

Phytochemical Screening and Antimicrobial Activity of Herbal Plant Extracts- *Achyranthus aspera*

Shahazadi Parveen

Shri Ram College of Pharmacy, Banmore, Ab Road, Madhya Pradesh 476444

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ABSTRACT

The plant species *Achyranthes aspera* from the family Amaranthaceae is a vital healthful and medicated herb found throughout in India. Although most of its components are employed in ancient systems of medicines such as seeds, roots and shoots are foremost necessary components that are used for treatment of disease. The article provides associate degree account on updated info about phyto-chemical and anti-microbial activity. Chloroform and methyl alcohol root and shoot extracts of *A. aspera* showed sensible quantity of medication activity against enterobacteria sp. Root extract showed the activity against *B. Subtilis* solely antifungal activity of roots was found in extracts with petroleum ether, chloroform and methyl alcohol against *fusarium* sp. *Phytophthora* and *Sclerouum* sp. Results recommend that extract has important medication and antifungal activities against tested microorganisms.

Keywords: Antimicrobial; *achyranthus aspera*; phytochemical screening; disc diffusion.

INTRODUCTION

An antimicrobial as an agent which is used to kill microorganisms and stop the growth. Antimicrobial medicine can grouped on basis on which microbes primarily acts against, such as, an antibacterial i.e used against bacterial growth and antifungal i.e. used against fungi growth. they can also be grouped on basis of their functional properties. Agents which kill microorganisms are called bacteriocidal, whereas that causes inhibition of growth are called as biostatic. The anti-bacterial chemotherapy done by using antimicrobial medicines which are used for treatment of infection, while those antimicrobial medicines used for prevention of inflectional diseases is called anti-microbial prophylaxis.

antimicrobial agents are mainly classified as the disinfectants ("non-selective anti-microbials", bleach), are used kill wide varieties of microorganisms and different microorganisms on non living things and the antiseptics are used for applied to livings tissues/bodies and the antibiotics destroy microorganisms inside the body. Antibacterial agents can also be further sub-divided in bactericidal agent, that are used to killing bacteria, and the bacteriostatic agent that slows down and still bacteria growth.

Achyranthes aspera (Amaranthaceae) is an important medicinal herbal plant found throughout in India. The parts which are used medicinally are seeds, roots and shoots. The information of updated data about the phytochemical properties and pharmacological properties of the herbal plant *achyranthes aspera* is all given by the present article. It reveal that wide types of the phytochemical constituents have been isolated from the plant which possesses many pharmacological activities and many various important

medicinal properties. For the last few decades or so, extensive research work has been carried out to prove the pharmacological activities and biological activities of extracts. oleonic acid, saponins, alkaloids, dihydroxy ketones, long chain compounds and many compounds have been isolated.

The plant *achyranthes aspera* extracts for antioxidant and antimicrobial activity consists of chemical constituents⁴. These extracts have greater minimal anti-bacterial activity⁵. For the work, *Achyranthes aspera*, local name: latjeera; family: Amaranthaceae was used⁶. *Achyranthes aspera* is mostly found and used as herbal plant in Bangladesh for treatment of infectious diseases. The study was conducted to approach antimicrobial activities against pathogenic bacteria when extraction was done with their leaf and stem parts in various organic solvents such as petroleum ether, methanol, ethyl acetate, chloroform.

REVIEW OF LITERATURE

Learning of herbs and plants utilizing from age to age for a long time as a major aspect of conventional time. These herbs and plants are utilized for drug on account of effortlessly accessible and cheap. *Achyranthes aspera* is an imperative restorative plant. The present research demonstrates refreshed data/edification for pharmacological exercises and against bacterial properties¹. In the field of clinical microbiology, the Nature has been a great wellspring of phyto-chemical operators². Phyto-chemicals contain an extensive variety of segments that is successful for bacterial sicknesses for instance urinary tract contaminations³. The utilization of plant extricate for therapeutic medicines is revering incredible prominence since 1990s. These phyto-chemical

Plant profile



Dried Roots Of Achyranthes Aspera

Table 1: Macroscopy of Achyranthesaspera Linn (leaf)

Particulars	Observation
Type	Simple
Apex	Slightly acuminate
Margin	Wavy
Veination	Pinnate
Shape	Obovate
Texture	Pubescent due to presence of thick coat of long simple hairs
Petiole	Subsessile, exstipulate
Phyllotaxy	Opposite

Table 2: extractive values of achyranthesaspera linn (leaf)

S.no.	Amount of powered drug	solvent used	% of extract
1	25g	chloroform	3.76% w/w
2	25g	Petroleum ether	3.16% w/w
3	25g	water	4.32% w/w
4	25g	ethanol	2.36% w/w

drugs are valuable when individual's abuse of conventional anti-infection agents that reason tranquilize protection.

