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Phytochemical estimation, *invitro* antioxidant and mutagenic activity of selected common agricultural weed plants

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Abstract
Weed plants are good source of herbal phyto pharmaceuticals because they contained several medicinal activities such antidiabetic, anticancer, antioxidant, hepatoprotective, antipruritic, antioxidant activities. In present study the hydroalcholic extract of 8 common agricultural weed plants of Indian subcontinental eg. *Fumaria indica*, *Celosia argentea*, *Chenopodium album*, *Corchorus olitorius*, *Digera muricata*, *Cyperus rotundus*, *Achyranthes aspera*, and *Cynodon dactylon* were tested for their antioxidant and mutagenic activity. The qualitative phytochemical estimation of all plant extracts were also performed. Antioxidant activity was tested by DPPH assay and mutagenic activity and cytotoxic activity was tested by *salmonella typhemurium reverse mutagen assay* (AMES assay)

Quantitative phytochemical estimation of all tested weed plant showed the presence of all common secondary metabolites which are responsible for their medicinal activities. Out of all tested 8 common weed plants *Cyperus rotundus* showed highest in vitro antioxidant activity (IC50- 676.18 µg/ml) in DPPH assay and all selected weed plants not showed mutagenic activity on all tested doses (10, 33, 100, 333, and 1000 µg/dish) in AMES assay.
Key words: Weed plant, AMES assay, Antioxidant activity, DPPH Assay

1. Introduction

Agricultural weed plants grow with crops without any human efforts and create nutrition deficiency for crop plants and decrease the crop production all over the world. To overcome the problem of weed plant eradication, farmers use different type of poisonous weedicide chemical that are hazard for human health as well as for environment because the high concentration of these weedicide chemicals effect the living organism of soil and water bodies, and ultimately effect the whole surrounding.

Weed plants are most important from medicinal point of view. These plant contains several secondary metabolites due to their wild type nature and these secondary metabolites are responsible for their medicinal activities eg. antidiabetic, anticancer, antioxidant, hepatoprotective, antipyretic, antioxidant activities. *Cynodone dectylone* was famous agricultural weed plant that contained numerous medicinal activities and use from the ancient era for the treatment of cancer, convulsions, cough, cramps,

diarrhea, dropsy, dysentery, epilepsy, headache, hemorrhage, hypertension, hysteria, measles, rubella, snakebite, sores, stones, tumors, urogenital disorders, warts and wounds.

2. Material and Method 2.1

2.1 Chemical Requirement:

Ethanol, H2SO4, Dragendroff's reagent, sodium hydroxide, hydrochloric acid, ferric chloride, chloroform, Methanol, Ascorbic acid, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Sodiumazide, Mitomycin C, 4-Nitro-o-phenylenediamine, 9-Aminoacridine, 2-Aminoanthracene, Magnesium sulphate heptahydrate (MgSO4·7H2O), Citric acid monohydrate, Potassium phosphate, dibasic(K2HPO4) (anhydrous), Sodium ammonium phosphate tetra hydrate (NaNH4HPO4·4H2O), D-biotin, L-histidine, Hydrochloric acid (HCI), Potassium chloride (KCl), Magnesium chloride hexa hydrate (MgCl2·6H2O), Sodium dihydrogen phosphate monohydrate (NaH2PO4·H2O) isodium hydrogen phosphate (Na2HPO4), NADP(sodium salt), D-glucose-6-phosphate (mono sodium salt), Ampicillin tri hydrate, Sodium hydroxide (NaOH) Crystal violet, Agar-Agar (Bacterial Agar powder), Nutrient broth, Tetracycline, Dimethyl sulfoxide (DMSO), Vogel-Bonner medium E(50x), 0.5mM his/bio solution, Salt solution (1.65MKCl+ 0.4MMgCl2), 0.2M sodium phosphate buffer, pH7.4, 1M Nicotinamide Adenine Dinucleotide Phosphate (NADP) solution, 1M glucose-6-phosphate, Ampicillin solution (4mg/ml), Crystal violet solution (0.1%) Minimal glucose plates, Histidine/Biotin plates, Ampicillin and tetracycline plates, LB agar plates.

