Annals of Biology **40** (1) : 1-8, 2024

Impact Score : 0.32 (Scopus)

Antimicrobial, Antioxidant and Phytochemical Analysis of Weed Plants of Amaranthaceae Family

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(Received: July 2, 2023; Accepted: August 7, 2023)

ABSTRACT

This study examined the antimicrobial activities, antioxidant properties and phytochemical composition of weed samples from the Amaranthaceae family. Antimicrobial activity was assessed using the disc diffusion method with weed extracts at 25 and 50 micrograms. The presence of phenolic compounds, indicating antioxidant activity, was measured at 517 nm. Phytochemical analysis was screened for glycosides, saponins, flavonoids, alkaloids, tannins, terpenoids, steroids and reducing sugars in the methanol extracts. Among the species tested, *Achyranthes aspera* and *Alternanthera sessilis* exhibited the highest antibacterial activity against *Bacillus rhizoplanae* and *Pseudomonas protogens*, respectively, with a 9 mm zone of inhibition. *Amaranthus deflexus* displayed the least antibacterial activity. *A. aspera* demonstrated the highest antifungal activity against *Phoma herbarum*, while *A. sessilis* showed the largest zone of inhibition (9 mm) against *Penicillium xyleborini*. *A. deflexus* exhibited the highest zone of inhibition (8 mm) against *Trichoderma reesei*. *Chenopodiastrum murale* showed the highest antioxidant activity, whereas *Chenopodium ambrosioides* exhibited the least. Phytochemical analysis revealed the presence of alkaloids, tannins, glycosides and flavonoids in most plants. These findings suggest that methanolic extracts of these weed species may serve as antimicrobial agents and sources of antibiotics for bacterial illnesses. Additionally, their strong antioxidant activity indicated potential as natural alternatives to synthetic antioxidants for food preservation.

Key words: Amaranthaceae, weeds, antimicrobial, antioxidant, phytochemical, disc diffusion

INTRODUCTION

Humans have been using plants to heal illnesses for centuries; many herbal medications have been developed (Kambale *et al.*, 2022). The World Health Organization (WHO) estimates that up to 80% of the global population receives its essential medical treatment through traditional medicine. WHO survey preliminary finding indicated that a sizable and expanding population, particularly young people, utilized medicinal herbs. Furthermore, according to the World Health Organization, medicinal plants are the best source for a wide range of drugs. Pesticides' toxic and persistent effects on helpful bacteria and the ecosystem have had a negative impact on the environment (Chowdhary *et al.*, 2018). Despite the fact that many weeds and their relatives are sometimes seen as unpleasant or inconvenient plants, they are essential to human survival because they constitute a rich natural supply of bioactive chemicals. Recently, interest in natural products has increased among those searching for novel antifungal therapies and pharmacological medications (Ferreira *et al.*, 2022). Such bioactive compounds are present in significant quantities in both weeds and cultivated plants (Khan and Javaid, 2019; Khan *et al.*, 2020).
Increased levels of several free radicals, including hydroxyl, peroxide and superoxide, which have the potential to be extremely harmful to macromolecules like DNA, lipids and proteins, are caused by the elevated reactive oxygen species (ROS) levels, which are the result of an imbalance in favour of oxidative stress. Carcinogenesis, immunosuppression, Parkinson's, Alzheimer's, atherosclerosis, inflammation, infectious disease, chronic obstructive pulmonary disease, cataract development, diabetes, hypertension, ageing and hair loss are all conditions that can be brought on by macromolecule dysfunction. Previous studies using animal models showed that synthetic antioxidants such butylated hydroxyl anisole, butylated hydroxyl toluene and propyl gallate caused internal and external

bleeding when used in high dosages (Xu *et al.*, 2021). Herbs and their byproducts, including fruits, vegetables and spices, have been utilized for treating a variety of illnesses since ancient times. It is possible to find novel antibacterial, antioxidant and anti-inflammatory medications from alternative sources due to the rapid emergence of multi-drug resistance against antimicrobials and anticancer treatments as well as the toxicities caused by impurities of synthetic compounds (Gupta and Birdi, 2017).

