



HISTOCHEMICAL STUDIES AND PHYTOCHEMICAL ANALYSIS OF SOME COMMON ALLELOPATHIC WEEDS OF PUNE DISTRICT IN MAHARASHTRA, INDIA

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ABSTRACT:

The present study reports about the presence of phytochemical constituents of aqueous extracts prepared from eight common and noxious allelopathic weeds belonging to seven families collected from 13 different Tahasils in Pune district of Maharashtra, India. Histochemical studies and qualitative phytochemical analysis were performed by using fresh healthy plant parts and extracts of the weeds for the presence of starch, proteins, tannins, saponins, fat, sugars, alkaloids, and glycosides. For the histochemical studies free hand cut sections about 20-25 μ of the root, stem and leaves of selected weeds were taken and tested with respective reagents for the detection and localization of chemical constituents. Each phytochemical analysis was carried out in duplicate, which resulted in a total of species of 4, 6, 4, 8, 7, 1, 8, 2 and 8 plant species were found positive results for Starch (50%), Proteins (75%), Tannins (50%), Saponins (100%), Fat (87%), Anthraquinones (12%), Alkaloids (100%), Flavonoids (25%) and Glycosides (100%) respectively. Most of the plants studied were reported to treat a variety of diseases in traditional system of medicine.

Key words: - *Allelopathic Weeds, Histochemistry, Phytochemicals; Pune, Maharashtra*

INTRODUCTION:

Molisch in 1937 first coined the term allelopathy, which refer to biochemical interactions between all types of plants including microorganisms. The term allelopathy was derived from Greek word, which means mutual harm. This term covers both the detrimental and beneficial reciprocal biochemical interactions. Rice in 1984 also defined allelopathy as any direct or indirect harmful or beneficial effect of plants, including microbes, on another plant through release of chemicals that escape into the environment.

Allelochemicals refer mostly to be the secondary metabolites produced by plants and are by-products of primary metabolic growth and development of the same plant or neighbouring plants. Some of them are accumulated at various

stages of growth, while some depends upon time of day or season.

The term weed in broader sense is a plant that is not valued where it is growing and is usually of vigorous growth. A weed is also defined as a plant that grows out of place and is competitive and persistent. Weeds are indigenous as well as exotic plants that grow and reproduce extensively. A weed in a general sense is a plant that is considered to be waste, and normally applied to unwanted plants in man-made ecosystems such as agricultural fields, gardens, barren lands, parks and other areas. Their competitive nature along with cultivated crops are due to the presence of phytochemical compounds and their resistance and non-susceptible nature to microbial attacks.

The problem of weeds is as old as cultivation of crop plants itself. The weeds are cosmopolitan in

distribution. They occur diverse habit as well as habitat. They create various problems in agriculture. Due to the dominant trait, they compete with other weeds and crops for water, space, light and nutrition and decrease the crop yield significantly.

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues. Starch deposition occurs widely in the plant body, but the particularly common places of its accumulation are seeds, the parenchyma of the secondary vascular tissues in the stem and root, tubers, rhizomes and corn (Kadam, 1999). Starch and proteins are the principal ergastic substances of the protoplast (Kuster, 1956). Tannin is the heterogeneous group of phenol derivatives, usually related to glucosides. Tannins are particularly abundant in the leaves (xylem) of many plants (Kadam et.al.,1996). Saponin are the rare occurrence. Fats are widely distributed in the plant body and they probably occur in small amount in every plant cell (Seifriz, 1934). Fats are common reserve material in seeds, spores, embryos and in meristematic cells. Glucosides and Alkaloids are the degradation product of the carbohydrates and proteins respectively. To find out their resistance nature to microbial attack and pests etc., the biochemical constituents of common allelopathic weeds were studied at preliminary level in this project.

MATERIALS AND METHODS

Collection of Plant material:

A healthy, mature and abundantly growing eight weed plant materials were collected from various Tahasils of Pune district, Maharashtra, India during July 2020-February 2021. Fresh plant parts of the weed plants were used for histochemical studies. A healthy root, stem and leaf of each weed plant sample was shade dried for 4-7 days and milled to a coarse powder for phytochemical analysis.

I. Histochemical study:

For the histochemical studies free hand cut sections about 20-25 μ of the root, stem and leaves of selected weeds were taken and tested with respective reagents for the detection and localization of chemical constituents such as starch, proteins, tannins, saponins, fat, sugars, alkaloids, and glycosides in the tissues (Johansen D A, 1940 and Krishnmurty K V, 1988). The results are depicted in table No.1.