2.2 Collection and extract preparation of plant material:

All weed plant were collect from the local agricultural fields near Tiwaripur village district Lucknow of India. The whole part of every weed plant were collected properly and wash with distilled water 2-3 times and under shade are to protect the medicinal value of these plants. The dry plant material was powdered with the help of mechanical grinder and convert in to fine powder. 500 gm Powdered plant material of each plant was mix with 70 % ethyl alcohol up to the whole powder was not soaked properly and mix the it in regular intervals. Filter the whole material after 48 hrs. and repeat the above step 2-3 times (Satyanarayana et al., 2022). The filtrate was concentrated with the help of rotary evaporator (Buchi rotavapor R-100) to convert it into semisolid crude plant extract and store it at 4°C in refrigerator for further biological activities. % yield of all plant extracts was also calculated

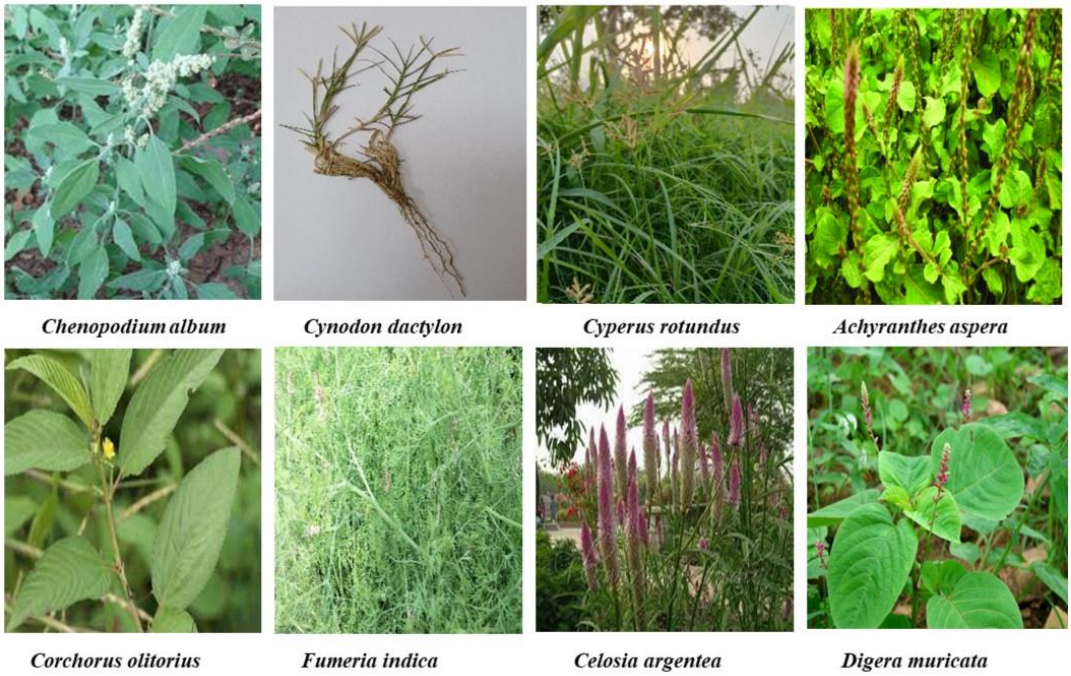

Figure:1 Showing the common agricultural weed plants used for estimation of phytochemical estimation, antioxidant and mutagenic effect.

2.3 Qualitative phytochemical screening:

Phytochemical screening of hydroalcholic extract of *Fumeria indica, Celosia argentea, Chenopodium album, Corchorus olitorius, Digera muricata, Cyperus rotundus, Achyranthes aspera, Cynodone dactylon* was performed through well-established standard procedures (Mansoori et al., 2020; Shah et al., 2014; Shrestha et al., 2024).

Alkaloids test: 200 mg of plant extract from each plant was mixed with 2% H_2SO_4 and boiled it for 2 min. Gently filtered and add few drops of Dragendroff's reagent in it and were mixed properly. If the precipitate convert orange-red in Colour shows presence of alkaloids in extract.

Flavonoids test: To test for flavonoids, 200 mg of each extract was dissolved in sodium hydroxide (NaOH), followed by the addition of hydrochloric acid (HCl). The solution changing from yellow to colorless confirmed the presence of flavonoids.

