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Research Article

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Antimicrobial, Antioxidant and Phytochemical Properties of Weeds of Poaceae Family

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Abstract

Weed plants are a significant therapeutic plant in India. Since the beginning of time, they have been utilized in almost all traditional medical practices, such as ayurveda, unani and siddahs. This study characterizes the antimicrobial activity, antioxidant activity and phytochemical analysis of five commonly identified weed plants of Poaceae family such as *Sorghum halepense, Dactyloctenium aegyptium, Setaria viridis, Cynodon dactylon,* and *Avena sativa.* Antibacterial activity was conducted against the *Bacillus rhizoplanae, Pseudomonas protogens* and antifungal against *Penicillium xyleborini, Phoma herbarum, Trichoderma reesei* by using the disc-diffusion method and antioxidant activity by using spectrophotometric assays. Results revealed that methanolic extracts of all the plants have phyto-constituents in them. For antibacterial activity, *S. halepense* shows a maximum zone of inhibition of 9 mm and *C. dactylon* shows maximum antifungal activity against fungi *P. herbarum* and *T. reesei.* The highest antioxidant capacity measured by DPPH (1.637, 1.362, 1.333, 1.212 respectively) method was also obtained in *S. viridis*, being the highest antioxidant activity with an absorbance of 1.791 at 517 nm. This amazing antioxidant activity in plants is attributed to various kinds of phytochemicals. These plant extracts can improve environmental quality and bio-valorization in addition to encouraging people's health. The phytochemical profile of weed plants becomes instrumental in creating functional and health-promoting food products. Incorporating these plants into the food industry can enhance the nutritional content of various products, providing consumers with not only flavorful options but also foods rich in bioactive compounds with potential health benefits.

Keywords

Antimicrobial, Antioxidant, Food industry, Phytochemical, Spectrophotometric, Therapeutic

Introduction

The grass family or Poaceae is one of the wide-reaching with majorly varied families of flowering plants. It is made up of around 11,000 species that may be found in almost every continent and climate zone. Medicinal plants are essential for the development of substitute medications that are free of the adverse side effects associated with synthetic pharmaceuticals [1]. The family of Poaceae is known for having fewer therapeutic applications and its predominance of alkaloid-free species [2]. Additionally, this family has limited chemical defenses to combat herbivory and is largely reliant on physical defenses, such as silicates and leaf hardness, to protect against it. This family plant contains kinds regarding natural products mainly tannins or lignin's, which are thought to be physiologically inactive or to be of low activity. Malnutrition and deficiency of antioxidants may

make people more sensitive to oxidative stress and raise their chance of developing cancer [3]. Long-term inflammation, as seen in conditions like long-lasting health problems, gastrointestinal illness, and neurotoxicity including heart disease can overwhelm antioxidant defenses [4]. There is a growing demand for natural, non-toxic preservatives because people become more aware of the dangers of synthetic preservatives. Many of these preservatives are expected to possess antioxidant and antimicrobial properties. As less solvent is required and extraction times are shorter than with the conventional extraction method, polyphenol extractions from plants using rapid and efficient methods are a cost-effective approach. Botanical insecticides have become more popular due to their efficacy, biodegradable nature, reduced toxicity, and extensive ability of raw materials [5]. Both ancient and modern medicinal products are derived from plants and natural products and the development of commercial pharmaceutical products currently relies heavily on these natural resources [6]. Furthermore, the development of replacement medications free from adverse effects caused by synthetic pharmaceuticals is significantly facilitated by medicinal plants [7]. Antibiotic resistance has recently been addressed using a variety of strategies. One of these methods has been the suggestion to mix ineffective medicines with other molecules to regain their intended antibacterial activity [8].

Phytochemicals, abundant in plants, are at present the basis of medication research [9]. Phytochemicals, in this case, have been found to have potent effects and numerous studies have been conducted to use natural resources against fungi and bacteria resistance. Polysaccharides from plants have been the focus of research due to their potential biological activities such as their antioxidant and antimicrobial with their alteration phytochemical changes. Numerous weed plants are characterized as the primary source of natural antioxidants. The function of secondary metabolites in plants plays a role in biotic defense against disease, herbivores, and insects as well as in abiotic survival in challenging environmental conditions [10]. It also looked at the extract of leaf, stem, root, and inflorescence of weed plants of Poaceae family. Plant extracts of methanol were tested for research purposes. The extracts were processed for qualitative determinations of certain phytochemical constituents.

Antioxidants have the potential to significantly affect human health. The search for plant bioactive compounds with different mechanisms of action to fight harmful microbes and natural antioxidants that save humans from redox imbalance and destruction spawned by free radicals has been sparked by the rise of antibiotic resistance among microbes and the various diseases that people suffer from due to reactive oxygen species [11, 12]. Free radicals have a strong-standing relationship with oxidative stress, which is when oxygen reacts with certain molecules. Various types of antioxidants in the secondary metabolites are produced by plants. These are substances that plants make during development as part of their defense system, and they act as antioxidants [13, 14]. As a consequence, their antioxidants, antimicrobials, colorants with flavoring characteristics, the secondary metabolites are classified as food by-products, making them a high-value food additive [15, 16].