The phytochemical components, organic bioactive substances, nutrients and fibre found in healing plants, fruits and vegetables protect us against a variety of diseases. Primary ingredients such amino acids, carbohydrates, proteins and chlorophyll are two important categories of phytochemicals. Alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, and phenolic compounds are examples of secondary ingredients. In addition, they have important medicinal properties (Niranjan and Prabhurajeshwar, 2020). In order to prevent hazardous germs from proliferating, medicinal plant extracts are frequently used as food preservatives (Stanislav *et al.*, 2019). Flavonoids and phenolic compounds are known to be potent antioxidants, anticancer, antibacterial, anti-inflammatory, immune system-stimulating and skin-protective agents. They also have potential as pharmaceutical and medicinal agents. Flavonoids and other phenolic compounds from medicinal plant species have been the subject of extensive investigation over the years due to their many benefits for human health (Tungmunnithum *et al.*, 2018).

In this study plants were used to examine the antimicrobial, antioxidant and phytochemical analysis from weed samples of Amaranthaceae family i.e. *Alternanthera sessilis* (Sessile joyweed), *Amaranthus deflexus* (Argentine amaranth), *Chenopodiastrum murale* (Nettle-leaf goosefoot), *Achyranthes aspera* (chaff-flower) and *Chenopodium ambrosioides* (American wormseeds). *A. sessilis* contains alkaloids, flavonoids, tannins, saponins, terpenoids, phenols and carbohydrates. It has febrifuge, galactagogue and cholagogue properties. *A. Aspera* has laxative, stomachic, carminative, astringent and good diuretic properties. It is used to treat kidney stones, piles, coughs, pneumonia and other conditions. *A. deflexus* is a rich source of amaranthine and phenolic compounds with high antioxidant and these molecules were used as reducing agents. *Chenopodiastrum murale*'s active components, which have been shown to be anti-fungal, or its abundance in flavonoids, particularly the active compounds of kaempferol glycosides and aglycone, which have been shown to be antifungal, may be responsible for the antimicrobial actions. *C. ambrosioides* is used as a leaf vegetable, herb, and herbal tea and is best harvested before going to seed.

MATERIALS AND METHODS

The five weed plant species of Amaranthaceae family were obtained from N.H. 21 roadside, near Hallomajran lights, Chandigarh. The botanical identification was carried out at Department of Botany, Panjab University, Chandigarh. The Accession Nos. for *Amaranthus deflexus, Achyranthus aspera, Alternanthera sessilis, Chenopodium ambrosioides* and *Chenopodium murale* are 22640, 22641, 22642, 22643 and 22644, respectively.

For antifungal activity, fungal strains used were: *Penicillium xyleborini, Phoma herbarum,* and *Trichoderma reesei.* For antibacterial activity, bacterial spp. used were: *Bacillus rhizoplanae* and *Pseudomonas protogens*.

After collection, the selected plant materials were chopped into little pieces, cleaned with distilled water and allowed to dry for four weeks in the shade. Direct sunlight was often avoided to lessen the increase of the chemical processes. However, it should be dried as quickly as possible in an open area at room temperature, with air circulation around the plant material, to prevent heat and moisture. Water and methanol were used as the extraction solvents to remove the phenolic components. For methanol extraction, 10 g of each powdered sample of the dried, powdered leaves, flowers, roots and seeds were added to 90 ml of methanol. The mixtures were sonicated for 30 min and then shaken in an incubator for 3-4 days. After thorough mixing of powdered weed with methanol, the mixture was taken out of the incubator and allowed to filter out using Whatman filter paper. The liquid filtrate was methanol extract.