Achyranthes aspera is a typical plant found all through India. This survey is engaged of union on the wide pharmacological exercises of *Achyranthes aspera* L. Prior to the presentation of current prescriptions, home grown cures was utilized for the treatment of maladies. It is evaluated that 80% of the total populace dwelling in the tremendous rustic regions of the creating and immature nations as yet depending principally on restorative plants.

It is very evident that in the conventional restorative framework plants are broadly utilized as a part of India and has been accounted for to have with hepatoprotective, mitigatin. *Achyranthes aspera* is a critical plant for huge uses as therapeutic properties and additionally restoratively vital chemicals for instance achyranthine, tritriacontane, ecdysterone, betaine, 6-pentatriacontanone, hexatriacontane and pentatriacontane. The plant demonstrates numerous pharmacological exercises like, against unfavorably susceptible, nephron-defensive, hostile to parasitic, cardiovascular, hypoglycemic, pain relieving and antipyretic.

Phytochemistry

Constituent examinations *Achyranthes aspera*'s seeds by V.Harihaaran, Mohd. Ali 1993 and S. Rangaswamy 1970 detailed disengagement and distinguishing proof of Saponins 'A' and 'B'^{16,18}. Saponin-A distinguished D-Glucouronic Acid. Saponins-B recognized as compound β -D-galactopyranosyl ester of D-Glucouronic.

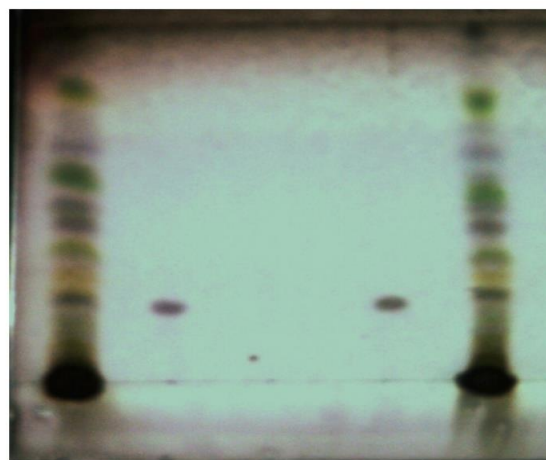
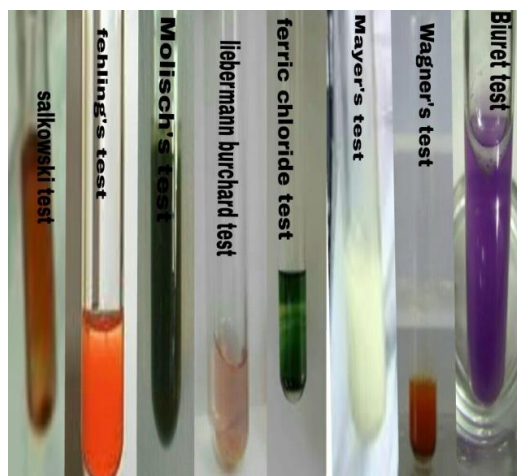
Investigation by R.D. Rameshwar uncovered three different oleonolic corrosive glycosides by seeds of *Achyranthes aspera* recognized as 1. α -L-rhamnopyranosyl-(1 \rightarrow 4)(β -D-glucopyranosyluronic corrosive) (1 \rightarrow 3) oleonolic corrosive, 2. α -L-rhamnopyranosyl-(1 \rightarrow 4)-(β -Dglucopyranosyluronic corrosive) (1 \rightarrow 3) oleonolic corrosive 28O- β -Dglucopyranoside and 3. α -L-rhamnopyranosyl(1 \rightarrow 4) (β -D-glucopyranosyluronic corrosive) (1 \rightarrow 3)- oleonolic corrosive 28O- β -Dglucopyranosyl (1 \rightarrow 4) β -Dglucopyranoside¹⁹.

Spermicidal Activity

Paul D et al. in 2010 examined impacts on different concentrates from underlying foundation of *A.aspera*, revealed spermicidal movement on human and rodent sperms. Chloroform, hydroethanol and n-hexane extricates

Table 3: Qualitative chemical analysis of the leaf extract of *Achyranthes aspera* linn.