Antimicrobials are chemicals that kill or inhibit the development of microorganisms like bacteria, fungi, protozoa, etc. An antimicrobial drug either kills the microorganism (microbiocide) or stops the growth of the microorganism (microbiostasis). An antimicrobial compound called a disinfectant is used on non-living things [17]. Plants have been used for centuries for their antimicrobial properties, which are made up of compounds that are produced during the secondary metabolism of plants. In addition to flavonoids and phenolics, other phytochemical antioxidants found in plants include carotenoids and tannins [18-20].

Phytochemicals, also known as plant chemicals, are the chemicals found in plants that are not edible but have a range of health benefits and anti-disease properties. Plant chemicals contain non-essential elements that your body does not need to function and maintain life [21]. Phytochemicals have both macronutrients and micronutrients. Phytochemical screening is a scientific process that involves the analysis, inspection, extraction, and experimentation of plant-derived substances including flavonoids, terpenoids, quinine and other phytochemicals. These play a role in a variety of metabolic processes that promote healing consequences, including anticancer, antimutation, and anti-inflammation with antioxidant properties [22]. The importance of phytochemical analysis and antioxidant activity of weed plants in food chemistry is gaining recognition as researchers and food scientists delve into the vast reservoir of bioactive compounds present in these often-underestimated plants. Weed plants, characterized by their adaptability and resilience, have been found to contain a myriad of phytochemicals that contribute significantly to their antioxidant properties, making them potential candidates for enhancing the nutritional value and shelf life of food products [23].

Phytochemical analysis is a key aspect of understanding the composition of weed plants. These analyses reveal the presence of various secondary metabolites, including flavonoids, alkaloids, terpenoids, and polyphenols. Each of these compounds plays a distinct role in the overall antioxidant potential of the plant. Flavonoids, for instance, are known for their ability to neutralize free radicals, while polyphenols exhibit strong antioxidant activity, contributing to the plant's defense mechanisms against environmental stressors [24, 25]. In addition to their direct use in food products, the antioxidant properties of weed plants can also be harnessed in food packaging. Developing packaging materials infused with weed-derived antioxidants helps prevent oxidation and spoilage of the packaged food, contributing to a reduction in food waste and enhancing the overall sustainability of the food industry.

Material and Methods

Botanical identification of plants used

The five weed plant species of Poaceae family were obtained from the fields of area Bhiwani, Haryana. The botanical identification was done by the Department of Botany, Panjab University, Chandigarh. The accession numbers for *A. sativa, C. dactylon, S. viridis, S. halepense,* and *D. aegyptium* are 22645, 22646, 22647, 22654, and 22655, respectively.

Sample collection

At first, the five species of weed plants of Poaceae family are collected from the fields of area Bhiwani, Haryana (*S. halepense, D. aegyptium, S. viridis, C. dactylon,* and *A. sativa*). Completely at, the plant parts were kept shaded for 10 - 20 days and then kept in a hot air oven for complete drying. Grinding was done to make fine powder with pestle mortar.

Drying and powdering of plant material

Procedure

The selected plant material was cut into very small pieces, cleaned with distilled water, and dried in a dark period of 3 - 4 weeks. To avoid overheating and humidity build-up, the plant material should be dried as quickly as possible under circumstances of airy space and room temperature. Plant material can be ground in a mortar and pestle, electric grinders, or both and even in spice grinders. Pre-treatment procedures like drying and grinding of plant material are frequently performed before extraction to increase extraction efficiency [26]. The particles must be as uniform in size as is practical because larger particles require more time to remove [27].

Preparation of methanol extract

The primary solvent employed was water or methanol. The best solvent for extraction was using methanol for maceration since methanol is regarded as the perfect solvent. For methanol extraction, 10 g of each powdered sample of dried plant extract was added to 90 ml of methanol. After that, the mixes were incubated in an incubator shaker for 3 - 4 days. Maceration aims to break down the cell wall of a plant to release emulsifiable phytochemicals [28]. By using an electronic analytical balance, the powdered material and chemicals for the test were weighed. Then, the cotton was infused with solution. Finally, residue and filtrate were obtained. The filtrates were centrifuged at 5000 rpm for 5 min to a pasty mass under low pressure after filtering the extracts with the Whatman number 1 filter paper. Chemical analysis was subjected to methanol extract to detect different secondary metabolites, antimicrobial and antioxidant activities.

Phytochemical screening

Generally, weeds have been disregarded, and their uses for medicinal applications have not been given much attention. Many qualitative assays used in this investigation are referred to as phytochemical tests done by using standard operating measures [29, 30].

Test for tannin/polyphenol

3 - 4 drops of 10% $FeCl_3$ were added to reconstituted extract. Gallic tannins changed the solution to a blue color, while catechol tannins changed it to a green color.

Test for reducing sugar

Test for reducing sugar involved, heat 0.5 ml of plant extract, 1 ml distilled water and 5 - 8 drops of Fehling's solution. A sign of the presence of reducing sugar result is brick-red precipitation.

Test for glycosides

Molisch's reagent test can be used to detect the presence of glycosides in plant extracts. 5 ml of Molisch's reagent and a little bit of concentration of H_2SO_4 were added to the plant's extract. Violet shade indicates glycoside presence.

Test for flavonoids

A small amount of magnesium was warmed along with 4 ml of plant extract solution was added. 1.5 ml of 50% methanol solution, and 4 ml of extract solution. After dropping 5 - 6 drops of concentrated HCl, flavonoids showed a crimson color.

Test for alkaloids

In 2 ml of plant extract add 1 ml of Mayer's reagent. The presence of alkaloids was revealed by pale-yellow precipitates.

