Phytochemical screening and antimicrobial activity of *Aerva lanata* (Gorakh ganja)

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Abstract- *Aerva lanata* is a perennial shrub which belongs to the family Amaranthaceae and is found throughout tropical India as a weed in the fields and wasteland area. This plant is a good source of phytochemicals like terpenoids, flavonoides, alkaloids, phenolic compounds, glycosides, gums, tannins, terpenes, carbohydrates and amino acids. Other chemical constituents of *Aerva lanata* include ferulic acid, syringic acid, narcissin and feruloyl tyramine etc which are responsible for their antibacterial, antifungal, antioxidant, anti-asthmatic and anthelmintic activities. The result of phytochemical study shows that the alcoholic extract of *Aerva lanata* contains alkaloids, saponins, tannin, polyphenolic compound, amino acid, proteins, flavonoids, steroids, cardio glycosides, terpenoids and carbohydrate and it can be mentioned that the alcoholic extract of *Aerva lanata* contains higher number of phytochemicals as compared to all other extract of *Aerva lanata* studied in the present investigation. The MIC of the different extracts of *Aerva lanata* was found in the range of 25 µl to 45 µl. The different extracts of *Aerva lanata* shows antimicrobial activity against tested microorganism with inhibition zone ranging from 22 mm to 24 mm. The ethanol extract of *Aerva lanata* shows maximum zone of inhibition and is comparable or better than standard drug against all tested microorganisms.

Key words: *Aerva lanata*, Phyto-chemical and Amaranthaceous.

Introduction

Aerva lanata is a perennial shrub which belongs to the family Amaranthaceae and found throughout tropical India as a weed in fields and wasteland area. This plant is a good source of phytochemicals like terpenoids, flavonoids, alkaloids, phenolic compounds, glycosides, gums, tannins, terpenes, carbohydrates and amino acids. Phytochemicals are naturally occurring biologically active chemical substances present in plants. Proteins, chlorophyll and regular sugars are the primary metabolites whereas alkaloids, terpenoids, phytosterols, flavonoids, glycosides, tannins and phenolic compounds are secondary metabolites. Research shows that phytochemicals play an important role in protecting/curing of human's diseases. Phytochemical screening includes the extraction, analysis, and identification of the biologically active substances found in plants. It is most commonly used as Antiurolithiatic and diuretic for urinary disorders and for kidney stones. Research studies show that betulin and quercetin of

Aerva lanata having inhibitory property on enzyme activity which is responsible for kidney stone formation.

Other chemical constituents of Aervalanata include ferulic acid, syringic acid, narcissin and feruloyltyramine etc which are responsible for its antibacterial, antifungal, antioxidant, anti-asthmaticand anthelmintic activities. The other use of *Aerva lanata* are cytotoxic, anti-HIV, anti-tumor, anti-diabetic and anticancer.

Material and method

The aerial part of the plant is collected from the Himalaya wellness company, Faridabad. Mayers reagent, Hager's reagent, Molisch's reagent, Benedict's reagent, Fehling's reagent, Schiffs reagent sodium nitroprusside, NaOH, ferric Chloride, benzene, H_2SO4, chloroform, lead acetate, gelatin, HNO3, ferric chloride, Ninhydrin reagent, copper acetate, sodium bicarbonate, hydrochloric acid, litmus papers, Tollens reagent, iodine solution are analytical grade. Microbiological culture media and pathogen are purchased from Hi Media Laboratory Pvt. Ltd.

Preparation of extract

Take 10 g of dry powder of *Aerva lanata* in 500 ml round bottom flask (RBF) and add 200 ml of solvent (Ethanol, Water, Ethyl acetate, Chloroform and Hexane) for which extraction will do. Extract the material on water bath for 3 hours remove the flask and allowed to cool. Filter the supernatant through whatman filter paper no. 1 into clean beaker and kept on water bath to concentrate them. Concentrate till we get 50 ml of final concentrated extract through which we will do further analysis.

Phytochemical screening of extract

Standard methods are used for the screening of extract prepared with different solvent

Test for alkaloid

Mayer's Test- Take 5 mL of extract and adds 5 mL of 1% HCl, boiled on a water bath and then filtered. Take 2 mL of the filtrate add two drops of Mayer's reagent. Appearance of yellow precipitate indicates the presence of Alkaloids.

Wagner's Test- Take 5 mL of extract and add 5 mL of 1% HCl, boiled on a water bath and then filtered. Take 2 mL of the filtrate add two drops of Wagener's reagent. Formation of brown color precipitate indicates the presence of alkaloids.