In a flask, 17.5 g of the dehydrated powder or lab-prepared MS media was added to 500 ml of

distilled or deionized water. The suspension was then heated to boil to dissolve the medium completely. The dissolved medium was then autoclaved at 15 lbs pressure (121°C) for 15 min. Once the autoclave process was complete, the beaker was taken out and cooled to a temperature of 40-45°C. The media was then poured into sterile Petri plates under sterile conditions. Once the media solidified, the plates were placed in the hot air oven on a lower heat setting for a few minutes to remove any moisture present on the plates before use. After the media got solidified, inoculation of bacteria was done. The bacterial culture was ready for further use.

19.5 g of PDA powder was poured in 500 ml of distilled water in a flask. The flask was heated to completely dissolve the medium. It was sterilized by autoclaving at 15 lbs pressure at 121°C for 15 min. It was mixed well before dispensing. Once the autoclave process was complete, the beaker was taken out and cooled to 40-45°C. The media was then poured into sterile Petri plates under sterile conditions.

At first 25% DMSO was made. Twenty-five µg of solid methanolic weed extract, 750 µl of 25% DMSO and 4 mm thick Whatman disc were added in one Petri plate. Similarly, in another Petri, plate 50 µg of solid methanolic weed extract, 500 µl of 25% DMSO and 4 mm thick Whatman disc were added. In third Petri plate, the control was prepared by adding 1000 µl of 25% DMSO and 4 mm thick Whatman disc. These two discs of different concentration of weed extract and control were allowed to absorb the content for about 24 h. After 24 h of absorption, two discs of different concentration of weed extract and control disc were placed in each Petri plate of freshly prepared bacterial and fungal culture in laminar air flow. For 24-48 h, the plates were incubated at 37°C. A metric ruler was used to measure the zone of inhibition in discs (Table 1).

One g of the powder sample was added to 10 ml of methanol or ethanol. The mixture was shaken for 30 min using a shaker or vortex mixer, and then filtered through a filter paper to remove any insoluble particles. The resulting extract was used for the DPPH assay. To perform the assay, 2 ml of the DPPH solution was added to 2 ml of the diluted weed plant extract at various concentrations. The mixture was allowed to react in the dark for 30 min at room temperature. The degree of discolouration of the DPPH solution, which was proportional to the antioxidant activity of the plant extract, was measured spectrophotometrically at 517 nm.

A phytochemical screening is a preliminary examination to discover both primary and secondary metabolites in an extract. The qualitative methods described below were used to identify alkaloids, flavonoids, tannins, saponins, flavones, sterols, terpenes, cardiac glycosides, protein, carbohydrates and lipids.

The diluted extract was treated with 3-4 drops of 10% $FeCl_3$; gallic tannins turned the solution blue, whereas catechol tannins turned it green. Heating 0.5 ml of plant extract with 1 ml of water and 5-8 drops of Fehling's solution was used as a test for decreasing sugar. Brick red precipitation emerged as a sign that reducing sugar was present. Molisch's Reagent Test was used to detect glycosides. The extract was mixed with concentrated H_2SO_4 and Molisch's reagent in a volume of 5 ml. A violet tint glycosides identified it.

Four ml of extract solution and 1.5 ml of 50% methanol solution was warmed with a little piece of magnesium. Five-six drops of strong HCl were added to turn it red indicating presence of flavonoids. Two ml of extract were combined with 1 ml of Meyer's reagent. The pale-yellow precipitate indicated the presence of alkaloids.

Twenty ml of distilled water was used to boil 1 g of the powdered sample. Ten ml of filtrate and 5 ml of distilled water were agitated ferociously. The emergence of foaming revealed the presence of saponins. 0.2 g of each

Table 1. Zones of inhibition of weed extracts against *Pseudomonas protegens*

Weeds	25 µg extract + 750 µl DMSO (mm)	50 µg extract + 500 µl DMSO (mm)	1000 µl DMSO (mm)
Achyranthes aspera	5	9	2
Alternanthera sessilis	7	9	3
Amaranthus deflexus	6	8	4
Chenopodiastrum murale	6	8	2
Chenopodium ambrosioides	6	9	2

sample was mixed with 3 ml of concentrated H_2SO_4 and 2 ml of chloroform. The reddish-brown colour suggested that terpenoids were present.