Test for saponins

To test saponin 1 g of powdered sample was boiled in 20 ml of distilled water. 5 ml distilled water with 10 ml filtrates were vigorously quenched. Development of foaming suggested saponin is present.

Test for terpenoids

0.2 g of each sample was added to 2 ml of chloroform and 3 ml of concentrated H_2SO_4. The reddish-brown coloring shows that terpenoids are present.

Test for steroids

The steroid test involved the use of dissolving 1 g of plant extract with a few drops of acetic acid and adding a drop of concentrated H_2SO_4. The green color detects the presence of steroids.

Antimicrobial activity

Microorganisms used

For fungal strains *P. xyleborini, P. herbarum,* and *T. reesei* are used and the bacteria spp. *B. rhizoplanae,* and *P. protogens* were used.

Preparation of bacterial culture with Murashige and Skoog media

In a flask, 17.5 g Murashige and Skoog media powder was dissolved in 500 ml distilled water. To fully dissolve, the mixture is then heated up to boiling. Then autoclave the dissolved mixture at 121 ˚C for 15 - 20 min. After autoclaving, a conical flask is taken out and left for 4 - 5 min at room temperature. Next, under sterile conditions, the medium is transferred into sterile petri dishes and left to solidify for nearly 5 - 10 min. After solidified, bacterial inoculation was done and after 24 h of incubation at 37 ˚C. The bacterial culture was ready for further use.

Preparation of fungal culture with PDA media

In a conical flask, pour 19.5 g of PDA powder into 500 ml of distilled water. To fully dissolve, heat the mixture. Then autoclave the medium at 121 ˚C for 15 - 20 min and mix well before dealing out. After autoclaving, the mixture is taken out

and left for 4 - 5 min to cool down at room temperature. Next, transfer the medium to sterile petri plates using ethanol for the sterilization process, and leave it to solidify. After solidification, inoculation of fungi was done. The fungal culture was ready for further use.

Disc-diffusion method

At first 25% dimethyl sulfoxide (DMSO) was made, 25 µg of solid methanolic weed extract, 750 µl of 25% DMSO and 4 mm thick Whatman disc were added to one petri plate. Similarly, in another petri plate 50 µg of solid methanolic weed extract, 500 µl of 25% DMSO and a 4 mm thick Whatman disc were added. In the third petri plate, the control was prepared by adding 1000 µl of 25% DMSO and a 4 mm thick Whatman disc. These two discs of different concentrations of weed extract and control were allowed to absorb the content for about 24 h. After 24 h of absorption, two discs of different concentrations of weed extract and a control disc were placed in each Petri plate of freshly prepared bacterial and fungal culture in laminar airflow. Before picking up the discs, first, sanitize the forceps with alcohol. Make sure the discs are placed to be spaced apart by an equal distance. For 24 - 48 h, the plate was incubated at 37 ˚C. Use a metric ruler to measure the zone of inhibition by discs after the 24 - 48 h of incubation period. Then compare the results of different zones of inhibitions by different concentrations of weed extract discs and control disc of bacterial and fungal culture.

Antioxidant activity

It is important to investigate the ways to calculate total antioxidant activity and total phenolic content Because they are quick, repeatable, affordable, simple, and drawn immersion in this field [31, 32]. The most well-known colorimetric test is DPPH because it has a strong correlation and can screen a lot of bioactive compounds and samples quickly.

Procedure

1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of aqueous extracts of Poaceae family (*S. halepense, D. aegyptium, S. viridis, C. dactylon,* and *A. sativa)* was performed.

Preparation of weed plant extract

The first step is to obtain a dry and powdery sample of weed plants. Next, add 1 g of powder to 10 ml of methanol or ethanol to prepare an extract of the plant. By using a shaker or vortex mixture, shake the mixture for 30 min. Then filter with filter paper by removing insoluble particles. The resulting extract was used for DPPH assay.

Preparation of DPPH solution

Dissolve 0.1 g of DPPH in 100 ml of methanol to prepare a 0.1 mM DPPH solution.

Assay procedure

To carry out the assay at various concentrations, add 2 ml of DPPH solution to 2 ml of diluted weed plant extract. Allow the reaction for thirty min in unlighted, at 37 ˚C. The color solution of DPPH, which is proportional to the antioxidant activity of plant extract, can be determined spectrophotometrically at a wavelength of 517 nm [33].

Results

Many chemical compounds found in medicinal plants have antibacterial and antifungal properties against human infections. Because of its potential effectiveness against antimicrobial properties and the benefit that infections cannot develop resistance at the same time, the search for alternative plant-derived antimicrobial medicines attracted a lot of attention [34]. The plant extracts were also found to have strong antioxidant effects and high inhibitory antimicrobial properties.

Analysis of antibacterial activity

In the present investigation, Gram-positive and Gram-negative microorganisms *B. rhizoplanae* and *P. protogens* were evaluated to test the performance of plant crude extract in methanol solvent. The antibacterial activity of several crude plant extracts and their combinations were studied by many scientists using a disc-diffusion technique [35]. The zone of inhibition for all the concentrations of five plant species extract shows antibacterial activity against both Gram-positive and Gram-negative microorganisms *B. rhizoplanae* and *P. protogens*.