Tannins test: A small amount of the plant extracts was mixed with distilled water and boiled it. After filtering the mixture, a few drops of ferric chloride were added to the filtrate. The appearance of a blackish-green color indicated the presence of tannins.

Terpenoids Test: To test for terpenoids, 200 mg of plant extract was mixed with 2 mL of chloroform. Then, 3 mL of concentrated sulfuric acid (H_2SO_4) was carefully added, forming a layer. A reddish-brown interface between the layers indicated the presence of terpenoids.

Saponins Test: To test for saponins, 200 mg of plant extract was mixed with 10 mL of distilled water and boiled. The formation of froth that persisted for more than three minutes confirmed the presence of saponins.

2.4 Antioxidant activity:

In-vitro antioxidant activity of all plant extract was analyzed by DPPH radical scavenging assay(Savadi et al., 2020) for the concentration 61.50, 125, 250, 500, 1000 & 2000 µg/ml. 180µl of 0.1mM solution of DPPH in methanol was mixed with 20µl solution of different plant in methanol of different concentration and mix property. The was stand at room temperature in dark for 30 minutes. The absorbance of mixture was measured at 517 nm using an ELISA Reader. Ascorbic acid was used as the reference compound. The capability of scavenging the DPPH radical was calculated by using the following formula. All the tests were performed in triplicates.

DPPH scavenging effect (% inhibition) = {(A0 –A1)/A0) *100}

A0 - Absorbance of the control reaction (DPPH SOLUTION)

A1- Absorbance of plant extract/reference item.

2.5 *Salmonella typhemurium* reverse mutation assay RIVES assay):

Mutagenic effect of all selected plant extracts was checked by Ames assay following OECD guideline 471 with some modifications. (Bouguellid et al., 2020; OECD, 2020)

Bacterial Strains:

Four lypolysed *Salmonella typhimurium* strains, TA97a, TA98, TA100, and TA102, were purchased from MOLTOX, USA.

Revival of bacterial strains:

The frozen glycerol stock was used for the revival of *Salmonella* strains. The *Salmonella* strains were inoculated in LB media for 16-17 hours at 37°C at 200 rpm. For revival of R-factor strains the media should contain ampicillin in a concentration of 8 mg/ml. For revival of TA-102 tetracycline 8 mg/ml was also added.

Spot assay

Spot assay was qualitative assay for the assessment of mutagenicity of drugs, chemicals and plant extracts etc. Mutagenic activity of all plant eight extract was checked by using AMES assay at dose 10, 33, 100, 333, and 1000 µg/dish in four *Salmonella typhimurium* tester strains, TA97a, TA98, TA100, and TA102. The tester strains were incubated 16-17 hrs in 5ml of LB medium at 180 rpm and 37°C in shaker incubator to obtained the 10^8 cells/ml. the experiment was performed without S9 mixture. Different Positive control drugs were used for different strains such as

9-Aminoacridine (50µg/plate) for TA-97a, 4-nitro-o-phenylenediamine, (2.5µg/plate) for TA-98, Sodiumazide (5µg/plate) for TA-100 and Mitomycin C (0.5µg/plate) for TA-102 without S9 fraction.

Take out the fresh overnight culture from the refrigerator and bring them on room temperature, to prevent thermal shock to the bacteria when they are added to the 43°C to 45°C top agar during the process. Take glucose minimal agar plates to biosafety cabinet. Make five partitions on the minimal glucose agar plates by drawing lines on the back side of the plates and label each partition as it indicates the concentration of the drug treatment. Take100µl of bacterial culture in a 5ml glass culture vial, add 200µl of 0.5mM of Histidine & Biotin solution and add 2ml of molten top agar (temp. about 45°C). Immediately mix the content thoroughly and pour on to the minimal glucose agar plate. Allow the plate for solidification (20-30minutes). Place a sterile filter paper disc at the center of each partition. Apply 10µl of the test substance having appropriate concentrations, and follow the same procedure for control and positive controls (no partitions required for control and positive control plates). Then incubate the plates in incubator at 37°C for 48 hours. Take out the plates from the incubator and check for the background lawn, zone of inhibition and revertant obtained.