One g of plant extract was dissolved in a few drops of acetic acid. Adding a drop of concentrated H_2SO_4, steroid usage was performed. The appearance of green colour served as a signal that steroids were present.

RESULTS AND DISCUSSION

Medicinal plants contain a variety of biochemical and phytochemical compounds that have antibacterial and antifungal effects on human ailments. The search for alternative antimicrobial medications from plant extract garnered a lot of attention because of its potential efficiency against microorganisms and the advantage that infection cannot concurrently evolve resistance (Kebede *et al.*, 2021).

Among the tested weed species, *Alternanthera sessilis* exhibited the highest values of zone of inhibition. When treated with 50 µg weed extract and 500 µl of DMSO, the zone of inhibition was measured 9 mm. This indicated that *A. sessilis* extract at this concentration had a strong inhibitory effect on *Pseudomonas protegens*. Following closely, *Achyranthes aspera* and *Chenopodium ambrosioides* also showed significant inhibitory activity. With 50 µg weed extract and 500 µl of DMSO, both weeds demonstrated a zone of inhibition measuring 9 mm. *Amaranthus deflexus* and *C. murale* exhibited slightly lower but still notable inhibitory effects, with zone of inhibition values ranging from 6 to 8 mm (Table 1). *A. sessilis* exhibited the second highest values of zone of inhibition, ranging from 6 to 8 mm when mixed with 25 µg of extract and 750 µl of DMSO. *C. ambrosioides* also showed notable inhibitory activity with a zone of inhibition ranging from 6 to 9 mm. *C. murale* exhibited a similar trend, with zone of inhibition values ranging from 5 to 7 mm. *A. deflexus* had relatively lower values, ranging from 4 to 7 mm. Based on these results, it can be concluded that *A. aspera* extract possessed the highest inhibitory potential against *B. rhizoplanae*, followed by *A. sessilis*, *C. ambrosioides*, *C. murale* and *A. deflexus* (Table 2).

Among the tested weed extracts against *Penicillium xyloborini*, the highest values of zone of inhibition were observed at both 25 µg and 50 µg concentrations. *A. sessilis* exhibited the highest zone of inhibition at both 25 µg and 50 µg concentrations, with values of 5 and 9 mm, respectively. Following closely behind, *A. aspera* displayed a zone of inhibition of 4 mm at 25 µg concentration and 8 mm at 50 µg concentration. *A. deflexus* and *C. murale* showed similar trends, with zone of inhibition values ranging from 5 to 7 mm and 3 to 8 mm, respectively, at both 25 µg and 50 µg concentrations. *C. ambrosioides* exhibited the lowest zone of inhibition values among the tested weeds, with values of 2 and 6 mm at 25 and 50 µg concentrations, respectively. When 1000 µl of DMSO was used as a control, all the weed extracts resulted in smaller zones of inhibition, ranging from 1 to 3 mm. Based on these findings, *A. sessilis* extract demonstrated the highest inhibitory potential against *P. xyloborini* at both 25 and 50 µg concentrations (Table 3).

