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# Phytochemical screening, antimicrobial, and antioxidant properties of *Helianthus annuus* and *Hyophila involuta*: A comparative account

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## Abstract

Angiosperms and bryophytes, though distantly related plant groups, have many similar ecological and economic implications, including medicinal value. Therefore, this study aimed to analyse the phenolic and flavonoids composition and antioxidant and antimicrobial activities of *Helianthus annuus* L. (Angiosperms-Asteraceae) and another plant, a moss (Bryophyta), *Hyophila involuta* (Hook.) Jaeg., aerial parts (leaves), prepared in four different extracts (methanol, chloroform, distilled water, and petroleum ether**).** Phytochemical screening was conducted using standard methods of precipitation and colouration reactions. The Folin-Ciocalteu method was employed to determine the total phenol content, while the Aluminium Chloride Colorimetric method was used for flavonoid content determination. The antioxidant activity was measured through two methods: DPPH and NOSA scavenging activity. The phytochemical screening detected the presence and absence of fixed oils and fats, flavonoids, saponins, terpenoids, tannins, polyphenols, carbohydrates, and glycosides in both plants. The antibacterial and antifungal activity of both plants' methanolic extracts was examined against bacterial and fungal pathogens, *i.e.*, *Escherichia coli* and *Bacillus subtilis* and fungal strains, *i.e.*, *Fusarium oxysporum* and *Aspergillus niger*. The results were compared to a regular antibiotic disc and negative control that served as a methanol solvent. The methanolic extract of *H. annuus* has higher total phenol and flavonoid content, as well as antioxidant and antimicrobial activity, than *H. involuta*. Based on these data, it can be concluded that, while *H. annuus* is more effective than *H. involuta*, both distantly related plant species have similar phytochemical profiles and should be included equally in future herbal compositions.

***Keywords:*** angiosperms; antimicrobial; antioxidant; bryophytes; phytochemicals

## Introduction

Humans have long relied on nature and its natural resources to meet their needs, with plants serving as the primary source of food, medicine, shelter, clothing, and other necessities. The knowledge of plants' nutritional and therapeutic benefits has been transmitted both within and between many civilizations. Because of this, plants are a crucial component of ethno-pharmacology and are used by various cultures around the globe to treat multiple illnesses. Plant-based products are of tremendous interest since they are affordable, have few side effects, and have a variety of uses (Chopra *et al.*, 1956; Pal and Jain, 1998; Joshi and Joshi, 2000; Jimoh

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*et al*., 2011). Plants are not only essential in health care, but they are also the most reliable source of sustainable future pharmaceuticals. Natural resources are the backbone of the traditional medical system around the globe, and they have long been a part of history and culture. The traditional Indian medical system known as Ayurveda (mother of all medicines) is thought to be the world's oldest healthcare system. It is still widely practiced in India, Sri Lanka, China, Tibet, and other countries (Jimoh *et al*., 2011).

The practice of Ayurveda was first carried out by Sages in India more than 4000 years ago. They had a profound understanding of the underlying causes of illnesses and were able to offer highly effective treatments. Ayurveda, as it was practiced then, was widely recognized for its excellence. However, due to great demand in the national and international markets, some medicinal plants are overexploited to such an extent that there is a concern about their extinction. Nearly 15,000 plant species are threatened due to overharvesting and habitat destruction (Ediriweera, 2007). Although the cultivation of these medicinal plants in an artificial environment can increase the yield of desired phytoconstituents to meet the demand of the healthcare sector, it is a very tedious, time-consuming, and costly affair. Hence, there is an urgent need to investigate more and more plants for their biological properties so that they can serve as a source of new photo drugs.

