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Evaluation of Phytochemical and Antimicrobial Activity of *Achyranthus aspera*

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Abstract:- Bioactive chemicals found in medicinal plants are used to treat a variety of illnesses. The medicinal herb *Achyranthus aspera* is the subject of the current inquiry. The aim of the current investigation was screening of phytochemicals and antibiotic activity of whole plant methanol extract against a few pathogenic fungi and bacteria. seven distinct solvents such as water, acetone, methanol, ethanol, diethyl ether, benzene, and chloroform were used for phytochemical screening of the plant's root, stem, and leaf. Using a conventional technique, the extracts were subjected to qualitative phytochemical screening. Alkaloids, Saponins, Tannins, Steroids, Glycosides, and Flavonoids are all detected by phytochemical screening. *Achyranthus aspera* demonstrated antimicrobial properties against microorganism. According to this investigation, the methanolic extract had the strongest antibacterial efficacy against practically all test microorganisms. The extract exhibited a maximum of 15 mm zone of inhibition when tested against Total Coliform, and a minimum of 8 mm zone of inhibition when tested against *Salmonela aureginosa*.

Keywords:- *Achyranthes aspera, Root, Stem and Leaf Phytochemical Screening, Antibacterial, Secondary Metabolites, Bioactive.*

I. INTRODUCTION

For thousands of years, people have utilized plants in traditional medicine. Because they contain a multitude of active ingredients with enormous therapeutic value, medicinal plants have been utilized for ages as an excellent source for alternative medicine to treat human problems. Many contemporary medications have been derived from natural sources, which have been a source of medicinal compounds for thousands of years.

Achyranthus aspera is an annual herb that grows to a height of 1-2 metres. It is stiff, upright, or procumbent and belongs to the Amaranthaceae family. It is usually found as a weed along roadsides and in waysides, with a woody base (Jain et al., 2006). The Indian languages Apang (Bengali), Nayurivi (Tamil), Agadha (Marathi), Aghedi (Gujarati), Kalalat (Malyalam), and Chirchita (Hindi) are among the many names for this wild tropical plant (Dwivedi et al., 2008). Commonly present in medicinal plants, secondary metabolites such as terpenoids, quinones, flavonoids, and tannins serve as a barrier against insects, bacteria, and other naturally occurring pests. Achyranthes aspera is said to contain a wide range of phytochemicals, including steroids, alkaloids, flavonoids, tannins, terpenoids, saponins, and glycosides (Yalavarthi et al. 2013). Lentigoderma, dropsy, piles, skin eruptions, colic, fractured bones, respiratory issues, asthma, laxatives, and snake bites are among the conditions for which the herb is utilised. In addition, it acts as a purgative, astringent, and snake bite remedy. Cough and hydrophobia can be treated with inflorescence. Fruit consumption in cases of hydrophobia, Ali Z. A. and Singh V. K. (1989). The seeds are used as a cough remedy, especially for asthma, gonorrhoea, hydrophobia, whooping cough, and bug stings. They have also been employed as a cathartic, purgative, and emetic. Typhoid, intermittent fever, dog bites, and urinary tract infections can all benefit from the leaves. Additionally, they are used to treat asthma. The root is used as an antiasthmatic, diuretic, diaphoretic, and antisyphilitic, and for whooping cough, tonsillitis, hemorrhage, cough, and hydrophobia. Londonkar and companions (2011).

II. MATERIAL AND METHOD

- *Procurement of plant*

Fresh & healthy plant parts, free from diseases of *Achyranthus aspera* and (root, stem and leaves) were collected in a separate sterile bag during the month of July to October from different locations of Chitrakoot, and Satna District, Madhya Pradesh, and identified by Dr. Manoj Kumar Tripathi SRO & Head, Dept. Of Taxonomy & Pharmacognosy (R&D Lab) Deendayal Research Institute, Arogya Dham, Chitrakoot, Satna (M.P.)