Among the tested weed extracts against *Phoma herbarum*, the highest values of zone of inhibition were observed at both 25 and 50 µg concentrations. *A. aspera* demonstrated the highest zone of inhibition at 25 µg concentration, with a value of 3 mm. *A. sessilis* and *C. ambrosioides* exhibited slightly higher values at 25 µg concentration, ranging from 4 to 3 mm. When the concentration was increased to 50 µg, *A. aspera* displayed a zone of inhibition of 7 mm, which was the highest among the tested weed extracts. *A. sessilis* and *A. deflexus* showed similar zone of inhibition

Table 2. Zones of inhibition of weed extracts against *B. rhizoplanae*

Weeds	25 µg extract + 750 µl DMSO (mm)	50 µg extract + 500 µl DMSO (mm)	1000 µl DMSO (mm)
Achyranthes aspera	5	9	2
Alternanthera sessilis	6	8	4
Amaranthus deflexus	4	7	2
Chenopodiastrum murale	5	7	3
Chenopodium ambrosioides	6	9	2

Table 3. Zones of inhibition of weed extracts against *P. xyloborini*

Weeds	25 µg extract + 750 µl DMSO (mm)	50 µg extract + 500 µl DMSO (mm)	1000 µl DMSO (mm)
Achyranthes aspera	4	8	1
Alternanthera sessilis	5	9	2
Amaranthus deflexus	5	7	3
Chenopodiastrum murale	3	8	2
Chenopodium ambrosioides	2	6	1

values at 50 µg concentration, ranging from 6 to 5 mm. *A. deflexus* and *C. murale* exhibited the lowest zone of inhibition values among the tested weeds at both 25 and 50 µg concentrations, ranging from 2 to 1 mm. When 1000 µl of DMSO was used as a control, all the weed extracts resulted in similar small zones of inhibition, ranging from 1 to 2 mm. Based on these results, *A. aspera* extract demonstrated the highest inhibitory potential against *P. herbarum* at both 25 and 50 µg concentrations, followed by *A. sessilis*, *C. ambrosioides, A. deflexus* and *C. murale* (Table 4).

When evaluating the zones of inhibition of weed extracts against *Trichoderma reesei*, the highest values of zone of inhibition were observed at both 25 and 50 µg concentrations. *A. deflexus* displayed the highest zone of inhibition at 25 µg concentration, with a value of 3 mm. *A. sessilis*, *A. aspera*, and *A. deflexus* exhibited slightly lower values at 25 µg concentration, ranging from 4 to 3 mm. When 1000 µl of DMSO was used as a control, all the weed extracts resulted in similar small zones of inhibition, ranging from 1 to 2 mm. Based on these results, *A. deflexus* extract demonstrated the highest inhibitory potential against *T. reesei* at both 25 and 50 µg concentrations, followed by *A. sessilis, A. aspera, C. ambrosioides* and *C. murale* (Table 5).

Among the tested weeds, *C. murale* exhibited the highest absorbance value of 1.804 at 517 nm. This indicated that the extract of *C. murale* had a higher capacity to absorb light at this specific wavelength. Following closely behind, *A. aspera* showed an absorbance value of 1.650, indicating a significant light absorption as well. *A. deflexus* displayed a relatively high absorbance value of 1.607, while *A. sessilis* exhibited a slightly lower value of 1.414. *C. ambrosioides* displayed the least absorbance value of 1.472 among the tested weed extracts at 517 nm. These absorbance values provided insights into the light-absorbing characteristics of the weed extracts at the specified wavelength, indicating the presence of specific compounds or pigments that contributed to their absorbance capabilities. *C. murale* had the highest light absorption capacity followed by *A. aspera, A. deflexus, A. sessilis* and *C. ambrosioides* (Table 6).

There was presence of blue or green colour in all species of weed plant extract except *A. sessilis*, which meant that in the plant species

Table 4. Zones of inhibition of weed extracts against *P. herbarum*

Weeds	25 µg extract + 750 µl DMSO (mm)	50 µg extract + 500 µl DMSO (mm)	1000 µl DMSO (mm)
Achyranthes aspera	3	7	2
Alternanthera sessilis	4	6	1
Amaranthus deflexus	2	6	1
Chenopodiastrum murale	2	5	1
Chenopodium ambrosioides	3	6	1