S.no.	Test for phytochemicals	Powder+ Water	Petroleum ether extract	Chloroform extract	Ethanol extract	Aqueous extracts
1.	Alkaloids: dragendroff test hager test Mayer test Wagner test	Negative	Negative	Positive	Negative	Positive
2.	Carbohydrates: fehling's test	Negative	Negative	Negative	Positive	Positive
3.	Proteins: Millon's test	Positive	Positive	Negative	Negative	Positive
4.	Glycosides: Balget's test Legal's test Keller-killiani test	Positive	Negative	Negative	Negative	Positive
5.	Tannins: Aq. Fecl3 test Alc. Fecl3 test	Negative	Negative	Negative	Positive	Positive
6.	Flavonoids: Lead acetate test shinoda test	Positive	Negative	Negative	Positive	Negative
7.	Saponins: Foam test Lead acetate test	Positive	Negative	Negative	Positive	Positive
8.	Steroids: Salkowski test libermann test	Positive	Positive	Negative	Negative	Negative

Phytochemical screening of roots of *Achyranthes aspera*TLC identification of terpenoids in *Achyranthes aspera*

observed to best sperm immobilisation, sperms suitability, achrosome status, 5' nucleotides action and atomic chromatinin decondensation. Vasudev and S. Sharma in 2006 announced ethanol concentrate of base of *A.aspera* L indicates post-coital Anti-fertility action on pale skinned person mices. separate displayed 83.4% against action administered via oral route 250 mg/kg. V. Shibashi et al in 2006 considered impacts on methanol concentrate of leaves, announced to the anti-fertility exercises, for example, estrogenicity, abortifacient, pituitary wgt and ovary hormones level, lipid profiles in mices. abortifacient impact of methanol concentrate of leaves of *A.aspera* controlled including dead hatchlings in-vivo. Impact in

estrogenicity surveyed taking proportion of uterine volume to body mass. Proportion of pituitary volume to body wt. additionally computed. impact of concentrate in level of ovary hormone and lipids profile assessed utilizing electrochemiluminescence immunobioassay. Pakrash and N. Bhattachary in 1977 revealed that constituent benzene concentrate of entire *A.aspera* indicates abortifacient movement in female rats. Paul.D et al in 2006 revealed half ethanol concentrate of leaf of *Achyranthus aspera* and *Stephania hernandifolia* demonstrates impact in sperms motility as well as capacity in proportion 1:3 ratio weight on various fixations.

AntiparasiticActivity

TLC result of methanolic extract

Stationary phase(silica gelcoated) mesh 60 to 100 size

Sample	Solvent system (7.8:2.2:0.75)	No. Of spot	Rf values
Root extract	Toluene:methanol:formic acid	7	0.19,0.20,0.45,0.5,0.66,0.82
Standard (oleanolic acid)	Toluene:methanol:formic acid	1	0.19
Standard (ursolic acid)	Toluene:methanol:formic acid	1	0.20

M. A. Zaheer et al in 2009 detailed that ethyl acetic acid derivation concentrates of *Achyranthus aspera* Linn indicates anti-parasitic movement (blossom, dried leaves and seeds extricate) against hatchlings to cows microplas (canestrine, 1887), tick *Rhipicephallus* (*Bophilus*), (*Acari-ixiodidae*), parasite *Paramphistomucervi* sheep. A. Baghavan et al in 2008 examined CH₃CO, ethyl acetic acid, chloroform, methanol and hexane leaf concentrates of *A.aspera* against mid fourth-insta hatchlings on *Aedesegypti*.L, *Culexquinque fasciatus*. larval mortality seen after 24 hours introduction. Concentrates indicated direct larvacidal impacts; be that as it may, the most astounding larvae mortality found in ethyl acetic acid derivation concentrate of *A.aspera* L. In present examination, bioassay fractionation on *A.aspera* prompted partition distinguishing proof of saponin as mosquito larvicidal component with LC₅₀ estimation 18.22 and 27.25 ppm against *C.quiuefasciatus* and *A.aegyptis* separately. ¹³C NMR, ¹H NMR, mass spectroscopy unearthly information affirmed recognizable proof of dynamic compounds. This is primary provide details regarding mosquito larvacidal action of saponins from ethyl acetic acid derivation concentrate of *Achyranthus aspera* L.

Hypoglycaemic Activity

Mohd.S. Akhtar and Mohd. Iqbal in 1991 considered fluid with methanol concentrates of powdered entire *A.aspera*, which indicates hypoglycemic movement. Blood-glucose level of typical and Alloxon instigated diabetes in rabbit was resolved by oral organization on different measurements.

Tumor Chemopreventive Activity

Chakraborty A et al in 2002 announced that methanol concentrates of powdered leaves, non-alkaloids, alkaloids and saponins portions demonstrates malignancy chemopreventive activity in Epsteins-Bar infection antigen initiation instigated tumors promoting 12'-O-tetradecanacylphorbol-13 acetic acid derivation inside Rajii cell.

Hepatotoxicity action

R. Bafna and S. Mishra in 2004 detailed that methanol concentrate of t ethereal parts of *A.aspera* demonstrates hepato(lever)-protective movement with rifampicin instigated toxicity pale skinned person rat. Methanol remove demonstrated dosage subordinate lessening in level of SGOT, SGPT, ALKP and aggregate bilirubin serum.