Test for Carbohydrates

Molisch's Test- Take 2 mL extract and add 5mL of distilled water and filtered. To the 2 mL of filtrate add 2 drops of Molisch's reagent now add concentrated sulphuric acid along the walls of the test tube. Formation of violet ring indicates the presence of carbohydrates.

Benedict's Test- Take 2 mL extract and add 5mL of distilled water and filtered. To the 2 mL of filtrate add 2 drops of Benedict's reagent isadded and heated gently for two Minutes. Formation of red precipitate indicates the presence of carbohydrates.

Fehling's Test- Take 2 mL extract and add 5mL of distilled water and filtered. Take 2mL of filtrate and add 1mL of each Fehling solution A and B and boiled on a water bath for 2 min. Formation of brown precipitate indicates the presence of carbohydrates.

Test for Glycosides

Legal's Test- 5 mL of extract is treated with 4mL of pyridine contained 2 mL of sodium nitroprusside solution. This is neutralized with 10% NaOH. Appearance of pink color shows the presence of glycosides.

Keller-kilani test- Keller-kilani test is carried out by mixing the 2 ml extract with 2 ml glacial acetic acid containing 1-2 drops of 2% FeCl3 solution. The mixture is then transferred into another test tube containing 2 mL concentrated H2SO4. Appearance of brown ring at the inter phase confirms the presence of cardiac glycosides.

Test for saponins

Foam Test- Take 5 mL of extract and add 20 mL of distilled water and shake vigorously in a 100 mL conical flask for 15min. Persistent foaming on shaking confirms the presence of saponins.

Test for Steroids

Salkowski's Test- Take 5mL of extract and add10 ml chloroform and filtered. To the filtrate add few drops of conc. H_2SO_4, shake and allowed standing. Appearance of golden yellow color indicates the presence of steroids.

Test for Phenolic compounds and Tannin

Ferric Chloride Test- Take 5 ml of extracts and adds 2-4 drops of 5% $FeCl_3$solution. Formation of deep green color indicates the presence of phenolic compounds.

Gelatin Test- Take 5 of extract and add 1% gelatin solution containing 10% NaCl. The formation of white precipitate indicates the presence of tannins.

Test for Flavonoides

Alkaline Reagent Test- Take 5mL of extract adds few drops of sodium hydroxide solution. Formation of an intense yellow color, which becomes colorless on addition of dilute acid indicate the presence of flavonoides.

Test for amino acid and proteins

Test for proteins- Take 5 ml extract add few drops of Conc. HNO_3. Formation of yellow color indicates the presence of proteins.

Ninhydrin Test- Take 5mL of extract add 15mL of distilled water. To this extract solution add 0.25% w/v Ninhydrin reagent and boiled for a few minutes. Appearance of blue color indicates the presence of amino acids.

Test for Terpenoids- Take 2 ml extract add dissolved in 2 ml chloroform and then evaporated to dryness. Now 2 ml of Concentrated H_2SO_4 is added to the resulting solid and heated for 2 minutes. The appearance of grayish color indicates the presence of terpenoids.

Anti-microbial activity

The following microorganisms were used for anti-microbial activity. *Bacillus subtilis*, *Streptomyces gresius*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*. All the microorganisms were maintained at 4°C on nutrient agar slants.

Determination of minimum inhibitory concentration (MIC) using micro-dilution method

The Minimum Inhibitory Concentrations (MICs) of different extracts were determined based on the macro-dilution method (Berghe and Vlietinck, 1991)

with some modifications as follows. The extracts were serially diluted (two-fold) in a series of test tubes using nutrient broth supplemented with 10% glucose and 0.05% phenol red (color indicator). These were later inoculated with 0.2ml suspension of the test organisms. The final concentrations were in the range 1000 to 10 µl/mL in the medium. Microbial growth was determined by observing for color change in the tube (red to yellow when there is growth). The lowest concentration that showed no change of color was considered as the MIC.

Anti-microbial activity

Cup plate method using Mueller-Hinton agar medium was employed to study the preliminary antibacterial activity of different extracts against different microbial strains. The Agar medium was purchased from HI media Laboratories Ltd., Mumbai, India. Preparation of nutrient broth, subculture, base layer

medium, agar medium and peptone water was done as per the standard procedure. The cups each of 9mm diameter were made by scooping out medium with a sterilized cork borer in a petri dish which was streaked with the organisms. The extracts (100 µL) was added separately in the cups and petri dishes were subsequently incubated. Kenamycin (30 µg) were used as standard reference drugs. Zone of inhibition produced by different extracts were measured in mm.