Bryophytes are employed as the first land plants and amphibians of the plant kingdom by the natives, to treat a wide range of diseases (Alam *et al*., 2015). Although its antimicrobial potential has long been known, there are not many convincing proofs in support of it up to this point. Researchers currently believe that these plants represent a reservoir of innovative medications as they are emerging as novel sources of natural treatments. As a result, their phytochemistry has been thoroughly investigated. Therefore, the potential of numerous bryophytes as antioxidants and antimicrobials as well as secondary metabolites has been evaluated (Asakawa, 2007). These plants have a variety of physiologically active molecules that actively contribute to the defensive mechanism, including microbial infections, as a result of the existence of exceptional phytochemicals (Beike *et al*., 2010). Many bryophytes (liverworts, hornworts, and mosses) have not yet been thoroughly investigated, despite numerous endeavors in this regard. Only a limited number of bryophytes publications are now available compared to angiosperms (Adebiyi *et al*., 2012).

Since the contribution of angiosperms to biodiversity and habitat is so significant that a considerable loss of angiosperms, the variety of food supplies and oxygen supplies in the ecosystem would decline, and the volume and distribution of precipitation over the planet would be significantly affected. There are undoubtedly many food and medicinal resources to be found in this family. Angiosperms outnumber all other plant groups on Earth's surface and in its vegetation, especially in terrestrial areas. As a result, angiosperms constitute the most important source of food for animals and birds, including humans. Additionally, the most economically significant group of green plants is the group of flowering plants, which are a source of goods like pharmaceuticals, fiber, hardwood, ornamentals, and other commercial goods. Other than this Angiosperms also shows many antioxidants, anti-inflammatory, hepato-protective, and antimicrobial activities, hence their phytochemistry is evaluated thoroughly.

Bryophytes, particularly mosses, are an important lineage of bryophytes with a distinct life history, antimicrobial properties, poikilohydric habit, the potential for soil management, and an indication of environmental pollution. These tiny plantlets act as an interface between air and substrate, where climatic conditions differ markedly from those recorded during routine meteorological observations. When compared to higher plants, mosses have several advantages: relatively simple structure than higher plants; haploid gametophytic dominant phase; less chromosome number (Glime, 1988); lack of variability of morphology through the growing season; wide distribution; possible detection of concentrations in the annual growth segments; and have high surface-to-volume ratio with ease of sampling. The rapidity with which they complete their life cycle in a short time adds a further advantage to the group in several ways (Sharma and Alam, 2022).

The Asteraceae family, also known as Compositae/aster family/family of sunflowers/daisy family, consists of about 10% of all the flowering plants and is regarded as the most advanced and prominent family of Angiosperms. This consists of over 32,000 accepted species and has 1800-1900 genera distributed worldwide, excluding Antarctica (Mandel *et al*., 2017). The species diversity of this family is compared only to the

Orchidaceae (Orchids) and Fabaceae (Legumes) families. Since the species of this family are present in different areas like sub-tropical, cold-temperate, and temperate regions, they are therefore regarded as a cosmopolitan family. Asteroidea, Bernadesioideae, and Cichorioideae are the three sub-families of the Asteraceae family (Medeiros-Neves *et al*., 2018). Asteraceae have medicinal properties and a vast history in conventional medicine: some have been cultivated for edible therapeutic reasons for over 3000 years. Members of the Asteraceae family have anti-inflammatory, antioxidant, antimicrobial, and hepato-protective properties (Achika *et al*., 2014). Due to its ability to withstand biotic and abiotic challenges by producing secondary metabolites, the *Asteraceae* family is widely dispersed in India. In members of this family, the phytochemical defense mechanism is particularly effective in protecting them and synthesizing exceedingly complex compounds with specialized stereochemistry and new pathways (Ugoh and Haruna, 2013).

Therefore, it is necessary to assess more and more angiosperms and bryophytes to identify biologically active constituents. The results of a comparison of two species from the kingdom Plantae, namely *Helianthus annuus* L. (Angiosperms-Asteraceae) (Figure 1) and *Hyophila involuta* (Hook.) Jaeg. (Bryophyta) (Figure 2) from Rajasthan (India), are fascinating. The work highlights the importance of these plants as a unique source of novel phytochemicals that might be studied further, particularly in pharmacology and ethnomedicine.