Mutagenic and non-mutagenic effect was checked by observing the increase of mutant colonies around the spot assay disc and compare it with positive control of same strain, higher the revertants showed the mutagenic activity of plant extract, if the plant extract is non mutagenic the revertants colonies are less as compare positive control and equal to control.

showed the mutagenic activity of plant extract, if the plant extract is non mutagenic the revertants colonies are less as compare positive control and equal to control,

3. Results

3.1 Collection, authentication, extract preparation of medicinal weed plants

Plant extracts were prepared from selected plants, the highest %yield showed was obtained from *Fumeria indica* plant which is 24.84% and the lowest one was *Corchorus olitorius* which was 8.57% (Table 1)

Table-1: % yield of plant extracts

Sr. No.	Plant Name	% Yield
1	*Cyprus rotundus*	14.46
2	*Corchorus olitorius*	8.57
3	*Chenopodium album*	17.33
4	*Fumeria indica*	24.84
5	*Celosia argentea*	23.33
6	*Acyranthes aspera*	18.67
7	*Digerea Muricata*	15.47
8	*Cynodone dactylon*	10.59

3.2 Phytochemical estimation:

Weed plant are unwanted plant from agricultural point of view, but these plant contain high amount of secondary metabolites with excellent antioxidant activity. Out of tested plant extracts Cyperus rotundus showed highest antioxidant activity due to the presence higher amount of secondary metabolites.

Table 2: Qualitative phytochemical test of hydroalcholic extracts of common indigenous common agricultural weed plants.

Name of Plant	Alkaloids test	Flavonoids test	Tannins test	Terpenoids test	Saponins test
Fumeria indica	+ve	+ve	+ve	+ve	-ve
Celosia argentea	-ve	+ve	+ve	+ve	+ve
Chenopodium album	+ve	+ve	-ve	+ve	+ve
Corchorus olitorius	+ve	+ve	+ve	+ve	+ve
Digera muricata	+ve	+ve	+ve	+ve	+ve
Cyperus rotundus	+ve	+ve	+ve	+ve	+ve
Achyranthes aspera	+ve	+ve	+ve	-ve	+ve
Cynodone dactylon	+ve	+ve	+ve	+ve	+ve

3.3 Antioxidant activity:

DPPH radical scavenging assay

Purple colored methanolic solution of DPPH induce stable free radical that loses its purple color when accepts an electron from an antioxidant molecule. DPPH free radical scavenging activities of 10 plants are shown in **Figure 2**.

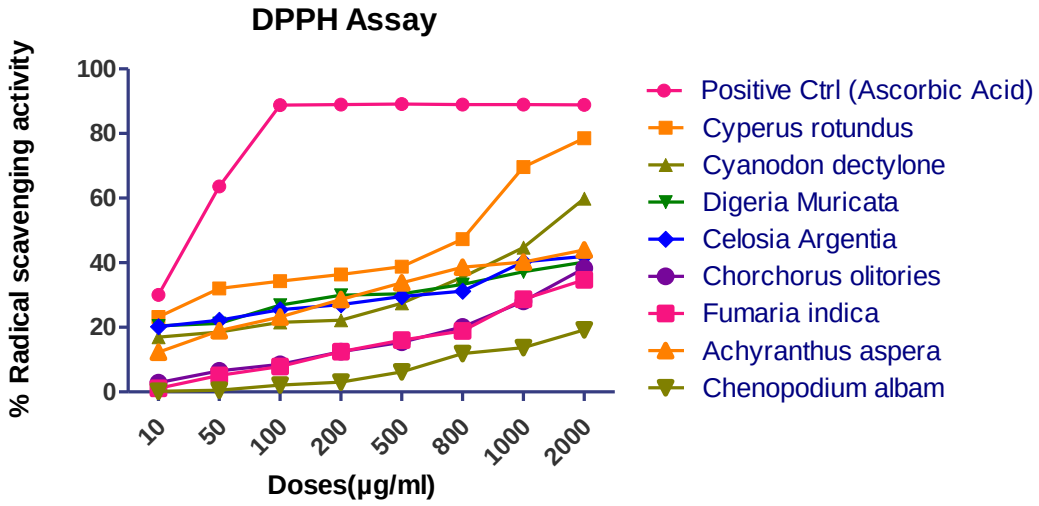

Figure: 2 Radical scavenging activity

The IC50 values of the ten plants were within the range 676.18- 6089.88 µg/ml. *Cyprus rotundus* showed the highest radical scavenging activity while *Chenopodium album* had the lowest DPPH radical scavenging activity. The order of DPPH activity for all plants are as - *Cyperus rotundus*> *Cynodon dactylon* > *Celosia argentea* >*Digera muricata* >*Corchorus olitorius* >*Fumeria indica* >*Achyranthes aspera* >*Chenopodium album* **(Table 3)**. The reference standard ascorbic acid showed the IC_{50} Value 29.37 µg/ml in DPPH assay.