1. Test for Starch: 0.3g of Iodine and 1.5g of KI were dissolved in 100.c.c. Distilled water. A drop of this solution was added on the section, washed with water and observed under the microscope.

2. Test for Proteins: Saturated aqueous solution of picric acid is an excellent precipitating agent for proteins. Staining them as an intensive yellow. It was allowed to react with the reagent for 24hrs. Dilute eosin, stains proteins red.

To detect the localization of proteins, reagent was prepared-1g of the potassium ferrocyanide plus 20 c.c. water and 100 c.c. Glacial acetic acid and sections were kept there in for 1 hr. The sections were washed with 60% alcohol and few drops of aqueous FeCl₃ were added. Blue colour indicates the presence of proteins.

3. Test for Tannins: Transverse sections were treated with dilute acidic FeCl₃ solution (0.5 to 1% of Ferric chloride in 0.1N HCl), mounted in a clove oil and observed under microscope for the presence of tannins. 10% aqueous FeCl₃ plus little Na₂CO₃, blue green colour was given by tannins.

4. Test for Saponins: Sections were placed directly in 1 drop of concentrated H₂SO₄ on a slide, which gives the characteristics sequence of colour reactions, beginning immediately with yellow, changing to red within 30 minutes and finally becoming violet or blue green in a short time. To determining localization of the saponins, sections were put in a saturated Barium hydroxide solution for 24 hrs. Sections were washed calcium chloride then placed in a

potassium dichromate. Yellow colour indicated the presence of the saponins.

5. Test for Fats: 0.5 g of dye Sudan III or Sudan IV was dissolved in 100 c.c. of 70% alcohol. Sections were kept in the stain for 20 minutes, washed carefully but quickly with 50% alcohol and transferred in glycerine for observations. Blue, red and pink precipitate indicated the presence of fats.

6. Test for Glucoside (Guignard's Test): Sections were immersed in 1% of aqueous picric acid for 30 minutes, washed with water and placed in 1 drop of 10% aqueous sodium carbonate on slide. A red colour of the sections appearing in hydrocyanic acid was released.

For the detection and localization of glucoside, sections were placed in KOH and solution composed of 20 parts of 20% aqueous KOH and 80 parts of 90% alcohol for few minutes. In a small watch glass, mixture of 2.5% aqueous FeSO_4 and 20% aqueous FeCl_3 solution taken in an equal proportion was heated to boiling and then the sections were transferred to a slide holding a drop of 20% HCL. A deep blue precipitate indicated the presence of hydro cyanic acids.

8. Test for Alkaloids: Transverse sections were treated with the following alkaloid reagents.

i) Mayer's Reagent: -Potassium mercuric iodide solution 13.55g of HgCl_2 and 50g KI, were dissolved in one litre of distilled water

ii) Wagner's Reagent: -Iodine 1g and Potassium iodide 2g were dissolved in 50ml of distilled water.

iii) Dragendorff's Reagent: -a) Solution A: - 0.85 g of basic bismuth nitrate was dissolved in mixture of 10ml of acetic acid and 40 ml of distilled water.

b) Solution B: - Solution was prepared by dissolving 8 g of KI in 20 ml of distilled water.

Stock Solution: -Equal volume of solution A and B were mixed together. The formation of precipitate or development of turbidity in the

sections clearly indicated the presence of alkaloids.

II. Phytochemical Analysis:

Preliminary qualitative phytochemical tests were carried out for the identification and confirmation of starch, proteins, tannins, saponins, and anthroquinones on water extractives, while alkaloids, glycosides and flavonoids in alcoholic extractives. The tests were carried out in duplicate. Results of these reactions were depicted in table No.1

1. Qualitative Tests for Starch: -The plant material was finely ground and extracted with boiling methanol (methanol removes fats, fatty acids, salts, chlorophyll and inactive enzymes). After drying, the plant tissues were centrifuged with cold water and tested with iodine in 2% aqueous potassium iodide (Peach and Tracy, 1955). Blue colour indicates the presence of starch.

2. Qualitative Tests for Proteins (Million's tests): -Millions reagent is a solution of mercuric nitrate in nitric acid (it react specifically with any phenolic compound in which 3 and 5 positions are unsubstituted). Proteins give red colouration with million's reagent.

Procedure: - 2 ml of the test solution was boiled with the few drops of Million's reagent and the colour was observed. (Trease and Evans, 1972).