**Figure 1.** *Helianthus annuus* L. (Angiosperm: Asteraceae) plant with leaves, stem, and flowers

**Figure 2.** *Hyophila involuta* (Hook.) Jaeg. (Bryophyta) population growing on the walls

## Materials and Methods

*Collection of plant sample*

Plant materials for this study were collected from the Banasthali University Campus in Tonk, India (Rajasthan) (Figure 3) and are deposited in the Banasthali University Rajasthan India (BURI) Herbarium {Specimen number: BURI17860318 [(*Hyophila involuta* (Hook.) Jaeg.)]; BURI-1620/2022 (*Helianthus annuus* L.)}.


**Figure 3.** Map showing collection sites

*Method of collection of plant sample*

The specimens were collected from the Banasthali Vidyapith campus, comprising *H. annuus* (Angiosperms) and *H. involuta* (Bryophytes). The collection process for Angiosperms was conducted with utmost care, encompassing the underground portions, including roots, trailing or underground stems, and storage organs. These parts were vital in identifying the specimens. To prevent damage or breakage to parent trees and specimens, twigs were removed cleanly using a sharp knife or pruners. Each sample was collected to maximize information content, following the standard herbarium sheet size. The ideal herbarium specimen included a stem with attached leaves, flowers, and/or fruits. Mosses were also collected in their entirety. In cases where they grew in mats, a good handful was taken, and pure specimens were preferred over mixed ones. Mosses growing in cushions or clumps or those closely growing on the substrate were cut away with some substrate. As a result, the specimen comprised bark, rotting wood, soil, humus, and other components, in addition to the plant. This approach ensured that the growth form of the plant was retained. Paper bags or envelopes were used to collect mosses, as they allowed for the drying of the plant within the same bag (John *et al*., 2010).

*Preparation of plant extract*

Collection of the plant parts was done and cleaned with running tap water, followed by autoclaved water to eliminate dirt. After drying for 10 days at room temperature, fresh leaves of plants were pulverized using an electronic grinder. The dirt-free plant powder was stored at 4 °C in an airtight container for future usage (Mehra and De, 2017).

*Extraction*

10 g of powdered leaves were loaded in a thimble made from Whatman filter paper (No. 1) and placed in the Soxhlet assembly's extraction chamber for 12 h, with 150 ml of four different extraction solvents, i.e., methanol, chloroform, distilled water, and petroleum ether, poured into the boiling flask. The flow of ice-cold water in the condenser portion of the Soxhlet apparatus was kept constant. With the help of a heating mantle, the temperature in the boiling flask was observed at 40-45 °C. A cyclic movement of the solvent was noticed between the extraction chamber, the boiling flask, and the thimble. This cyclic flow was maintained for 20 to 25 cycles until the solvent in the extraction chamber was colorless.

*Phytochemical screening*

The phytochemicals were qualitatively screened using extracts prepared with various solvents, including methanol, chloroform, petroleum ether, and distilled water. The solvents were evaporated (at 40 °C) with the support of a heating mantle. Using conventional specified techniques, several extracts from each solvent were utilized for qualitative phytochemical screening to identify the various classes of active chemical components. Positive tests were defined as (+) a significant amount present and (-) completely absent (Table 1).

*Total phenolic content (TPC)*

The Folin-Ciocalteu method was used to determine the plant extract's total phenolic content (Aiyegoro and Okoh, 2010). Gallic acid and a methanolic solution of the samples were prepared at 1 mg/ml each. The reaction mixture was produced by mixing 0.5 mL of methanolic extract solution, 2.5 mL of water-dissolved 10% Folin-Ciocalteu reagent, and 2.5 mL of 7.5% $NaHCO_3$. Instead of the plant samples, 0.5 mL of methanol was used for the Blank. The samples were incubated for 45 minutes at 45 °C on a thermostat. The absorbance was measured at 765 nm. Results were presented as gallic acid equivalent (GAE) of mg/g of dry weight.