The 25 and 50 µg concentration of *S. halepense* against *P. protogens* bacteria has zone of inhibition with 6 mm and 8 mm and the value for 25% DMSO is 4 mm. But the value for *S. viridis,* which has the least antibacterial activity against the *P. protogens* bacteria value zone of inhibition is 5 mm for 25 µg, 9 mm for 50 µg plant extract concentration and 2 mm for 25% DMSO. The value for zone of inhibition of 25 and 50 µg concentration of *D. aegyptium* against *P. protogens* bacteria is 6 mm and 8 mm and the value for 25% DMSO is 4 mm (Figure 1). *C. dactylon* consists of zone of inhibition of 5 mm and 7 mm of 25 and 50 µg concentration and 2 mm for 25% DMSO



Figure 1: Disc-diffusion assay to measure the antibacterial activity of methanolic extract of *D. aegyptium* against *P. protogens* bacteria.

Table 1: Zones of inhibition of weed extract against *P. protogens.*

Name of plant species	25 µg extract + 750 µl DMSO (mm)	50 µg extract + 500 µl DMSO (mm)	1000 µl DMSO (mm)
C. dactylon	5	7	2
D. aegyptium	6	8	4
S. viridis	5	9	2
A. sativa	7	9	3
S. halepense	6	8	4

against *P. protogens*. *A. sativa* consists of 7 mm and 9 mm of 25 and 50 µg concentration and 3 mm for 25% DMSO against *P. protogens* (Table 1).

The 25 and 50 µg concentration of *S. halepense* against *B. rhizoplanae* bacteria is 7 mm and 9 mm and the value for 25% DMSO is less which is 3 mm (Figure 2). But the value for *S. viridis*, which has the least zone of inhibition against the *B. rhizoplanae* bacteria is 6 mm for 25 µg; 8 mm for 50 µg plant extract concentration and 4 mm for 25% DMSO. But the values for *D. aegyptium* against *B. rhizoplanae* bacteria have zone of inhibition with 5 mm and 7 mm and the value for 25% DMSO is less which is 2 mm. *C. dactylon* consists of zone of inhibition of 5 mm and 8 mm of 25 and 50 µg concentration and 2 mm for 25% DMSO against *B. rhizoplanae*. *A. sativa* consists of a zone of inhibition of 5 mm and 7 mm of 25 and 50 µg concentration and 3 mm for 25% DMSO against *B. rhizoplanae* (Table 2).

The dilute concentration of 100 ml of 25% DMSO shows the least activity or fewer zones of inhibition but plant extract with 25 and 50 µg concentration shows high antibacterial activity and has high values of zone of inhibition. 50 µg concentration of all plant species shows the highest antibacterial activity. *S. halepense* shows the maximum zone of inhibition of

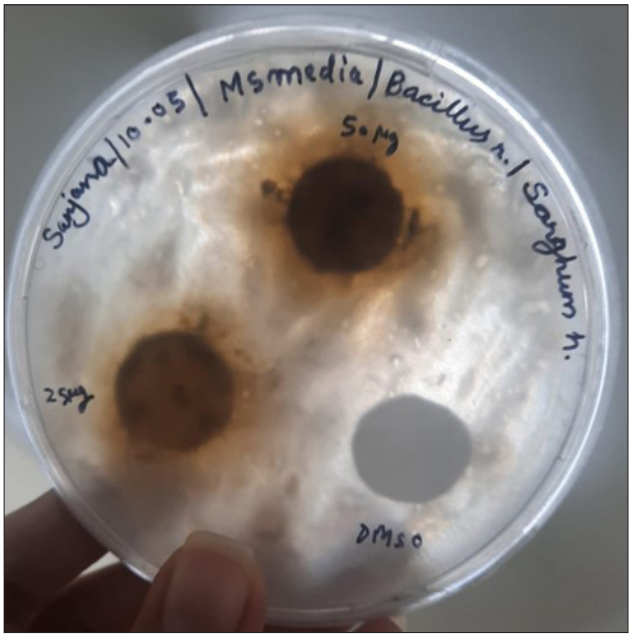

Figure 2: Disc-diffusion assay to measure the antibacterial activity of methanolic extract of *S. halepense* against *B. rhizoplanae* bacteria.

Table 2: Zones of inhibition of weed extract against *B. rhizoplanae.*

Name of plant species	25 µg extract + 750 µl DMSO (mm)	50 µg extract + 500 µl DMSO (mm)	1000 µl DMSO (mm)
C. dactylon	5	8	2
D. aegyptium	5	7	2
S. viridis	6	8	4
A. sativa	5	7	3
S. halepense	7	9	3

the antibacterial activity among the other species (Figure 2) and *S. viridis* shows the least antibacterial activity against the bacteria. *D. aegyptium, C. dactylon, A. sativa* show average antibacterial activity against both bacteria. The concentrations of *S. halepense* are more active against *B. rhizoplanae* rather than *P. protogens*. The concentrations of *D. aegyptium* have more zone of inhibition active against *B. rhizoplanae* rather than *P. protogens*. *C. dactylon* with 50 µg has more activity against *B. rhizoplanae* rather than *P. protogens*. *A. sativa* has more antibacterial activity against *P. protogens* rather than *B. rhizoplanae*

Analysis for antifungal activity

The plant species used for antifungal activity were *S. halepense, D. aegyptium, S. viridis, C. dactylon,* and *A. sativa* against three fungus species (*P. xyleborini, P. herbarum,* and *T. reesei).*