3. Qualitative Tests for Tannins: -Plant part water extracts were treated with Ferric chloride (Acidic) and observed for the presence of tannins (Trease and Evans, 1972).

4. Qualitative Tests for Saponins: -Water extracts of the plant material were vigorously shaken with few drops of neutral water. A permanent lather (foam) indicates the presence of saponins (Trease and Evans, 1972).

A portion of the residue obtained after evaporating the ethanol extracts was dissolved in water and shaken vigorously. A honeycomb, froth persisting for 15 min indicated the presence of saponins. A portion was dissolved in chloroform

and filtered. A few drops of concentrated sulphuric acid and 1 ml. of acetic anhydride were added to 1 ml of iced filtrate. The appearance of blue or bluish green or reddish-brown colour showed the presence of saponins (Fransworth et al., 1960).

5. Qualitative Tests for Free Anthroquinones:

- 5 ml of the plant extract was shaken with 10ml of benzene and filtered. A 10% ammonium hydroxide solution (about 5ml) was added to the filtrate and the mixture was shaken. The presence of pink, red or violet colour in the ammonium phase indicated the presence of free anthroquinones (Fransworth et al., 1960).

6. Qualitative Tests for Flavonoids: -To 1ml of ethanol extracts, few drops of concentrated HCl and Mg turning were added. The development of pink or magenta colour indicated the presence of flavonoids. (Fransworth et al., 1960).

7. Qualitative Estimation of Alkaloids: - Precipitation of alkaloids can be obtained with a variety of inorganic & organic reagents. Sometimes even from dilute solution. Among the inorganic precipitation reagents, to mention a few potassium mercuric iodide. (Mayer's reagent), bismuth potassium iodide (Dragendorff's reagent) and iodine potassium iodide (Wagner's reagent), were used.

Characteristic colour reactions were obtained with the acid of dehydrating agents such as concentrated sulphuric acid, with oxidizing agent such as Nitric acid, with the combination of these two or other reagents, which will dehydrate and oxidize simultaneously and finally by treating with aldehyde or like compounds in the presence of dehydrating agents. The exact mechanism of precipitation reactions of alkaloids was not clearly understood. However, these reactions have proved to be an efficient tool in detection of alkaloids in plant tissues.

Reagents:

1) Mayer's reagent: - 1.3 g of HgCl₂ and 5ml of KI were dissolved separately in 60 ml and 10 ml of distilled water respectively and both the solutions were mixed and diluted to 100ml.

2) Dragendorff's reagent: - 8g of Bismuth nitrate was dissolved in 20 ml of concentrated HNO₃ and 27.2g of KI in 50 ml of distilled water. Both the solutions were allowed to stand till KIO₃ crystallized out. Supernatant was decanted and final volume was adjusted to 100 ml.

3) Wagner's reagent: - 1.27 g of iodine and 2g of KI were dissolved in distilled water and diluted to 100ml. This reagent gives brown fluorescent precipitate especially with colchicine.

8. Qualitative Tests for Glycosides: -10ml of filtrate in a test tube was taken. Slowly warm benzene added in the filtrate through the sides of test tube. Formation of white ppt at the junction of both the filtrate and benzene indicates the presence of glycosides.

RESULT & DISCUSSION:

When the hand cut sections of different plant parts were treated with the respective reagents and subsequently observed under microscope, revealed localization of different phytochemical constituents in different tissues. Out of the investigated plants, a total of species of 4, 6, 4, 8, 7, 1, 8, 2 and 8 plant species were found positive results for starch (50%), Proteins (75%), Tannins (50%), Saponins (100%), Fat (87%), Anthraquinones (12%), Alkaloids (100%), Flavonoids (25%) and Glycosides (100%) respectively. Interestingly, the common weeds plants tested for phytochemical presence given maximum positive indications for proteins, tannins, alkaloids and glycosides, but only 12% of weeds showed the presence of Anthraquinones and 25% of weeds showed the presence of Flavonoids. This obtained information will be helpful as a primary platform for further phytochemical and pharmacological studies.

Most of the plants studied were reported to treat a variety of diseases in traditional system of medicine. The phytochemical analysis for the presence of alkaloids, Saponins flavonoids, Tannins and Glycosides was carried out on aforesaid extracts and the results are reported in Table. In the present investigation, aqueous extract showed the presence of flavonoids which may be accounting for the anti-inflammatory and analgesic activities (Chakraborty et al., 2004). The presence of alkaloid and saponins in the plant indicates that the plant extracts could be used for the antifungal activity (Rani and Murthy, 2006). The extracts belonging to the following species listed below given positive indication for Starch, Proteins, Tannins, Saponins, Fat, Anthraquinones, Alkaloids, Flavonoids, and Glycosides respectively.