Results and Discussions

Phytochemical screening of different extracts shows that all the phytochemicals are present in the alcoholic extract. Aqueous extract contains all the phytochemicals excluding steroids. Ethyl acetate extract contains all listed phytochemicals excluding carbohydrates and cardio glycosides. Chloroform extract contains only steroids and cardio glycosides. Hexane extract contain only steroids, cardio glycosides and carbohydrates as shown in the table given below.

Table Pytochemical Screening

Type of extract	Alkaloid	Saponins	Tannins & Polyphenolic compound	Amino acid & proteins	Flavonoids	Steroids	Cardio glycosides	Terpenoids	Carbohydrates
Ethyl acetate	+	+	+	+	+	+	-	+	-
Chloroform	-	-	-	-	-	+	+	-	-
Ethanol	+	+	+	+	+	+	+	+	+
Hexane	-	-	-	-	-	+	+	-	+
Water	+	+	+	+	+	-	+	+	+

Antimicrobial activity

Minimum inhibitory concentration of *Aerva lanata*

The MIC of the different extract of *Aerva lanata* are found in the range of 25µl to 45µl. The lowest MIC of ethyl acetate extract is found in the range of 30 to 35 µl against *Bacillus subtillis* and maximum MIC is in the range of 40-45 µl against *Streptomyces gresius*. The lowest MIC of chloroform extract is found in the range of 30 to 35 µl against *salmonella typhi* and *E. coli* whereas maximum MIC is in the range of 40-45 µl against *Stephylococcus aureus*. The lowest MIC of ethanol extract is found in the range of 25 to 30µl against *salmonella typhi* and *E. coli* whereas maximum MIC is in the range of 35-40µl against *Stephylococcus aureus*. The lowest MIC of Hexane extract is found in the range of 30 to 35 µl against *salmonella typhi, E. coli, Bacillus subtillus* and *Streptomyces gresius* whereas maximum MIC is in the range of 35-40 µl against *Stephylococcus aureus*. The lowest MIC of Water extract is found in the range of 35 to 40µl against *salmonella typhi, Bacillus subtillus* and *Streptomyces gresius* whereas maximum MIC is in the range of 35-40 µl against *Stephylococcus aureus* and *E. coli* as shown below in the (**Table-1)**.

Table-1 Minimum inhibitory concentration (MIC in µl)

Sr. No	Type of extract	Minimum inhibitory concentration (MIC in µl)				
		Bacillus subtillis	***Salmonella typhi***	***Staphylococcus aureus***	***Streptomyces gresius***	***Escherichia coli***
1	**Ethyl acetate**	30-35	35-40	35-40	40-45	35-40
2	**Chloroform**	35-40	30-35	40-45	35-40	30-35
3	**Ethanol**	35-40	25-30	35-40	30-35	25-30
4	**Hexane**	30-35	30-35	35-40	30-35	30-35
5	**Water**	35-40	35-40	40-45	35-40	40-45

The different extracts of *Aerva lanata* show antimicrobial activity against tested microorganism with inhibition zone ranging from 22 mm to 24 mm. the comparable or better results of different *Aerva lanata* extract with Kenamycin as standard drugs are as: ethyl acetate extract shows against *staphylococcus aureus* and *Streptomyces gresius*. The chloroform extract shows against *Streptomyces gresius*. The ethanol extract shows better result against most of all tested microorganisms. The hexane extract shows against *Streptomyces gresius*. The water extracts shows against *staphylococcus aureus* as shown in below **(Table-2).**

Table-2 The water extracts shows against *staphylococcus aureus*

Sr. No	Type of extract & Std Drug	Zone of inhibition (mm)				
		Bacillus subtillis	***Salmonella typhi***	***Staphylococcus aureus***	***Streptomyces gresius***	***Escherichia coli***
1	**Ethyl acetate**	18	19	20	18	17
2	**Chloroform**	14	15	15	17	14
3	**Ethanol**	22	25	23	21	22
4	**Hexane**	12	13	14	18	14
5	**Water**	13	15	19	14	15
6	**Kenamycin**	20	25	20	18	21

Conclusion

The result of phytochemical study shows that the alcoholic extract of *Aerva lanata* contains alkaloids, saponins, tannin, polyphenolic compound, amino acid, proteins, flavonoides, steroids, cardio glycosides, terpenoids and carbohydrate and we can say this type of extract of *Aerva lanata* contains more numbers of phytochemicals as compared to all other extract of *Aerva lanata* studied in the research under study. The ethanol extract of *Aerva lanata* shows maximum zone of inhibition and comparable or better than standard drug against all tested microorganism.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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