For *P. xyleborini* fungus *A. sativa* has 5 mm and 9 mm of the zone of inhibition of 25 and 50 µg concentration and 2 mm for 25% DMSO against *P. xyleborini* but *S. viridis* has 2 mm and 5 mm of zone of inhibition of 25 and 50 µg concentration and very least zone 1 mm for 25% DMSO against *P. xyleborini.* The zone of inhibition for *C. dactylon* shows 4 mm and 8 mm for 25 and 50 µg concentration and 1 mm for 25% DMSO against *P. xyleborini.* Zone of inhibition for *S. halepense* shows 5 mm and 7 mm for 25 and 50 µg concentration and 3 mm for 25% DMSO against *P. xyleborini.* Zone of inhibition for *D. aegyptium* shows 4 mm and 6 mm for 25 and 50 µg concentration and 1 mm for 25% DMSO against *P. xyleborini.* For *P. xyleborini* fungus *A. sativa* shows maximum zone of inhibition for antifungal activity as compared to other plant species and *S. viridis* shows least antifungal activity from other plant species (Table 3).

For *P. herbarum* fungus *C. dactylon* has 5 mm and 7 mm of zone of inhibition of 25 and 50 µg concentration and 3 mm for 25% DMSO against *P. herbarum* but *A. sativa* has 2 mm and 5 mm of the zone of inhibition of 25 and 50 µg concentration

Table 3: Zones of inhibition of weed extract against *P. xyleborini.*

Name of plant species	25 µg extract + 750 µl DMSO (mm)	50 µg extract + 500 µl DMSO (mm)	1000 µl DMSO (mm)
C. dactylon	4	8	1
D. aegyptium	4	6	1
S. viridis	2	5	1
A. sativa	5	9	2
S. halepense	5	7	3

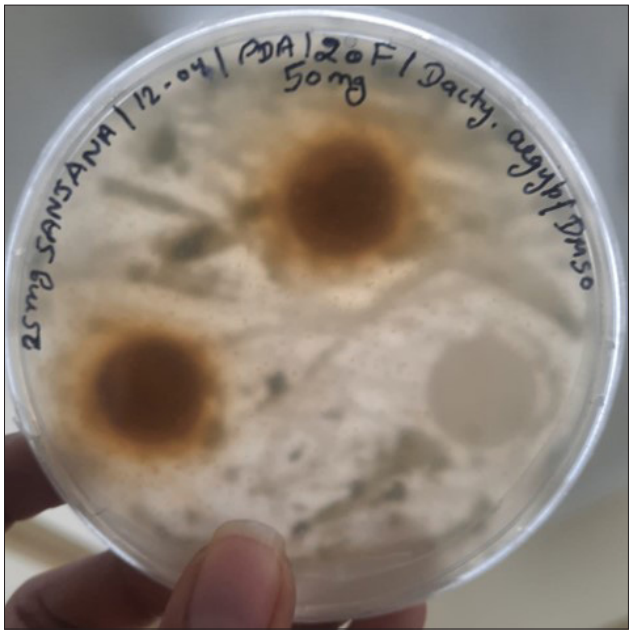

Figure 3: Disc-diffusion assay to measure antifungal property of methanolic extract of *D. aegyptium* against *P. herbarum* fungi.

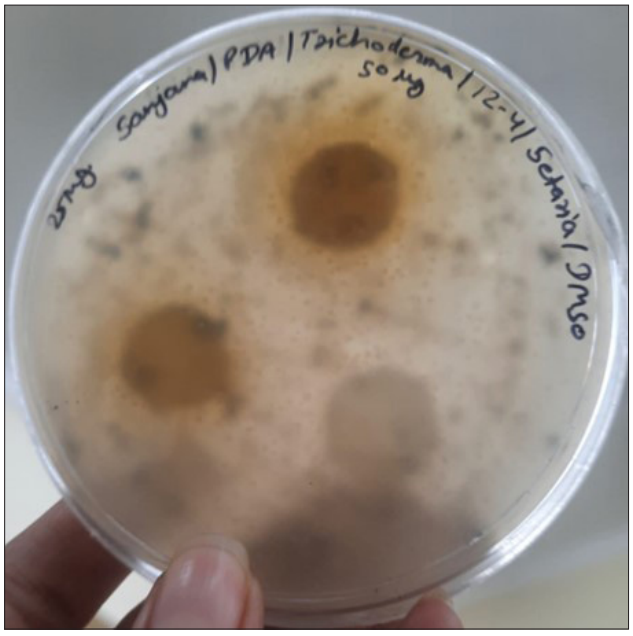

Figure 4: Disc-diffusion assay to measure the antifungal activity of methanolic extract of *S. viridis* against *T. reesei* fungi.

and very least zone 1 mm for 25% DMSO against *P. herbarum*. Zone of inhibition for *S. viridis* shows 5 mm and 9 mm for 25 and 50 µg concentration and 2 mm for 25% DMSO against *P. herbarum*. Zone of inhibition for *S. halepense* and *D. aegyptium* (Figure 3) both show the same values of 4 mm and 6 mm for 25 and 50 µg concentration and 1 mm for 25% DMSO against *P. herbarum*. *P. herbarum* fungus *C. dactylon* shows the highest antifungal activity as compared to other plant species and *A. sativa* shows the least antifungal activity from other plant species (Table 4).

