India up to an altitude of 3000ft. The plant was studied pharmacognostically and observed an average stomata index of 6.6, average palisade ratio of 9.2, average vein islet number 9 and average epidermal cell count 360 [19],[20].

1.4 Traditional Uses:

Achyranthes aspera L. (Family: Amaranthaceae) is a common plant of the study area abundantly found in wastelands.



Fig.4. Achyranthes aspera

It is known as “Prickly chaff flower” in English and “Chirchita”, “Onga”, “Latjeera” or “Apamarga” in local language and dialects. The plant is highly esteemed by traditional healers and used in treatment of asthma, bleeding, in facilitating delivery, boils, bronchitis, cold, cough, debility, dropsy, dog bite, dysentery, ear complications, headache,

leucoderma, pneumonia, renal complications, scorpion bite, snake bite and skin disease etc[21]. Traditional healers claim that addition of *A. aspera* would enhance the efficacy of any drug of plant origin.

II. MATERIALS AND METHODS

2.1 Drugs and chemicals:

Ethanol and diethyl ether (Best scientific Pvt. Ltd. Dharmapuri), were used in this study. All the reagents were prepared freshly before use and all the reagents used were of analytical grade.

2.2 Plant materials:

Achyranthes aspera was selected for the current work. The stems and leaves of the plant were collected from the fields of Periyampatti in the month of Nov. 2022. It was identified and authenticated by Dr. K. Gowrishankar, Head and Assistant professor, PG & Research & Department of Botany, Sri Vijaya Vidyalaya College of Arts and Science, Nallampalli. A voucher specimen (mvm-H/Am-01) has been deposited at the herbarium of the same institute. The collected stems and leaves were washed, shade dried and milled into coarse powder using electric grinder and stored in an air-tight container at 25°C.

2.3 Preparation of extracts:

Method 1:

The leaves were shade dried at room temperature and milled into a coarse powder. The ethanolic extract of *A. aspera* leaves were prepared by Maceration after defatting with petroleum ether. The extract was filtered through a cotton cloth and evaporated under 40°C up to one third of initial volume, remaining solvent were evaporate. The brownish residue (yield 16.74% w/w) designated as ethanolic extract of *A. aspera* (EEAA) was employed for the experimental studies.

Method 2:

Dried powdered materials were placed in the round bottom flask to obtain sequential extracts of different solvents ranging from non-polar to polar-petroleum ether, benzene, chloroform, ethyl acetate, ethanol and distilled water by placing them in in 250ml round bottom flask. The materials were kept with the solvents for about 12-14 hours. Extracts were collected and kept at room temperature and poured in glass petri dishes and the evaporated at 40°C using hot air oven. Dried extracts were kept in desiccators for 2days and stored at 5°C in air tight container. Extraction was made by using variety of solvents such as alcohol, petroleum ether, benzene, chloroform, ethyl acetate etc, in this research work, extraction was made by using

ethanol and ethyl acetate and the current investigation was planned to evaluate the phytochemical screening of different sequential extracts of stems and leaves of *A. aspera*.

III. EXPERIMENTAL ANALYSIS

3.1 Chemicals and reagents:

Ferric chloride, gelatin, HCl, dragendroff's reagent, methanol, gallic acid, sulfuric acid, tannic acid, acetic acid, acetic anhydride, fehling solutions were all purchased from Best scientific, Dharmapuri. All other unlabeled chemicals and reagents were of analytical grade and of high purity.

3.2 Qualitative Phytochemical Screening:

The phytochemical screening was performed by standard methods by using dried powdered materials of *A. aspera*.

3.2.1 Test for alkaloids:

a) Mayer's test:

Test solution (1ml) was taken in test tube and few drops of Mayer's reagent (Potassium mercuric iodide solution) were added into it and cream color precipitate was observed.

b) Dragendroff's test:

Test solution (1ml) was taken in test tube and few drops of Dragendroff's reagent (potassium bismuth iodide solution) were added into it and observed for reddish brown precipitate.

c) Tannic acid test:

Test solution (1ml) was taken in the test tube and few drops of 10% tannic acid solution was added to it and observed for buff coloration.

3.2.2 Test for tannins:

a) Gelatin test:

About 1ml of test solution was taken in a dried test tube and 1% gelatin solution was added followed by 10% sodium chloride solution and observed for white precipitate to form.

b) FeCl₃ test:

About 0.5mg of dried powdered sample was boiled in 20ml water in test tubes and filtered. A few drops of 0.1% ferric chloride was added and observed for blue-black coloration.

c) Vanillin hydrochloride test:

Test solution was treated with few drops of vanillin hydrochloride reagent and observed for purplish-red color.