Antipyretic and analgesic Activity

N.G Sutar et al. 2008 revealed methanol concentrate of stems and leaves to pain relieving & antipyretic exercises utilizing hot plate technique and brewer's yeast actuated techniques utilizing headache medicine as standard medication. A. Mehta et al. 2009 examined seeds and

leaves of *A.aspera* indicate pain relieving movement. Two leaves and seeds demonstrate pain relieving action in mice utilizing acidic corrosive instigated squirming reaction & eddy's hot-plate strategy. Hari Kumar et al. 2009 detailed for hydro- alcohol concentrate of root and leaves of *A.aspera* indicate midway pain relieving action on grown-up pale skinned person rat utilizing tail flicking, hot-plate method and acidic corrosive instigated squirming strategy for incidentally acting pain relieving movement utilizing headache medicine as standard medication.

Anti-Inflammatory Activity

S.Vijay et al. 2009 considered alcohol concentrate of *A.aspera* indicates calming movement in rats utilizing carragenan-incited paw-edeema technique & cotton-pellet granuloma method. Alcohol concentrates of seeds and leaves demonstrate calming action on rat utilizing carragenan-instigated paw edema technique & formlin display. T. Vetrichevan and Jegadesan 2003 detailed liquor concentrate of *A.aspera* tried on carragenin-actuated rear paw edema & cotton-pellet granuloma method in pale skinned rats (male). Paw volume estimated plethysmometrically on 0h, 1h, 2h, 3h, 4h and 5 h and diclofenac Na utilized as standard medication. Liquor separate (350mg/kg and 400 mg/kg) demonstrated greatest restraint oedema on 65.37% and 72.38%, individually. the concentrate displayed 40.02% and 45.33% diminishment on granuloma weight test. A. Gokhal et al. 2002 revealed that ethanol concentrates of *A.aspera* at measurements of 50mg/kg, 100mg/kg & 200 mg/kg screened for impact in intense, perpetual irritation prompted in rat & mice utilizing carragenan and Freund's entire adjuvant technique.

Anti-Biotic Activity

Mohammed J. Khan et al.2010 detailed that chloroform and ethanolic concentrates of seed of *A. aspera* L indicates gentle direct anti-toxin movement against *E.coli*, *B. subtilis*, *E. aeruginosa*. S.R. Prashad et al. 2009 examined about different concentrates of callus and leaf likewise demonstrates antibacterial movement. P.Saravnan et al. 2008 detailed dissolvable leaves removes was tried for anti-bacteria and anti-fungi exercises across *P.aeruginosa*, *E. coli*, *P. vulgaris*, *P.aureus*, *Klebsiella*. N. Mishra et al. 1992 detailed 17-pentatriconetanol as main constituents separated fundamental oil of shoot of plant, oil indicates antifungi movement against *Aspergilluscarneus*. S. Sharma 2006 contemplated alcohol concentrate which demonstrates nearness of triterpenoidal saponin dosage subordinate inhibition movement against *S.aureus*. Least inhibition fixation observed most elevated 0.15 milligram purged part. Manjulika et al. 2009 examined concentrates of *A. aspera* judging antimicrobial movement against several pathogenic strains, For example, *E.coli*, *P.aeruginosa* and *Citrobacter* species, *B.subtilis* and

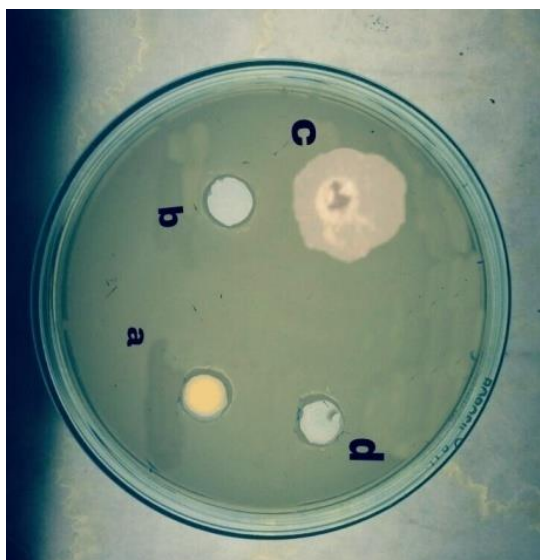


Figure 1: Anti-bacterial activity against *Pseudomona*.

- (a) Negative control
- (b) Positive control (Imipenem)
- (c) Methanolic extract
- (d) Pet. ether extract



Figure 2: Anti-bacterial activity against *Proteus*.

- (a) -tive Controls
- (b) Resistance-Polymyxin
- (c) +tive controls Ampicillin
- (d) Ethanolic extracts

Micrococcus species utilizing circle dispersion plate method. Phytochemical screening by using thin layer chromatography system. concentrates of *A. aspera* demonstrates the greatest restraint of *Escherichia coli*. *A. aspera* indicates dominating restraint in contrast to gram negative microscopic organisms at higher centralization of 55µg/ml. In plate technique restraint zone varies from 8mm to 17mm against pathogen strain.