For *T. reesei* fungus *C. dactylon* has 6 mm and 9 mm zones of inhibition of 25 and 50 µg concentration and 3 mm for 25% DMSO against *T. reesei* but *S. viridis* has a 3 mm and 6 mm zone of inhibition of 25 and 50 µg concentration and very least zone 1 mm for 25% DMSO against *T. reesei* (Figure 4) zone of inhibition for, *A. sativa* shows 4 mm and 7 mm for 25 and 50 µg concentration and 1 mm for 25% DMSO against *T. reesei*. The zone of inhibition for *S. halepense* shows 5 mm and 9 mm for 25 and 50 µg concentration and 2 mm for 25% DMSO against *T. reesei*. Zone of inhibition for *D. aegyptium* shows 5 mm and 7 mm for 25 and 50 µg concentration and 3 mm for 25% DMSO against *T. reesei*. *T. reesei* fungus *C. dactylon* shows maximum zone of inhibition for antifungal activity as compared to other plant species and *S. viridis* shows least antifungal activity from other plant species extract (Table 5).

Table 4: Zones of inhibition of weed extract against *P. herbarum*.

Name of plant species	25 µg extract + 750 µl DMSO (mm)	50 µg extract + 500 µl DMSO (mm)	1000 µl DMSO (mm)
C. dactylon	5	7	3
D. aegyptium	4	6	1
S. viridis	5	9	2
A. sativa	2	5	1
S. halepense	4	6	1

All the concentrations of five plant species extract show a zone of inhibition for antifungal activity against all three fungi i.e., *P. xyleborini, P. herbarum,* and *T. reesei*. The dilute concentration of 100 ml of 25% DMSO shows the least activity but the plant extract zone of inhibition with 25 and 50 µg concentrations shows high antifungal activity. 50 µg concentration of all plant species shows the highest antibacterial activity.

Analysis of antioxidant activity

DPPH has a heavy purple appearance. After receiving a proton from a proton donor (e.g., phenolic compounds), it loses its chromophore and becomes yellow [36]. It consists of a high wavelength at 517 nm. The reduction of color was measured by spectrophotometer; λ max 517 [37].

In the present investigation, *A. sativa* shows antioxidant activity of absorbance 1.637 at 517 nm. *C. dactylon* shows an absorbance of 1.212 at 517 nm of antioxidant activity. *D. aegyptium* has an absorbance of 1.333 at 517 nm for antioxidant activity. *S. halepense* shows antioxidant activity of value 1.362 at 517 nm. In this study, the methanol extract of *S. viridis* plant exhibited high antioxidant activity with an absorbance of 1.791 at 517 nm. As comparing all species, *C. dactylon* consists of the least antioxidant activity with an absorbance of 517 nm (Table 6).

Analysis for phytochemical screening

Test for tannin/polyphenol

3 - 4 drops of 10% $FeCl_3$ were added to reconstituted extract. Gallic tannins changed the solution to a blue color, while catechol tannins changed it to a green color. There was a presence of blue or green color appears in *C. dactylon, A. sativa* weeds which means that in the plant species, there is the presence of gallic acid or catechol tannins. Other plant species show a negative result for this test (Table 7).

Table 5: Zones of inhibition of weed extract against *T. reesei*.

Name of plant species	25 µg extract + 750 µl DMSO (mm)	50 µg extract + 500 µl DMSO (mm)	1000 µl DMSO (mm)
C. dactylon	6	8	3
D. aegyptium	5	7	3
S. viridis	3	6	1
A. sativa	4	8	1
S. halepense	5	9	2

Table 6: Absorbance of different weed extracts at 517 nm.

Weeds	Absorbance at 517 nm
C. dactylon	1.212
D. aegyptium	1.333
S. halepense	1.362
S. viridis	1.791
A. sativa	1.637

Table 7: Phytochemical analysis of different weeds.

Phytochemical tests	*A. sativa*	*C. dactylon*	*D. aegyptium*	*S. viridis*	*S. halepense*
Tannin/Polyphenol	+	+	-	-	-
Reducing sugar	-	-	-	-	-
Glycosides	-	+	-	-	-
Flavonoids	+	+	+	+	+
Alkaloids	+	+	+	+	+
Saponins	+	-	+	+	-
Terpenoids	+	-	-	-	-
Steroids	-	-	-	-	-

Note: Negative (-) sign means, phytochemicals are not present. A positive (+) sign means phytochemicals are present in weed plants of the Poaceae family.

Test for reducing sugar

Test for reducing sugar involved, 0.5 ml of plant extract, 1 ml distilled water and 5 - 8 drops Fehling's solution and heat, the result was only very light color appears and observation of brick red ppt is not which means there is no reducing sugar present (Table 7).

Test for glycosides

Molisch's reagent test can be used to detect the presence of glycosides in plant extracts. 5 ml of Molisch's reagent and a little bit of concentrated of H_2SO_4 were added to the plant's extract. The violet hue indicates the presence of glycosides. Only *C. dactylon* shows positive results for this test (Table 7).

Test for flavonoids

After a little amount of magnesium was warmed along with 4 ml of plant extract solution added. 1.5 ml of 50% methanol solution, and 4 ml of extract solution. After dropping 5 - 6 drops of concentrated HCl, crimson color observed, flavonoids showed a crimson color which means here also there is the presence of flavonoids in all plant species (Table 7).

Test for alkaloids

In 2 ml of plant extract add 1 ml of Mayer's reagent. Pale-yellow precipitates revealed alkaloids. There is the presence of alkaloids in all species of plants (Table 7).

Test for saponins

To this, 1 g powdered sample was heated in 20 ml distilled water. 5 ml distilled water with 10 ml filtrates were vigorously quenched. The development of foaming was suggested by the presence of saponins. *C. dactylon* and *S. halepense* showed negative results and all other three plant species showed positive results for this test (Table 7).