Table: 3 IC_{50} Values of Different Extract Plants in DPPH Assay

Plant	IC50(µg/ml)
Positive Ctrl (Ascorbic Acid)	29.37
Cyprus rotundus	676.18
Cynodone dectylone	1314.40
Celosia Argentia	1871.33
Digeria Muricata	1920.71
chorchorus olitories	3940.64
Fumaria indica	4091.09
Achyranthus aspera	4837.80
Chenopodium album	6089.88

3.4 *Salmonella typhemurium* reverse mutation assay (AMES assay):
Hydroalcoholic extracts of *Fumeria indica, Celosia argentea, Chenopodium album, Corchorus olitorius, Digera muricata, Cyperus rotundus, Achyranthes aspera, Cynodon dactylon* were tested for their mutagenic and cytotoxic activity by using *Salmonella typhimurium* reverse mutation assay (AMES test). All plant extracts were found non mutagenic and non-cytotoxic at tested concentrations eg 10- 1000 µg/dish in for bacterial stairs eg. TA97a, TA98, TA100, and TA102 (Table 4 & Table 5).

Table-4: Showing mutagenic response of different tester strains to control and positive control in Spot assay

Groups	TA-97a	TA-98	TA-100	TA-102
Control	-ve	-ve	-ve	-ve
Positive Control	+ve	+ve	+ve	+ve

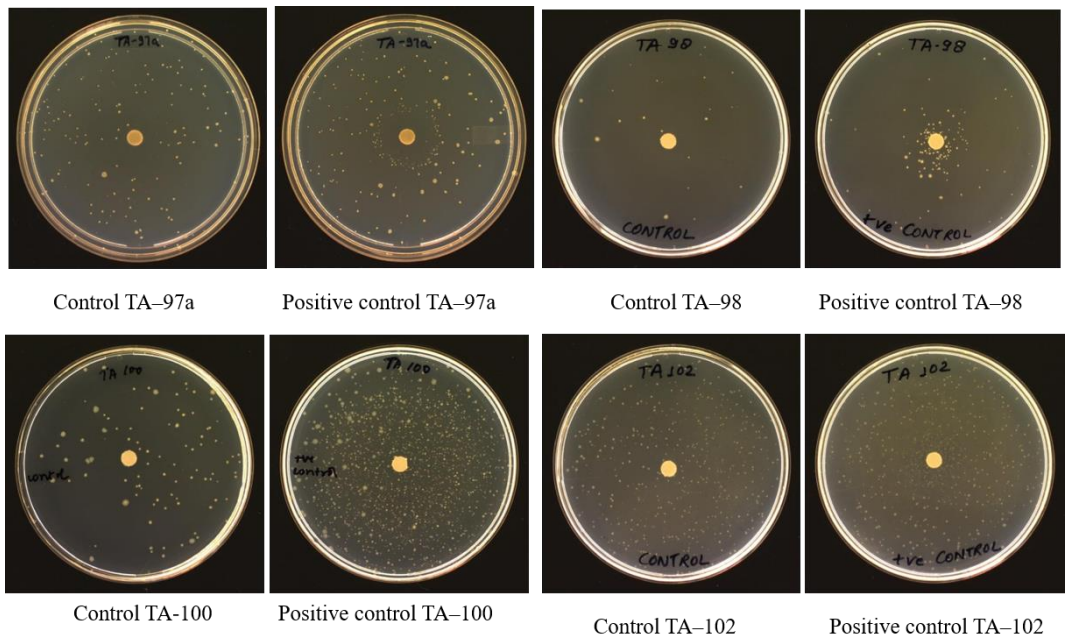

Figure: 3 Showed the control and positive control of TA97a, TA98, TA100 and TA102