*Total flavonoid content (TFC)*

The total flavonoid content was determined using the method described by Srinivasan *et al*. (2014). Utilizing the aluminium chloride colorimetric method, quercetin was chosen as a benchmark to assess the flavonoid concentration. In methanol (1 mg/mL), extracts and quercetin were produced. To 1 mL of plant extract, 0.2 mL of aluminium chloride (10%), and 1M potassium acetate (0.2 mL) were added followed by double distilled water. The mixture of the samples was vortexed and kept in the dark for 60 min at room temperature. A spectrophotometer was used to measure the absorbance at 415 nm. Results were given in milligrams of quercetin equivalent (mg/g QE) per gram of dry weight.

*Antioxidant activity*

2, 2-diphenyl 1-picryl –hydrazyl Assay (DPPH)

The 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) test was used to determine the free radical scavenging activity of the plant samples. 1mg/ml stock solutions of leaf extract in their respective solvents were diluted from 10-100 g/ml to test antioxidant activity by DPPH assay. 1 ml of 0.3 mM DPPH solution was added to 1 ml of leaf extracts of various strengths and the standard (Ascorbic acid). For 30 minutes, the samples were incubated in the dark. A UV-Vis spectrophotometer was used to measure the absorbance at 517 nm (Saklani

*et al*., 2017). The assays were carried out in triplicates, and the mean of the data was recorded. According to the formula below, the extracts' scavenging activity was determined in percent inhibition:

% Inhibition = (Absorbance control − Absorbance sample)/ Absorbance control × 100

Nitric oxide scavenging assay (NOSA)

NOSA was evaluated by using spectrophotometrically according to Srinivasan *et al*. (2014) with slight modifications. Different concentrations (20-100 µg/mL) were added to different test tubes. Ascorbic acid was used as a control. Each test tube was filled with sodium nitroprusside (5 mM) in phosphate buffer to a volume of 1.5 mL. At 25 °C, the solutions were incubated for 30 min; a mixture of the filtrate was taken in another boiling tube and diluted with Griess reagent (diluted to equal volume, 1% sulphanilamide, 3% phosphoric acid, and 0.1% naphthylethylene diamine dichloride in water) was added to it. The sample was properly mixed, and the OD of the colored sample was taken at 546 nm against a blank. All the experiments were carried out thrice to determine the mean and standard deviations.

Percentage inhibition was measured by the formula given below.

% Inhibition = (Absorbance control − (Absorbance sample/Absorbance control) × 100

*Estimation of $IC_{50}$ value*

The volume of plant extract needed to quench 50% of the Nitric oxide scavenging test and DPPH free radicals is referred to as $IC_{50}$. A graph showing percentage inhibition was used to determine the $IC_{50}$ value (µg/mL) against different concentrations of plant extracts.

*Antimicrobial activity*

The antibacterial and antifungal activities of the plant extracts prepared in methanol were tested using the method of disc diffusion against two pathogenic bacterial strains and two fungal strains.

*Antibacterial activity*

*Microbial strains*

The antibacterial efficacies of all selected plant extracts were assessed using the bacterial species**,** ***viz*.,** *Escherichia coli* (MTCC-119) and *Bacillus subtilis* (MTCC- 619).

Preparation of bacterial culture

Mueller Hinton Agar (MHA) media (HiMedia) was used to maintain and prepare bacterial cultures. To sterilize the needed amount of medium, it was autoclaved for 15 min at 15 psi pressure and 121 °C temperature. A loopful of powdered bacterial species was lyophilized and spread on sterilized Mueller Hinton Agar plates. For the revival of bacterial culture, 1mL of culture (from stock culture) was inoculated in 250 mL of nutrient broth aseptically was kept on a shaker maintained at 37 °C for 24 h. After the proper development of bacterial colonies, the culture flasks were stored and maintained at 4 °C.