Test for terpenoids

0.2 g each sample was added to 2 ml chloroform and 3 ml concentrated H_2SO_4. The reddish-brown coloring shows the presence of terpenoids. But as a result, *C. dactylon* and *S. halepense* show a black color appearance *S. viridis* and *D. aegyptium* show a light color appearance which means terpenoids are not present in any of the plant species, except *A. sativa* which shows reddish-brown coloring (Table 7).

Test for steroids

The steroid test involved the use of dissolving 1 g of plant extract with a few drops of acetic acid and adding a drop of concentrated H_2SO_4. The green color detects the presence of steroids. Only light color was observed in plant species except the black color of *Avena* sp. and the result showed that there is no presence of steroids in the all-plant species (Table 7).

Discussion

Antimicrobial activity

In this study, the methanolic extract of *S. halepense, S. viridis, D. aegyptium, A. sativa,* and *C. dactylon* antibacterial properties were assessed. For this, several strains of fungi (*P. xyleborini, P. herbarum,* and *T. reesei*), and bacteria (*B. rhizoplane,* and *P. protogens*) were selected, and their non-growth halo diameter (in mm) and disc-diffusion technique were used to analyze the effects of the extract on the non-growth of these microorganisms. Bacterial growths with the development of the non-growth halo were both successfully inhibited by the methanolic extract of all plant species.

Fungi were affected by the methanolic extract of the different components of plant species, and a halo with a diameter of 5 – 9 mm developed. In a similar study, the same methods were used (Disc -diffusion) to appraise antimicrobial properties, and hydro-alcoholic extract of *C. dactylon* against bacteria (*S. aureus, S. albus, E. coli,* and *P. aeruginosa*). The result demonstrates that hydroalcoholic extract of *C. dactylon* has strong antibacterial properties. In anticipation of bacteria, *C. dactylon* demonstrated high antibacterial activity at the lowest inhibitory concentration [38].

One of the studies involved, plant extract of *C. dactylon* obtained from seven solvents, by using disc diffusion meth-

od to find out antibacterial. Outcomes examined that ethanol 7 – 10 mm and ethyl acetate 7 – 12 mm extracts had remarkable antibacterial activity in opposition to all infections. With inhibition zones of 2 – 5 mm and 5 - 9 mm, respectively, methanol extracts demonstrated acceptable antibacterial efficacy. Using the agar disk-diffusion method, the leaf extract of *C. dactylon* against some pathogens, investigated antibacterial activity [39]. The result showed that the tested microorganisms significantly inhibited the leaf extract of *C. dactylon* from chloroform extract. In this study, *C. dactylon* shows reasonable antibacterial and antifungal activity against *B. rhizoplanae, P. protogens, P. xyleborini, P. herbarum,* and *T. reesei*.

Strong antifungal activity was shown by the methanolic plant extracts of *S. halepense* against fungi and bacteria. Similarly in a study, strong antifungal activity was demonstrated against *Cladosporium cladosporioides* and *Trichoderma viride* by the methanolic plant extracts of *S. halepense.* Because *S. halepense*, contains lots of phytochemicals like sterols, is considered to be good against antimicrobial agents [40].

In a study, the entire plant of *Dichanthium annulatum* is used for the treatment of diseases like dysentery and menorrhagia, while the stem of *Saccharum spontaneum* is sometimes eaten to reduce stomach issues [41, 42]. Similarly, *Eleusine indica, D. aegyptium, Chrysopogon zizanioides* and *Imperata cylindrica*, the majority of biological activities of bulk of these plantsare not studied, despite their relevance in ethnomedical research [43]. Several types of research prove most species consist of different kinds of secondary metabolites [44]. The present study of *D. aegyptium, A. sativa*, *S. viridis* shows antimicrobial activity similar to these studies and contains various secondary metabolites. Additionally, the *P. protogens* strain was well inhibited by the extracts of *S. halepense* and *A. sativa*.

Antioxidants properties

The capability of various solutions is measured using the activity of stationary DPPH radicals. The methanolic extract of plant parts of all five species of antioxidants was measured by DPPH. The reducing agent DPPH is transformed into the colorless molecule hydrazine when it accepts electrons from reducing substances like phenols. Reduced absorbance is related to this structural modification. Antioxidants are substances that possess this property [45]. With increasing quantities of the methanolic extract of plant species, it is evident that the free radical scavenging action considerably increases. DPPH, tests were performed to determine the ability of a methanolic extract of Poaceae family (*S. halepense, D. aegyptium, S. viridis, C. dactylon,* and *A. sativa)* fractions to scavenge free radicals. The DPPH (a-diphenyl-b-picrylhydrazyl) test determined spectrophotometrically the scavenging potential of plant extract by converting DPPH into an a-diphenyl-b-picrylhydrazyl and altering its color to violet.

In this study, the methanol extract of *S. viridis* plant exhibited high antioxidant activity with an absorbance of 1.791 at 517 nm. Different methods of antioxidant activity were accessed from *C. dactylon* hydroalcoholic extract. The extract results in antioxidant properties in each of the assays. In a similar study, in *C. dactylon*, the extracts of ethyl acetate and methanol were used continuously and then concentrated. To determine the antioxidant activity, DPPH tests were done on 4 cancer lines and a normal cell line, resulting in plants with substantial anti-proliferative and antioxidant properties [46]. The present study showed an antioxidant value of 1.212 for *C. dactylon* which indicates that the plant has a significant number of phenolic components in it. Similarly, components of *A. viridis* produced in methanol and ethyl acetate fractions were found the antioxidant properties [47].