Table 5. Zones of inhibition of weed extracts against *T. reesei*

Weeds	25 µg extract + 750 µl DMSO (mm)	50 µg extract + 500 µl DMSO (mm)	1000 µl DMSO (mm)
Achyranthes aspera	3	6	1
Alternanthera sessilis	4	6	2
Amaranthus deflexus	3	8	2
Chenopodiastrum murale	2	8	2
Chenopodium ambrosioides	2	7	2

Table 6. Absorbance of different weeds extract at 517 nm

Weeds	Absorbance at 517 nm
Achyranthes aspera	1.65
Alternanthera sessilis	1.414
Amaranthus deflexus	1.607
Chenopodiastrum murale	1.804
Chenopodium ambrosioides	1.472

there was presence of gallic acid or catechol tannins (Table 7). A very light colour appeared and observation of brick red ppt was not which meant there was no presence of reducing sugars. Glycosides were recognized by a violet colour appearance in all weed plants except *C. ambrosioides*. Flavonoids showed a crimson colour which meant there was presence of flavonoids in all weed plant species (Table 7). The presence of alkaloids was revealed by the pale-yellow precipitate. The presence of saponins was suggested by the development of foaming. All weed plant species showed positive result for this test. The presence of terpenoids was suggested by the reddish-brown colouring in all weed plant species except *C. murale*.

The presence of steroids was detected by the emergence of green colour. Only light colour was observed in plant species except black colour of *A. deflexus* and *C. murale* showing absence of steroid in other plant species.

In this study, antimicrobial properties in the methanolic extracts were assessed of *A. deflexus, A. aspera, A. sessilis, C. ambrosioides* and *C. murale*'s. Fungi and bacteria were affected by the methanolic extract of the different parts of all plant species as development of a halo with a diameter of 0.5-0.9 cm. In a similar study, the methanol and chloroform extracts of the plant *A. sessilis* were subjected to antibacterial and antifungal activities in terms of zone of inhibition. The zone of inhibition study revealed that methanol extract showed 16, 9, 12 and 14 mm on *Escherichia coli, Staphylococcus aureus, Candida albicans* and *Aspergillus fumigates,* respectively. The chloroform extract seemed to be effective with 10 mm on *E. coli*. The control of both methanol and chloroform did not show any remarkable zone of inhibition (less than 7 mm) therefore their values were ignored (Nayak and Bhatta, 2020).

Mishra *et al.* (2020) observed that the antibacterial activities of different concentrations of methanolic and petroleum-ether leaf extracts of *A. aspera* against three gram-positive bacteria (*Micrococcus luteus, Bacillus subtilis* and *Streptococcus mitis*) and six gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella typhi, Salmonella paratyphi* A (MTCC-3220) and S*higella flexneri*). The methanolic extract at 0.0781 mg/ml concentration showed least antibacterial activity against all tested bacteria producing a zone of inhibition between 10 to 12 mm, while petroleum ether extract of same concentration had moderate antibacterial activity against *S. flexneri* (15 mm zone of inhibition). The present results showed similar antimicrobial activity against several strains of fungi (*P. xyleborini, P. herbarum, T. reesei*), Gram-positive bacteria (*B. rhizoplanae*) and Gram-negative bacteria (*P. protogens*) with zone of inhibition ranging from 0.5- 0.9 cm for methanol extracts of different weed plant species.

In this study, DPPH test was performed to determine the ability of a methanolic extract of Amaranthaceae family fractions to scavenge free radicals. The antioxidant components in the plants extract scavenged the free radicals generated during these experiments. The DPPH test measured spectrophotometrically the scavenging potential of the plants extract

Table 7. Phytochemical analysis of different weeds of Amaranthaceae family

Phytochemical tests	*Achyranthes aspera*	*Alternanthera sessilis*	*Amaranthus deflexus*	*Chenopodiastrum murale*	*Chenopodium ambrosioides*
Tannin/Polyphenol	+	-	+	+	+
Reducing Sugar	-	-	-	-	-
Glycosides	+	+	+	+	-
Flavonoids	+	+	+	+	+
Alkaloids	+	+	+	+	+
Saponins	+	+	+	+	+
Terpenoids	+	+	+	-	+
Steroids	-	-	+	+	-