Fungal strains

Two fungal strains, *viz*., *Aspergillus niger* (MTCC 282) and *Fusarium oxysporum* (MTCC 8608), were used.

Preparation of fungal culture

Potato dextrose agar media (HiMedia) was used to maintain and prepare fungal strains. For the preparation of fungal culture, 100 and 200 µg/mL of sterilized media were poured into the Petri plates under sterilized conditions maintained in laminar airflow. The Petri plates are then incubated for 7 days at 28 °C.

After the proper development of fungal colonies, the culture flask was stored and maintained at 4 °C. The fungal colonies obtained were sub-cultured regularly.

Media preparation

The autoclaved media (Mueller Hinton Agar for bacterial culture and Potato Dextrose Agar for fungal cultures) was poured into the Petri-plates under sterilized conditions in laminar air flow and left to solidify. After the solidification of the media, the Petri-plates were ready to be used for further experiments.

Preparation of Whatman paper discs

A paper punching machine was used to prepare a Whatman filter paper (No. 3) disc with a diameter of 5 mm. The discs were autoclaved and kept in sterilized storage vials. Plant extracts at a 100 mg/mL concentration were used in the disc diffusion assay. The positive control was Streptomycin (0.1 mg/mL), and for fungal strains Clotrimazole (0.1 mg/mL) were used as positive control. Methanol was used as a negative control for both strains.

*Disc diffusion method*

The assay was carried out by the slightly modified method of Mostafa *et al*. (2018). 100-200 µL of sterilized media was poured into the sterilized Petri plates in aseptic conditions and allowed to solidify. A disc of 100 mg/mL concentration of different plant extracts was loaded into the Petri plates placed over the media. A disc of positive control (Streptomycin) and negative control were loaded into the Mueller Hinton Agar plates. The plates were sealed with the paraffin wax film and left at 37 °C for 24 h. In the case of fungal strains, a disc of positive control (Clotrimazole) and negative control (methanol) was loaded into the potato dextrose agar media. The plates were sealed with the paraffin wax film and maintained at 28 °C for 24 h. The inhibitory zone was calculated after the incubation time and compared to the zones of inhibition of positive controls using a scale. The method was performed in triplicates and the mean value was recorded.

*Statistical analysis*

Each experiment was carried out in replicates and data were expressed as mean ± standard deviation. A means test was applied to differentiate the phenol and flavonoid content and antioxidant and antimicrobial activity of *H. annuus* and *H. involuta* extracts. Three-way interactions were executed between the chosen variables. The obtained data for both plants were subjected to a one-way analysis of variance (ANOVA) and a multiple range test (Tukey's test) with IBM SPSS statistics 20 software (SPSS Inc., Chicago, USA). Results were considered statistically significant at the $p < 0.05$. Graphs were plotted and statistical calculations were carried out using Origin Pro-8 and Microsoft Excel.

## Results

*Phytochemical analysis*

The presence of several phytoconstituents was examined in leaf extracts of *H. annuus* and *H. involuta* prepared in different solvents. Methanolic leaf extracts of both plants contained the highest number of phenols, carbohydrates, alkaloids, sterols, fixed oils and fats, amino acids, terpenoids, phenols, and flavonoids. Glycosides and anthraquinones are absent in the methanolic extract of *H. annuus*, whereas it was present in *H. involuta*. Saponins were present only in the methanolic extract of *H. annuus* and absent in *H. involuta*. At the same time, extracts of leaves prepared in chloroform, distilled water, and petroleum ether showed little to no residues of the variables chosen. Finally, the ideal outcome was achieved in the methanol extract (Table 1).