- *Preparation of extract*

Extraction is an essential step in phytochemical processing for the finding of bioactive secondary metabolite from plant materials. Extracts of sample of *Achyranthus aspera* (root, stem and leaf) 5gm was soaked into 100ml organic solvents like Acetone, Methanol, ethanol, water, benzene, diethyl ether and Chloroform by Soxhlet Extraction apparatus separately. All the extracts were concentrated by distilling the solvents and the extracts were dried in water bath.

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➢ *Test Organisms used for antibacterial activity*

Six bacterial species were used in screening for antibacterial activities which were procured from Deendayal Research Institute, Arogya Dham, Chitrakoot, Satna (M.P.)

Table 1: Microorganisms used for antibacterial activity

S. No.	Name of microorganisms
1	*Salmonella*
2	*Staphyloccus*
3	*Pseudomonas*
4	*Escherichia coli*
5	*Total coli form*
6	*Yeast & moulds*

➢ *Phytochemical screening*

- **Test for Alkaloids- Mayer's test:** To 1 millilitre of the plant extract, add a few drops of Mayer's reagents. It creates hues like pale yellow or white.
- **Wagner's Test (Iodine-potassium iodine):** Acidify 1.5% v/v of HCl, 1 ml of the alcoholic extract, and a few drops of the Wagner's reagent. A brown or yellow ppt. forms
- **Test for Carbohydrate- Anthrone's test:** Add 0.5 millilitre of plant extract to two millilitres of Anthrone's test solution. Carbohydrates are indicated by a green or blue hue.
- **Fehling's test (for reducing sugar):** To 2 ml of extract of plants, add 1 ml of Fehling's solution, A and Fehling's solution B and boil the content of the test tube for few minutes. A red or brick red ppt. is formed.
- **Test for Proteins-Biuret's test:** To 1 ml of hot extract of plant, add 5 – 8 drops of 10% NaOH (W/V) solution followed by 1 or 2 drops of 3% w/v $CuSO_4$ solutions. A red or violet colour is obtained.
- **Millon's test (Mercuric nitrate solution):** Dissolve small quantity of extract of plants in 1 ml of distilled water and add 5 - 6 drops of millon's reagent. A white ppt. is formed which turns red on heating.
- **Test for Resins-** Dissolve the 1 ml of plant extracts in 1 ml of acetone and pour the solution into 5 ml distil water. Turbidity indicates the presence of resins.
- **Test for Saponins-Foam test:** In test tube containing about 5 ml of an extract of plant, add drops of sodium bicarbonate. Shake it vigorously and left for few minutes. Honey comb – like structure is formed.
- **Test for flavonoids- Shinoda test:** In the test tube containing 0.5 ml of extract of plant and add few drops of concentrated HCl followed by 0.5g of magnesium metal. Appearance of pink, crimson or magenta colour within minutes or two indicate the presence of flavonoids.
- **Test for Steroids- Salkowski's reaction:** Add 1 ml of concentrate H_2SO_4 to 2 ml of chloroform extract of the drug carefully, from the side of test tube. A red colour is produced in the chloroform layer.
- **Test for tannins:** The test residue of each extract was taken separately in water, warmed and filtered. Tests were carried out with the filtrate using following reagents.
- **Lead acetate Test:** To the filtrate, few drops of 10 percent w/v solution of aqueous basic lead acetate solution was added. Development of precipitate indicates the presence of tannins.
- **Test for Glycosides:** 2ml of alcoholic extract was subjected to the following test:
- **Borntrager's Test:** 1 ml of benzene and 0.5 ml of diluted. NH3 solutions were added to the plant extract and were observed for the formation of reddish pink colour.
- **Test for Triterpenoids- Libermann's Burchard's test:** Add 2 ml of acetic anhydrate solution to 1 ml of petroleum ether extract of plant in chloroform followed by 1 ml of concentrate H_2SO_4. A violet colour coloured ring is formed indicate the presence of triterpenoids. (Abhay kumar Kamble 2018) (Harborne J.B, 1973)