Punia et al. [48] observed that *S. halepense* can operate as an antioxidant agent because of the existence of biologically active phytochemicals and enriched antioxidants. In one more study, for antioxidant properties there were different *S. halepense* extracts were tested [49]. From previous studies, *S. halepense* may have cytotoxic, antioxidant, and anti-diabetic properties related to several disorders [50]. In this study, *S. halepense* shows antioxidant activity, methanol *S. halepense* extracts were tested for their antioxidant capacities and it shows absorption similarly with a value of 1.362. The present study shows the same significant radical scavenging activity with all the five plant species of Poaceae family and shows antioxidant activity, indicating a significant light absorption by the specific compounds present in the weed extract.

Phytochemical screening

The various antimicrobial properties are attributed as a consequence of a variety of phytoconstituents, which might have antimicrobial and change cell membranes [51].

Leaf extracted from *C. dactylon* and obtained 24 different compounds. Similarly, earlier research by Nischitha et al. [52] proposed that the methanolic extract of the same plant *C. dactylon* shows the presence of alkaloids, flavonoids, tannins, and glycosides that support the present result for *C. dactylon* while saponin showed negative result. Depending on their solubility in different solvents, plant materials' bioactive compounds vary, as given by David et al. [53]. Truong et al. [54] used to determine qualitative analysis for the existence of different phytochemical compounds in *C. dactylon*, steroids, alkaloids, and phenols, also with macroscopic and microscopic characteristics of plants. This study of *C. dactylon* shows remarkable flavonoids, tannins, alkaloids, and glycosides.

Kumar and Ssami [55] investigated plant extract of *A. sativa* and found phytochemicals; organic acids, alkaloids, tannins, polyphenolics, carbs, flavonoids, sterols, tocols, terpenoids, lignans and carotenoids. In this investigation, *A. sativa* shows positive results for alkaloids, tannins, terpenoids, saponins and flavonoids. A previous study in plant *D. aegyptium* phytochemical examination found alkaloids and saponins [56]. Similar to this investigation, *D. aegyptium* shows positive results for alkaloids, flavonoids and saponins. Flavonoids are present in trace amounts in *D. aegyptium.*

In this study, *S. halepense,* was revealed to contain the existence of proteins, alkaloids, terpenes, steroids, cardiac glycosides and carbohydrates and devoid of saponins. *S. viridis* shows results found alkaloids, flavonoids and saponins in the plant. In a similar study of *S. halepense*, plant extract consists of cytotoxic, anti-diabetic and antioxidant properties due to the prevalence of aforementioned phytochemicals in plant extract and its fractions linked with many illnesses [57]. Similarly in

this study, plant extract of *S. halepense* was found with alkaloids and flavonoids.

The plant extract that contains saponin can be treated for various diseases; inflammation, ultra violet damage, stomach ulcers, cerebrovascular and cardiovascular disorders [58]. Furthermore, saponins are utilized as assisting to intensify the taking up of medicinal drugs and bioactive compounds [59]. Alkaloids and saponins in plants make their plant extract potentially powerful antimicrobial agents [60]. To examine the grasses exhibit phytotoxicity there is the presence of certain phytoconstituents that can be utilized as natural herbicides [61, 62].

The plant extract of various species used in this study can have been used in traditional medicine because of the presence of various phytochemicals in them [63]. One may conclude that the methanolic extract of all parts of plant species of Poaceae family including *S. halepense, D. aegyptium, S. viridis, C. dactylon,* and *A. sativa* can prevent harmful microbes, those reported in other research. As a result, it is advised to utilize these in culinary items as a natural preservative and flavoring ingredient.

Conclusion

The antimicrobial, antioxidant and phytochemical screening of tested plant samples were measured. The investigation concludes that plants used are good origin for antimicrobial, antioxidant and phytoconstituent which may be used for treating different illnesses. For antibacterial activity, *S. halepense* shows the maximum zone of inhibition of the antibacterial activity among the other species and *S. viridis* shows the least antibacterial activity against the bacteria. But *C. dactylon* shows maximum antifungal activity against fungus *P. herbarum* and *T. reesei*. For *P. xyleborini* fungus *A. sativa* shows maximum zone of inhibition for antifungal activity. In antioxidant properties, the methanol extract of the *S. viridis* plant exhibited high antioxidant activity and *C. dactylon* had the least antioxidant activity with an absorbance of 517 nm. Along with strong DPPH activity, this plant exhibits significant phenolic and flavonoid contents. For phytochemicals, *C. dactylon* shows the existence of phytochemicals in it. *A. sativa* shows positive results for alkaloids, tannins, terpenoids, saponins and flavonoids. In this study's findings, all the plants have Phytoconstituents in them. These plant extracts can be utilized as an effective substitute for eatable ingredients because of their strong antioxidant activity. The importance of phytochemical analysis and antioxidant activity of weed plants in food chemistry is evident in their potential to revolutionize the way we approach nutrition, preservation, and sustainability in the food industry. As researchers continue to unveil the diverse array of bioactive compounds present in weed plants, the integration of these plants into food formulations holds promise for creating healthier, more resilient, and environmentally friendly food products.

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Conflict of Interest

None.

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