Anti-oxidant Activit

A. Kar and P. Tahilani in 2000 examined different concentrates of leaves against oxidation movement. D. Gayatri in 2009 likewise revealed cell reinforcement movement of roots and leaves. T. Malarveli and Ghomati 2009 detailed cell reinforcement movement of seeds. *A. aspera* all around reported for nearness of phytochemical-active components. Diminishment on lipids peroxidation rate and upgrade of free ion radicals searching action home grown seeds powdered because quality of phytochemical active components. Edwin S. et al. 2008 announced free ion radicals searching movement of ethanol and fluid concentrates. The two concentrates were surveyed utilizing two techniques, DPPH radical rummaging movement, and superoxide searching action. The plant displayed great cancer prevention agent impact by keeping the development of free-ions second models contemplated.

Nephroprotective Activity

T. Jaykumar in 2009 revealed methanol concentrate of entire *A. aspera* L. demonstrates nephro-protective movement against acetic acid of lead salts derivation prompted nephrotoxicity in male pale skinned person rat.

Anti depressant Activity

C.K. Barau et al. 2009 demonstrated that Methanol concentrate of leaves of *A. aspera* indicates upper impact in rat and mices utilizing constrained swim test of mices and tail suspension test of rat.

Diuretic Activity

S. Gupta 1972 detailed saponin disengaged from seeds of *A. aspera* indicates huge diuretic impact on grown-up pale skinned male-rat. Achyranthine (orally given) have diuretic movement in male-rat.

Broncho-protective Action

B.R. Goyal in 2007 detailed ethanol concentrate of *A. aspera* indicates bronchial protection impact on toluene di-isocyanate prompted word related to asthma on rats. Aggregate WBCs were checked inside broncho alveolar serum and blood. Liver hemogenate were used to check evaluation of oxidation pressure and histology of lungs was perform to research aviation routestatus.

Achyranthine, a water-dissolvable alkaloid separated from *Achyranthes aspera*, diminished pulse and heart rate, enlarged veins, and expanded rate and adequacy breath of frog and canines. Contractile impact of alkaloid (0.5 mg per ml) in frog rectum muscular strength were not as much as of acetylcholine (0.2 mg per ml), its spasmogenic impact not hindered on tubocurrarine.

Antiallergic action

S.V. Dateer in 2009 announced that oil extricate of plant demonstrates anti-allergy movement in drain instigated leukocytosis and drain actuated eosinophillia in rat. In this way antiallergy movement might because of non-polar compounds. Phytochemical screening for ether-oils separate demonstrates nearness to steroidal compounds like ecodysone, β-sitosterol and ecodysterone.

Wound Healing Action

Edwin S. in 2008 explored ethanol and watery concentrates of *A. aspera* for recuperating wound movement. Injury mending movement were examined utilizing injury model, cut injury show and wound extraction model.

Immunostimulant action

R.Y. Vasudev and R. Chakrabarti demonstrated immunostimulant movement in catlas. *A. aspera* altogether $P < .05$ improved

BSA-particular immune response than non-treated sample bunch all through investigation time frame. Effectiveness of the antigens likewise improved.

Hyperlipidemic movement

A.Khanna in 1992 examined alcoholic concentrate of *Achyranthes aspera*, 100 mgperkg measurements brought down serum cholesterol, phospholipid, triglyceride and aggregate phospholipid level up 60%, 52%, 34% and 52% individually on trion instigated hyper-lipidemic mice. constant organization of medication on similar measurements to ordinary mice for 25 days, brought down by 55%, 61%, 66% and 68% individually took after noteworthy diminishment in level of lipid. feecal discharge of deoxycholic & cholic corrosive expanded by 41% and 25% individually under activity medication. Conceivable component activity of cholesterols bringing down movement because of quick discharge of bile acids causing low absorption of cholesterol.

Aim and Objective

The aim of present study is

To standardize the parts of *Achyranthes aspera* by carrying out phytochemical studies.

To evaluate antimicrobial activity of various extracts of plant *achyranthus aspera*

To evaluate the in-vitro antimicrobial activity of the various extracts of plant of *achyranthus aspera*

Plant Introduction

Biological name: *Achyranthus aspera* Linn

Synonyms: *Centrostachys indica* (L.) Standl

Stachyarpagophora aspera maza

Family: Amaranthaceae

Vernacular Names

Latin - *Achyranthes aspera*

Sanskrit - Aghata

Hindi - Latjira, Chirchira

Gujarati - SafadAghedo

Telugu - Uttaraene

Tamil- Shiru-kadaladi

Punjabi - Kutri

Malayalam-Kadaladi

Ayurvedic -chirchita, Apaamaarga, Shikhari

Unani- Chirchitaa

Persian - Khare-vazhun

French - *Achyranth a feuilles rudes*, gendarme, collant,

Spanish - Mosotillo, rabode chango rabo de gato, rabo de raton

Arbain - Atkumah

Taxonomy

Kingdom – Plantae

Subkingdom - Tracheobionota

Super Division - Spermatophyta

Division - Mangoliophyta

Class - Mangoliophsida

Subclass - Caryophyllidae

Order - Caryophyllales

Family - Amaranthaceae

Genus - *Achyranthes*

Species - *Aspera*

Geographical Source

Found at many places like, on roadsides, waste places and field boundaries as a weed throughout India up to the altitude 2100m, in Southern Andaman Islands^{8,10}. plant is widespread in countries like Tropical Aisa, Ceylon, america, Australia and Africa

Habitat

elevations about 2000m (open dry places) in Nepal.