➢ *Antimicrobial Activity:*

One millilitre of diluted inoculums was added to a sterile Petridis. Next, roughly 15 millilitres of autoclave-liquefied medium were added and thoroughly blended. The dishes were then left in the refrigerator for around two hours to solidify. Following that, sterile cook borer was used to create wells based on the hardened agar plates. The well was filled with 50µl of drug extract, and a disc containing the antibiotics amoxyclave and clotrimazole was placed on the agar surface. In order to allow the extract in the agar to vary, all of the plates were then left to stand at room temperature for one hour. The plates were then all incubated for 24 hours at 37°C. Clotrimazole was utilised as a recognized antibiotic in the case of fungus culture. During 72 hours, the fungus culture plates were incubated at 25°C. Following the incubation period, the plates were checked for antibacterial activity and the diameter of the zone where the growth of microorganisms was inhibited was measured. (Tullanithi K Met.al., 2010)

III. RESULTS AND DISCUSSION

The present study showed the presence of Alkaloids, resins, saponins, flavonoids and proteins in extract of all parts of *Achyranthus aspera*. The leaves extracts possess almost all the phytochemicals that were tested when compared to stem extract and root extract of *Achyranthes aspera.* From the antimicrobial study, it was confirmed that the methanol extract of *Achyranthus aspera* shown the maximum zone of inhibition in total coli form (15mm) while the lowest level of zone of inhibition was observed in the methanol extract of *Achyranthus aspera* against *Salmonela aureginosa* (8mm).

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Table-2: PhytoChemical Screening of *Achyranthes Aspera* (Root Stem And Leaf)
(+ Present; -Absent)

S. No	Phytochemical	Acetone			Methanol			Ethanol			Water			Benzene			Diethyl ether			Chloroform		
		R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L
1.	**Alkaloids**																					
	Mayer' reagent	-	-	-	+	+	+	-	-	-	+	+	+	-	-	+	-	-	-	-	+	+
	Wagner's reagent	-	-	-	+	+	+	-	-	+	+	+	+	+	-	+	-	-	-	-	+	-
2.	**Carbohydrate**																					
	Anthrone's test	-	-	-	+	-	+	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-
	Fehling's test	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-
3.	**Proteins**																					
	Bieuret's test	+	-	-	-	-	-	+	-	-	+	+	+	-	-	-	+	-	-	-	-	-
	Millon's test	-	-	+	-	-	+	-	-	-	+	+	+	-	-	+	-	-	-	-	-	-
4.	**Resins**	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
5.	**Saponins**																					
	Froth test	-	-	-	-	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-
6.	**Flavonoid**																					
	Shinoda test	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
	Fluroscence test	+		+	+		+	+		+	+	+	+				-	-	-	-	-	-
7.	**Steroid**																					
	Salkowski's test	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8.	**Glycoside**																					
	Borntrager's Test	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
9.	**Tannin**																					
	Lead acetate Test		+	+	+	+		+	+	+		+	+	-	-	-	-	-	-	-	-	-
10	**Terpenoid**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table-3: Antimicrobial Activity of methanolic extract of *Achyranthus aspera*

S. No	Name of Pathogens	Name of Antibiotics	Zone of Inhibition in mm	
			Antibiotics	Methanolic extract of Achyranthus aspara
1	*Staphyococcus aureus*	Amoxyclave	19	13
2	*Pseudomonas aeruginosa*	Amoxyclave	21	12
3	*E.Coli*	Amoxyclave	19	14
4	*Salmonela aeruginosa*	Amoxyclave	30	08
5	*Total Coliform*	Amoxyclave	21	15
6	*Total Bacterial Count*	Amoxyclave	17	11
7	*Yeast and Mould*	Clotrimazole	08	12

IV. CONCLUSION

Achyranthus aspera's many parts are the subject of the current investigation. Plant extracts may include chemicals with antibacterial characteristics, according to a screening for antibacterial activity conducted to support traditional plant uses. When developing new medications to treat infectious disorders brought on by human pathogenic bacteria, it might be utilized as an antibacterial agent. The growth of numerous harmful microorganisms is inhibited by extracts from A. aspera. Flavonoids, triterpenoids, alkaloids, and naturally occurring phenolic compounds all of which are categorized as potent antibacterial compounds may be responsible for this effect. High levels of antibacterial activity have been observed for *Achyranthus aspera*. Additional pharmacological assessment and active component isolation can be performed on the most active extracts.

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