Starch: *Aristolochia bracteolata* Lam., *Bidens biternate* Merr. & Sheriff., *Oxalis corniculata* L., and *Cullen corylifolia* Medik.

Proteins: *Aristolochia bracteolata* Lam., *Bidens biternate* Merr. & Sheriff., *Cyathocline purpurea* O. Ktze., *Fagonia bruguieri* DC., *Oxalis corniculata* L. and *Cullen corylifolia* Medik.

Tannins: *Aristolochia bracteolata* Lam., *Bidens biternate* Merr. & Sheriff., *Cyathocline purpurea* O. Ktze. and *Cullen corylifolia* Medik.

Saponins: *Aristolochia bracteolata* Lam., *Bidens biternate* Merr. & Sheriff., *Cyathocline purpurea* O. Ktze., *Euphorbia parviflora* L., *Fagonia bruguieri* DC., *Oxalis corniculata* L., *Cullen corylifolia* Medik., and *Thlaspi arvense* L.

Fat: *Aristolochia bracteolata* Lam., *Cyathocline purpurea* O. Ktze., *Euphorbia parviflora* L., *Fagonia bruguieri* DC., *Oxalis corniculata* L., *Cullen corylifolia* Medik., and *Thlaspi arvense* L.

Anthraquinones: *Bidens biternate* Merr. & Sheriff.

Alkaloids: *Aristolochia bracteolata* Lam., *Bidens biternate* Merr. & Sheriff., *Cyathocline purpurea* O. Ktze., *Euphorbia parviflora* L., *Fagonia*

bruguieri DC., *Oxalis corniculata* L., *Cullen corylifolia* Medik., and *Thlaspi arvense* L.

Flavonoids: *Euphorbia parviflora* L. and *Fagonia bruguieri* DC.

Glycosides: *Aristolochia bracteolata* Lam., *Bidens biternate* Merr. & Sheriff., *Cyathocline purpurea* O. Ktze., *Euphorbia parviflora* L., *Fagonia bruguieri* DC., *Oxalis corniculata* L., *Cullen corylifolia* Medik., and *Thlaspi arvense* L.

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Table 1: Histochemical studies and Phytochemical Analysis of Some Common Allelopathic Weeds of Pune District in Maharashtra, India

Name of the Plant Species	Phytochemicals								
	Sta	Pro	Tan	Sap	Fat	Anth	Alk	Gly	Fla
<i>Aristolochia bracteolata</i> Lam.	+	+	+	+	+	-	+	+	-
<i>Bidens biternata</i> Merr. & heriff.	+	+	+	+	-	+	+	+	-
<i>Cyathocline purpurea</i> O. Ktze.	-	+	+	+	+	-	+	+	-
<i>Euphorbia parviflora</i> L.	-	-	-	+	+	-	+	+	+
<i>Fagoni abruquieri</i> DC.	-	+	-	+	+	-	+	+	+
<i>Oxalis corniculata</i> L.	+	+	-	+	+	-	+	+	-
<i>Cullen corylifolia</i> Medik.	+	+	+	+	+	-	+	+	-
<i>Thlaspi arvense</i> L.	-	-	-	+	+	-	+	+	-

Sta= Starch; Pro= Proteins; Tan= Tannins; Fat= Fats; Anth= Anthraquinones; Alk= Alkaloids; Gly= Glycosides; Fla= Flavonoids

(+) Indicate the presence of phytochemicals and (-) Indicate the absence of phytochemicals

Fig. 1: Common Allelopathic Weeds from Pune District used for Histochemical and Phytochemical Analysis



Aristolochia bracteolata Lam.



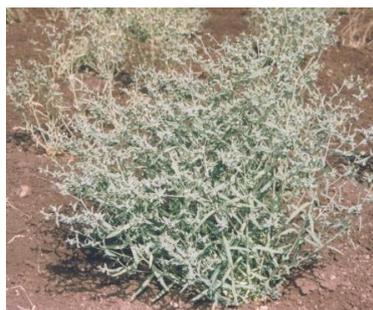
Bidens biternata Merr. & Sheriff.



Cyathocline purpurea O. Ktze.



Fagonia bruguieri DC.



Euphorbia parviflora L.



Oxalis corniculata L.



Cullen corylifolia Medik.



Thlaspi arvense L.