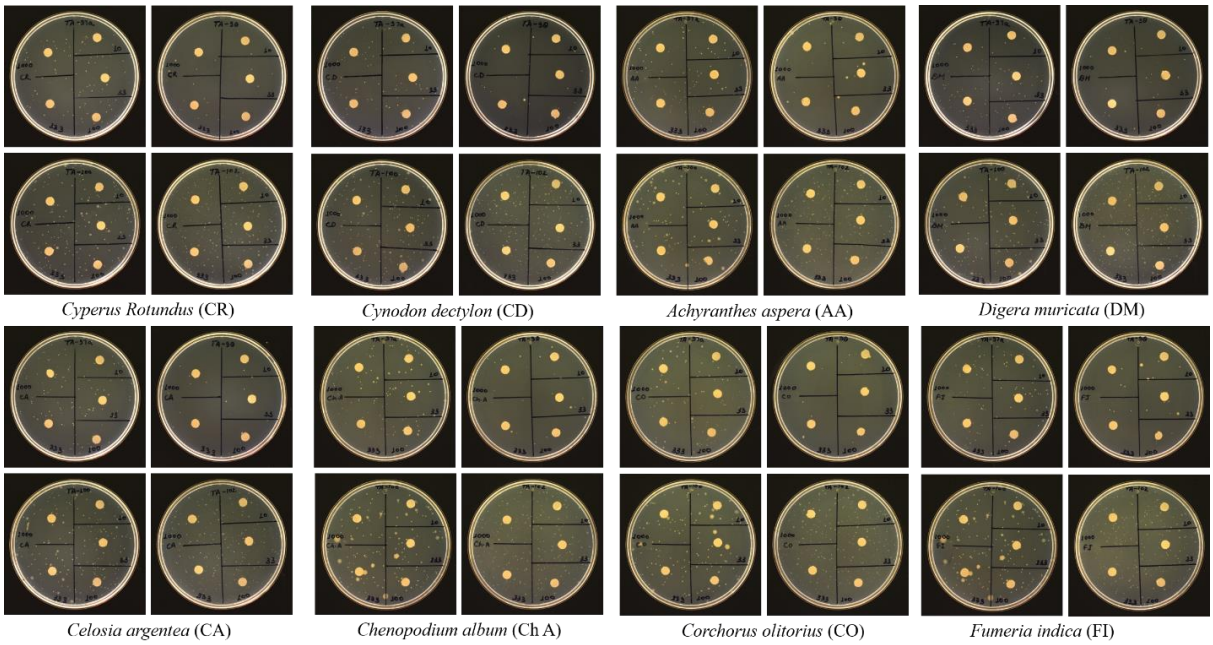

Figure: 4 Showed the non-mutagenic effect of *Cyperus rotundus, Cynodone dectylone, Achyranthes aspera, Digera muricata, Celosia argentea, Chenopodium album, Corchorus olitorius, Fumeria indica.*

Table-5: Showing mutagenic response of different tester strains to different concentrations of *Fumeria indica, Celosia argentea, Chenopodium album, Corchorus olitorius, Digera muricata, Cyperus rotundus, Achyranthes aspera, Cynodon dactylon* in Spot assay

Name of Medicinal plants	Bacterial strain	10 µg /plate	33 µg /plate	100 µg / plate	333 µg / plate	1000mg/ plate
Cyperus rotundus	TA-97a	-ve	-ve	-ve	-ve	-ve
	TA-98	-ve	-ve	-ve	-ve	-ve
	TA-100	-ve	-ve	-ve	-ve	-ve
	TA-102	-ve	-ve	-ve	-ve	-ve
Cynodone dectylone	TA-97a	-ve	-ve	-ve	-ve	-ve
	TA-98	-ve	-ve	-ve	-ve	-ve
	TA-100	-ve	-ve	-ve	-ve	-ve