Rang

East Asia: Himalayas-Australia.

Morphology

Achyranthes aspera L. (Latjeera) is procumbent or an erect. The herb is annual/ perennial about 1m- 2m in height, generally, woody-base. Stems are ribbed, angular sometimes branched from the base, light purple in colour⁸. Branches are absolutely quadrangular or terete, pubescent, striate⁹. The leaves are thick 3.8cm - 6.3cm × 22.5cm - 4.5cm^{8,9}, obovate-rounded or ovate-elliptic⁸. They are finely and softly pubescent on both sides, entire, petiolate, petiole 6 – 20 mm long⁹. The flowers are greenish-white colour. These are numerous axillary and terminal spikes about 75cm long. Seeds are truncate at apex, sub-cylindric, rounded at base and reddish brown colour.

Part Used

Generally Leaves, stem, seeds, root are used. i.e, whole plant.

Chemical Constituents

Achyranthes aspera consists of tri-terpenoid saponins possess Ecdysterone which is an insect-moulting hormone, oleanolic acid as aglycone. The long-chain of alcohols are also found in plant²¹. Other chemical constituents are also present in plant for example betaine, achyranthine, hextriacontane, pentatriacontane, tritriacontane, 6-pentatriacontanone²¹.

Ethnomedical Uses

It is Used in treatment of pneumonia, cough, cold, bronchitis.

It is also Used as an antimicrobial agent.

Side Effects

It may cause vomiting when given in high dose.

It is not suitable for using in pregnant ladies.

For men, who is undergoing infertility treatment should better avoid long term usage of apamarga.

Methodology

Collection of Specimen

Fresh leaves from the plant *Achyranthus aspera* was collected from rajaji national park, Dehradun in the month of January 2018. The leaves was collected from plant by means of plucking. The plant was identified and authenticated by Prof. Sangeeta Gupta (pharmacognosy professor). The plant material was certified as *Achyranthes aspera* of the family Amaranthaceae and certificate number is (PARC/2018/2054)

Phytochemical Screening

The powder of the entire plant *achyranthes aspera* was air dried. It was weighed 100gm and successively extracted in Soxhlet apparatus with the solvents on the basis of increasing polarity for example petroleum ether (600 – 800 C), chloroform, acetone, methanol & water. The extracts were dried by the means of using rotary evaporator. The percentage extractive value was determined. After this,

Table 1: Inhibition of growth of bacteria for fraction extract sample & zones measurement.

Medicinal plant	Target organism	Zone of the inhibition produced in different organic solvent				
		Pet.ether	Methanolic solvent	Ethanollic solvent	Ethyll acetate	Chloroform solvent
<i>A. aspera</i> (leaf)	<i>Pseudomonas</i>	12	12	13	14	12
	<i>Proteus</i>	21	14	10	12	17
	<i>E. faecalis</i>	22	7	9	7	8
<i>A. aspera</i> (stem)	<i>Pseudomonas</i>	14	6	17	21	21
	<i>Proteus</i>	12	8	14	18	17
	<i>E. faecalis</i>	–	–	–	–	–

The dry extracts were screened for the presence of various phyto-constituents / secondary metabolites which are responsible for the therapeutic values of the drug for example presence of tannins, alkaloids, glycosides, carbohydrate, tannins, proteins & amino-acids, gums & mucilage, flavours & flavonoides, saponins and steroids & Sterols etc^{13,14}. The resulting data obtained were recorded in the table.

Preparation of Extracts

Extraction is the preliminary *step* involved in the phytochemical studies. It brings out the metabolic into the extracting solvent depends upon its polarity. Hot percolation method is used for the extraction.

Hot Percolation Method

About 200gm coarsely powdered bark of *Achyranthes aspera* was first extracted with petroleum ether in Soxhlet apparatus and then with solvents with increasing polarity like 1.chloroform, 2. ethyl acetate and 3.ethanol at 60-70 degree Celsius. Each extract was concentrated using rotary vacuum evaporator. The percentage yield, colour and consistency of these extracts were recorded and proceed for further detailed phytochemical and pharmacological screening.