- shows the absence of phytochemicals and + shows presence of phytochemical compounds.

by converting DPPH (a, a-diphenyl-b-picrylhydrazyl) into a, a-diphenyl-b-picrylhydrazine and altering its colour. In the present study, methanol extract of *C. murale* exhibited the highest absorbance value of 1.804 at 517 nm. This showed that plant methanolic extracts, especially at higher concentrations, had a strong scavenging action. The concentration of this parameter was in line with the concentration of phenolic compounds in various plant species' methanolic extracts. These findings suggest that the antioxidant activity of the plant extract may be due to the phenolic components. Higher amounts of phenolic components enhance the chance of hydrogen donation to free radicals, increasing the scavenging activity of the extract since there are more hydroxyl groups in the reaction media (Sharaibi *et al.*, 2023).

The presence of phytochemical compounds such as phenolics, flavonoids, alkaloids, tannins, saponins, steroids and triterpenes, which had antimicrobial properties to disrupt the cell membrane, could be the cause of the various antimicrobial activities of plant extracts. The findings of this study are in accordance with those of Sureshkumar (2021) who showed results of phytochemical screening of both methanol and aqueous extract of *A. aspera*. The results revealed the presence of various phytochemical constituents including alkaloids, flavonoids, amino acids, carbohydrates, saponins, tannins and phenolic compounds. Similar study on *A. aspera* showed similar result as in their result, almost all tested phytochemicals were found in methanolic plant extracts except coumarin in *A. aspera* (Yashoda *et al.*, 2021). Similar study revealed that there was presence of alkaloids, flavonoids, terpenoids, tannins but no steroids, reducing sugars and saponins in methanolic extract of *A. aspera* (Karunakaran and Niranjana, 2023).

According to other sp. of Amaranthus, phytochemical screening of the extract from leaves of *A. viridis* L. indicated the presence of biologically active constituent's saponins, tannins, phenols, flavonoids, alkaloids, cardiac glycoside, steroids and triterpenoids (Kumar *et al.*, 2022). Similar study of *C. ambrosioides* showed that in methanolic leaf extract alkaloids, flavonoids, saponins, tannins, phenolic groups, terpenoids, fats and oils were present (Oumarou *et al.*, 2021). Tchani *et al.* (2021) conducted experiments with aqueous extracts of *C. ambrosioides*, showing the presence of certain biologically active molecules such as flavonoids, terpenoids, tannins and alkaloids. Similarly, recent study with methanolic extract of *C. ambrosioides* revealed the presence of alkaloids, terpenoids, flavonoids, tannins, saponins and phenols (Mulugeta and Daka, 2023).

CONCLUSION

From the study, it could be concluded that plants used were great source of antimicrobial, antioxidant and phytochemicals that could be utilized in curing various ailments. For antibacterial activity, *Achyranthes aspera* and *Alternanthera sessilis* showed maximum zone of inhibition against *Bacillus rhizoplanae* and *Pseudomonas protogens,* respectively. *A. aspera* showed maximum antifungal activity against fungus *Phoma herbarum.* In case of *Trichoderma* fungus, *Amaranthus deflexus* displayed the highest zone of inhibition. In antioxidant properties, methanol extract of *Chenopodiastrum murale* plant exhibited highest antioxidant activity. For phytochemical, almost all the plants showed the presence of alkaloids, tannins, glycosides and flavonoids. According to the study's findings, all of the plants had phyto-constituents. Methanolic extract of all plant species may operate as an antimicrobial agent and a source of antibiotics for the treatment of bacterial illnesses. These plants extract can be utilized as an effective substitute for synthetic antioxidants for preserving food ingredients and edible oils because of its strong antioxidant activity.

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