	TA-102	-ve	-ve	-ve	-ve	-ve
Achyranthes aspera	TA-97a	-ve	-ve	-ve	-ve	-ve
	TA-98	-ve	-ve	-ve	-ve	-ve
	TA-100	-ve	-ve	-ve	-ve	-ve
	TA-102	-ve	-ve	-ve	-ve	-ve
Digera muricata	TA-97a	-ve	-ve	-ve	-ve	-ve
	TA-98	-ve	-ve	-ve	-ve	-ve
	TA-100	-ve	-ve	-ve	-ve	-ve
	TA-102	-ve	-ve	-ve	-ve	-ve
Celosia argentea	TA-97a	-ve	-ve	-ve	-ve	-ve
	TA-98	-ve	-ve	-ve	-ve	-ve
	TA-100	-ve	-ve	-ve	-ve	-ve
	TA-102	-ve	-ve	-ve	-ve	-ve
Chenopodium album	TA-97a	-ve	-ve	-ve	-ve	-ve
	TA-98	-ve	-ve	-ve	-ve	-ve
	TA-100	-ve	-ve	-ve	-ve	-ve
	TA-102	-ve	-ve	-ve	-ve	-ve
Corchorus olitorius	TA-97a	-ve	-ve	-ve	-ve	-ve
	TA-98	-ve	-ve	-ve	-ve	-ve
	TA-100	-ve	-ve	-ve	-ve	-ve
	TA-102	-ve	-ve	-ve	-ve	-ve
Fumeria indica	TA-97a	-ve	-ve	-ve	-ve	-ve
	TA-98	-ve	-ve	-ve	-ve	-ve
	TA-100	-ve	-ve	-ve	-ve	-ve
	TA-102	-ve	-ve	-ve	-ve	-ve

4. Discussion:

Weeds are nature's ultimate survivors. They thrive in conditions where other plants fail, making them resilient and adaptable. This adaptability is due to their rapid growth, efficient reproduction, and ability to withstand harsh environments. For instance, *Cynodon dactylon* (Bermuda grass) is known for its ability to cover barren soil, preventing erosion and contributing to soil fertility. Weeds are often viewed as unwelcome intruders in gardens, farms, and landscapes. However, a closer look reveals that many weed plants have fascinating roles in ecosystems and even provide significant benefits to humans. Far from being mere pests, weeds like *Fumaria indica*, *Chenopodium album*, *Cynodon dactylon*, and others possess remarkable qualities that make them valuable in medicine, and environmental conservation. Many weeds considered nuisances have long been used in traditional medicine (Ko & Kwahk, 2024; Kumari et al., 2018). Chenopodium album (Bathua), a common weed, is packed with nutrients like vitamins A, C, and iron(Saini & Saini, 2020). It also shows antioxidant and anti-inflammatory properties. Fumaria indica is used in Ayurveda for detoxifying the liver and improving skin health(Gupta et al., 2012). Achyranthes aspera is a known anti-inflammatory agent(Mengie et al., 2021), often used in herbal remedies for joint pain and digestive issues. These examples remind us that weeds are not just competitors for crops but also a source of healing compounds.

An imbalance between free radicals and antioxidants in the body is responsible for several chronic diseases such as diabetes, cancer, and cardiovascular disorders. Nature, with its vast diversity of plant species, offers a treasury of compounds with antioxidant properties. Among these, *Fumaria indica*, *Celosia argentea*, *Chenopodium album*, *Corchorus olitorius*, *Digera muricata*, *Cyperus rotundus*, *Achyranthes aspera*, and *Cynodon dactylon* stand out for their rich phytochemical profiles and health-promoting potential. Out of all plant extract *Cyperus rotundus* showed the highest antioxidant activity in DPPH assay in this study. *Cyperus rotundus* contains high amount of sesquiterpenoids (such as cyperene, α-cyperone, and β-selinene), flavonoids, phenolic acids, and saponins. Other important bioactive molecules include cyperotundone, valencene, and nootkatone. These compounds not only underpin the plant's antioxidant activity but also exhibit anti-inflammatory and anticancer properties.

All plant extracts were analyzed for their mutagenic effect through Ames assay, the results of Ames assay showed that all plant extracts were non mutagenic up to tested dose level of 1000 µg/ml in spot assay. The similar results were also observed by

5. Conclusion

The results of qualitative phytochemical estimations showed the presence of all common secondary metabolites. The AMES assay result showed that all tested weed plants are non-mutagenic up 1000 µg/ml. Out of all tested ten hydro alcoholic extract of different weed plants, *Cyperus rotundus* showed the highest in vitro antioxidant activity.

6. Acknowledgments

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7. Conflicts of Interest

Author declare no conflict of interest.

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