Preliminary Phytochemical Screening

The chemical tests was carried out for various phyto-constituents in the dried powder and extracts of bark of *Achyranthes aspera* were carried out as described below.

Detection of Alkaloids

The Methanolic extract warmed with H₂SO₄ of 2% for 2 minutes. It was filtered. then Few drops of reagents was added which indicated the presence of alkaloids.

Dragendroff's Test

To extract (5ml) few drops of Dragendroff's reagent added. It given formation of orange color precipitate. A orange precipitation indicated the positive test for presence of alkaloids.

Mayer's test

To extract (5ml) few drops Mayer's reagent were added which given formation of creamy-white colour precipitate. The creamy-white precipitation indicated presence of alkaloids.

Wagner's test

To extract (5ml) few drops of Wagner's reagent added which formed Reddish-brown color precipitate. reddish brown coloured precipitation indicated presence of alkaloids.

Hager's test

To extract (3ml) few drops of Hager's reagent Picric Acid (1%) was added. formation of prominent yellow color

precipitate took place. presence of yellow color precipitation indicated positive test for alkaloid.

Test For Flavonoids

A small quantity extract was heated with ethyl acetate (10ml). It was done boiling water for about 3 minutes. The mixture was filtered. filtrates were used for following test.

Ammonium Test

filtrate was shaken with dilute ammonia solution (1ml of 1%). separation of layer was allowed by keeping it stable. A yellow color was observed at ammonium layer in test tube. these Indicated the presence of flavonoids.

Aluminium-Chloride Test

The filtrates were shaken with aluminum chloride solution (1ml of 1%). then observed the formation of light-yellow color. this indicated presence of Flavonoids. further dil.NaOH and HCL added . A yellow solution turns colorless indicating positive test for presence of flavonoids.

Test For Terpenoids

Salkowski Test

chloroform (2ml) and concentrated H₂SO₄ (3ml) was mixed with extract and was added. formation of layer. Reddish-brown color of interface is formed positive result of terpenoids.

Test For Tannins

To the small quantity of extract (5ml) of 45% solution of ethanol was boiled with for 5 minutes. the mixture was cooled. Filtration was done. Filtrates were used for following test.

Lead Sub Acetate Test

To the different filtrate(1ml), three drop of lead acetate solution was added. cream gelatinous precipitation was observed which shows positive test for Tannins.

Ferric Chloride Test

Each of filtrate (1ml) was diluted by using distilled water. 2 drops ferric chloride was added. Transient greenish-black color formation indicated presence for Tannins.

Test For Sterols

Liebermann-Burchard test

To small amount of extract chloroform (3-5 drops), acetic anhydride and H₂SO₄ were added along sides of test tube to observe formation of dark red/pink color. The observation of dark red/pink colour formation indicated presence of sterols.

Test For Proteins

Ninhydrin test

To the test solution (1ml) ninhydrin solution (2%) was added. violet color indicated presence for protein in sample of extract.

Biuret test

To extract (3ml) few drops of sodium chloride (10%) and copper sulphate (1%) was added which given formation of violet/purple color. And On adding alkali, becomes dark purple.

Xanthoprotein test

To extract (3ml) few drops HNO₃ was added. formation of intensely yellow color indicated the presence of proteins in the sample extract.

Test For Carbohydrates**Molisch's test**

To small amount of extract Molisch's reagent (5-6 drops) was added follow by addition of concentrated H₂SO₄ along sides of test tube. mixture was allow to kept for two minute. it was diluted with distilled water(5ml). the Forming of red/dull violet colour at interial phase of 2 layer indicates presence of the carbohydrates.

Fehling's test

The extract (5 ml) Fehling's reagent and Fehling's reagent B was added. kept for boiling in water-bath. formation of yellow/red color precipitate indicated presence of reducing carbohydrate.

Test For Glycosides

diluted sulphuric acid (5ml) was added to extracts in test tube. It was boiled in a water bath for 15 minutes. It was cooled and potassium hydroxides solution (20%) neutralized. mixture of equal parts of Fehling's solution A and B (10ml) was added. It was again boiled for few min. more dense red color precipitate formed indicates presence of glycosides.

Baljet's Test

To extract (5ml), sodium picrate (5 drops) were added. It was observed change in yellow to orange colour. It indicated the presence of glycosides.

Keller-Killiani test

To extract (5ml), ferric chloride solution (5-7 drops) were added. It was stirr properly by stirring, then sulphuric acid and ferric chloride solution were added forming 2 layer, brownish-red and upper layer greenish blue indicated presence for glycosides.

Test For Saponins**Foam test**

To extract add distilled water and vigorous shaken until foam observed.

TLC identification of two terpenoids from methanolic root extract

Phytochemical identification of terpenoids from extract by thin layer chromatography was performed as per the given method. Briefly, the extract was drawn into tubes of capillary. It was apply 1 cm above on stationary phase silica gel coated plate from the base. plates were dipped in a solvent system, mobile phase. It was then kept in a chromatographic tank. plates were dried and R_f value was calculated and color spot (separated compounds) identity was done.