3.3 Test for cardiac glycosides:

3.3.1 keller killiam test:

Test solution 1ml was taken in a test tube and 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride was added to it.

Carefully added 0.5ml of concentrated sulphuric acid by the side of the test tube and observed for blue color to be appearing in the acetic acid layer.

3.3.2 salkowski test:

Test solution 1ml was taken in a clean and dried test tube and 2ml chloroform and few drops of sulphuric acid were added to it. Shaken well and allowed to stand for some time and observed for reddish brown color at interface.

3.4 Test for flavonoids:

3.4.1. Alkaline reagent test:

About 1ml test solution was treated with few drops of sodium hydroxide solution and observed for intense yellow colouration which disappears on the addition of dil. HCl.

3.4.2. Lead acetate test:

Test solution 1ml is taken in a test tube and few drops of lead acetate solution was added to it and observed for yellow colored precipitate.

3.5. Test for terpenoids:

Test solution 1ml was taken in clean and dried test tube and 2ml chloroform and few drops of sulphuric acid was added into it. Shaken well and allowed to stand for some times and observed for reddish brown color at interface.

3.6. Test for proteins:

3.6.1. Ninhydrin test:

Test solution were boiled with 0.2% solution of Ninhydrin and observed for violet color to appear.

3.7. Test for reducing sugar:

3.7.1. Fehling test:

Test sample 1ml was taken into a clean and dried test tube and 0.5ml of Fehling A and B solution were added to it, boiled and observed for brick red coloration.

IV. RESULTS

Mobile phase of different composition was tested for IR-Fingerprinting analysis of *Achyranthes aspera* samples and oleanolic acid in high resolution and reproducible results. The desired objective was achieved by use of toluene: ethyl acetate: formic acid (4.5:0.5:0.1 v/v) as mobile phase, which gave a peak at RF value 0.54. the peak obtained in IR spectroscopical analysis of leaf extract of *Achyranthes aspera* using ethanol, aqueous and ethyl acetate as a organic solvent was shown in the figure 5,6 and 7.

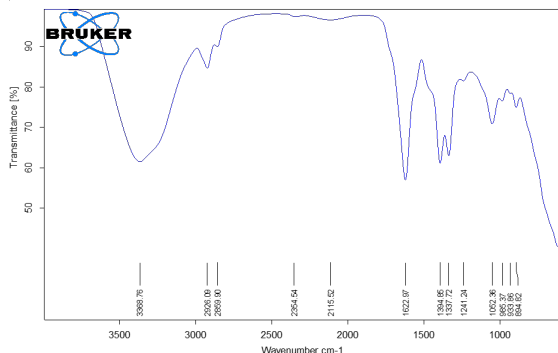


Fig.5. IR spectroscopy- ethanolic leaf extract of Achyranthus aspera

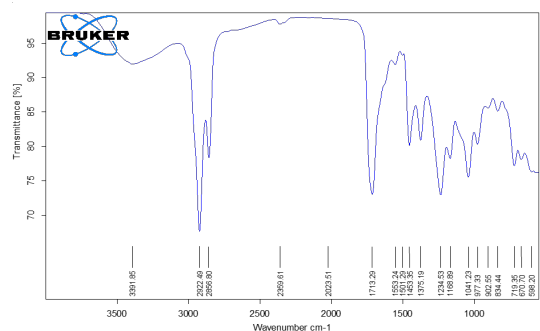


Fig.6. Organic solvent- Ethyl acetate leaf extract of Achyranthus aspera

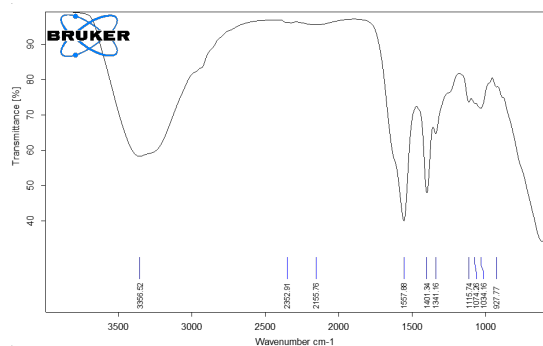


Fig.7. Aqueous leaf extract of Achyranthus aspera

V. CONCLUSION

Evaluated phytochemical investigation of an aqueous extracts, alcoholic extract and organic solvent extract of Achyranthus aspera in IR spectroscopy. The desired objective was achieved by use of toluene: ethyl acetate: formic acid (4.5:0.5:0.1 v/v) as mobile phase, which gave a peak at RF value 0.54. the peak obtained in IR spectroscopical analysis of leaf extract of Achyranthus aspera using ethanol, aqueous and ethyl acetate as an organic solvent.

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