$R_f \text{ value} = \frac{\text{Dist. moved by compound}}{\text{Dist. moved by solvent front}}$

MATERIALS AND METHODS (IN-VITRO)**Procurement of Material**

Achyranthes aspera leaves, bark and roots was collect in January 2018 from roadsides of rajaji national park, dehradun, uttrakhand. plant was taxonomically identified in Kreeshi Vigyaan Kendr, dehradun, uttrakhand.

Preparation of extracts using sequential extraction process

leaves, stem and root was separated from plant. They were dried and with the help of mixer grinder powder separately. Powder parts was extracted with the help of soxhlet apparatus. petroleum ether, chloroform, benzene, ethanol and ethyl acetate were used sequentially for 16 hours. Maceration method was used for Aqueous extract preparation. The extracts were than concentrated by the means of on a rotary evaporator. The temperature was maintained below 50°C. later the extract was kept in air tight container at temperature 4°C for further studies.

Selection of active extract

Plants are rich in phyto-constituents. These phyto-constituents with antioxidant properties posses anti-microbial effect too.

Hence, all the extracts of bark of Achyranthes aspera were subjected to in-vitro antimicrobial and inhibition of growth of different microbial studies to help in the selection of the best extract which would be taken up for further studies.

Microbial strains

Pseudomonas aeruginosa- MTCC No. 1688, Bacillus cereus- MTCC No. 430, Escherichia coli- MTCC No. 119, Staphylococcus epidermidis- MTCC No. 435, Shigella flexneri- MTCC No. 1457 used as bacterial strains.

Trichoderma viridae- MTCC No.166, Fusarium oxysporum- MTCC No.2086, Aspergillus niger- MTCC No. 281 used as fungal strains.

All these strains obtained from IMTECH, Chandigarh.

Preparation of Culture medium & Inoculums

Nutrient broth agar was used as media for culture of bacteria and Potato dextrose agar for culturing fungi strain. To culture (1ml), bacteria strain were inoculate in nutrient agar providing aseptic condition then incubate at temperature 37°C for 24 hours respectively. a loop of fungi strains was inoculate in Potato dextrose agar separately, then incubated at room temperature for 2 weeks for culturing fungi strain.

Preparation of Test Sample

Three concentrations (a) 10 mg/ml, (b) 12.5 mg/ml and (c) 25 mg/ml of six extracts that is 1. petroleum ether, 2. benzene, 3. chloroform, 4. ethyl acetate, 5. Ethanol, 6. aqueous were prepared for screening anti-bacterial activity and anti-fungal activity. The antimicrobial activity was screen by using disc-diffusion method.

Disc diffusion method

the sterile extract disc was prepared using filter paper whatmann No.1. It was soak in different prepared concentrations extracts. Bacterial strains and fungal strains was spread on respective media. Test extracts load discs was placed on bacterial lawn, fungal lawn and incubated on suitable temp. After the incubation process zone of different inhibitions was measured. Data was record. Gentamycin used as standard disc for bacteria.

ketoconazole as standard anti-biotic for fungi was used for comparing anti-microbial activity study of test sample.

RESULTS AND DISCUSSION

The Pharmacognostical studies comprises the macroscopical parameters and phytochemical constants which correlate with the standard monographs available in "The Ayurvedic Pharmacopoeia of India" for the leaf of the plant. The behaviour of the power with glacial acetic acid and methanolic sodium hydroxide showed orange fluorescence. Preliminary qualitative phytochemical screening showed presence of tannins, alkaloids, saponins, Flavonoids, Steroids, Carbohydrates and Proteins.

Percentage Yield of the Extracts

Result of anti-microbial activity determination of all organic extract of *Achyranthes aspera* (stem & leaf) against bacteria are experimented by disc diffusion method.

Figures I and II of illustrate plate shows medicinal activity of ethanol extract and methanol extract from *Achyranthes aspera* which made zone of inhibition against bacteria proteus and pseudomona.

CONCLUSION

Discovery of remedies by herbal plant are going to be good advantage for microbial infection treatment and therapy. Result for investigation indicates that plant have antimicrobial property against various microorganism. The study of leaf and stem extract incontestable people medication iseffectivetreatment to infective microbes. Plant function is helpful source of anti-microbialdrugs^{11,12}.

The selected Ethanomedicinal plant is supply of secondary and tertiary metabolites for example, Flavanoids, alkaloids, terpenoids, steroids, glycosides and carbohydrates. Medicinal plant play important role for prevention numerous diseases. Anti-analgesic, antiviral, anticancer, anti-malarial, anti-microbial activities of plants are result of the presence of secondary metabolite. Herbal plant used screening for phytochemical that are used for producing medication.

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