**Table** 1. Qualitative analysis of *Helianthus annuus* and *Hyophila involuta* in four different solvents

| No | Phytoconstituents | Leaf extract of *Helianthus annuus* | | | | Leaf extract of *Hyophila involuta* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Methanol | Chloroform | Petroleum ether | Water | Methanol | Chloroform | Petroleum ether | Water |
| 1. | **Phenol** Ellagic acid test | + | + | - | + | + | + | - | + |
| 2. | **Amino acids** Milon's test | + | - | - | - | + | - | - | - |
| 3. | **Alkaloids** Wagner's test<br>Mayer's test | +<br>+ | -<br>- | -<br>- | -<br>- | +<br>+ | -<br>- | -<br>- | -<br>- |
| 4. | **Flavonoids** Shinoda test | + | + | - | - | + | - | - | - |
| 5. | **Glycosides** Kellar-Killani test | - | - | - | - | + | - | - | - |
| 6. | **Fixed oils and fats** Saponification test | + | - | - | - | + | - | - | - |
| 7. | **Carbohydrates** Benedicts test | + | - | - | - | + | - | - | - |
| 8. | **Sterols** Liebermann-Burchard test | + | - | - | - | + | - | - | - |
| 9. | **Saponins** Froth formation test | + | + | + | + | - | - | - | - |
| 10. | **Anthraquinone** Borntrager's test | - | - | - | - | + | - | - | - |
| 11. | **Terpenoids** Salkowski test | + | - | - | - | + | - | - | - |

+ (Present), - (Absent)

*Total phenol and flavonoid content*

Total phenols and flavonoid concentration in *H. annuus*, and *H. involuta* leaf extracts expressed as gallic acid and quercetin, respectively (Tables 2 and 3). These tables depict the total phenolic and flavonoid content of extracts prepared in various solvents. The total phenolic content was more in the methanolic extract of *H. annuus* (44.66±0.003 mg/g GAE) in comparison to other solvents (Chloroform, Petroleum ether, and water). The flavonoid content is found to be higher in the methanolic extract of *H. involuta* (32.07±0.005 mg/g QE) as compared to *H. annuus* (Figures 4 and 5). Methanol proved to be the most promising solvent.

**Table 2**. Quantitative analysis of leaf extract of *Helianthus annuus* in different solvents

| Variable | Methanol | Chloroform | Water | Petroleum ether |
|---|---|---|---|---|
| Total phenolic content (mg/g GAE) | 44.66±0.003[a] | 23.84±0.002[a] | 19.79±0.007[b] | 14.71±0.003[a] |
| Total flavonoid content (mg/g QE) | 18.96±0.002[a] | 17.87±0.0011[a] | 16.81±0.0014[b] | 12.33±0.0008[a] |

The averages and standard deviations of n = 3 independent experiments are the data.

**Table 3.** Quantitative analysis of leaf extract of *Hyophila involuta* in different solvents

| Variable | Methanol | Chloroform | Water | Petroleum ether |
|---|---|---|---|---|
| Total phenolic content (mg/g GAE) | 22.05±0.02[a] | 18.07±.04[b] | 10.08±0.02[b] | 06.05±0.001[b] |
| Total flavonoid content (mg/g QE) | 32.07±0.005[c] | 20.03±0.03[c] | 12.05±0.02[b] | 08.02±0.007[b] |

The averages and standard deviations of n = 3 independent experiments are the data.

*DPPH assays*

DPPH assay is used mainly for the determination of the antioxidant potential of phenolic as well as plant extracts (Fukumoto and Mazza, 2000). When different extracts of *H. annuus* were evaluated for their antioxidant activity, methanol exhibited the highest % scavenging activity, followed by chloroform, distilled

water, and petroleum ether. Correspondingly, the lowest $IC_{50}$ value (50.60 ± 0.05 µg/mL) was obtained with DPPH radical by methanol followed by chloroform (56.87 ± 0.011 µg/mL), distilled water (59.86±0.09 µg/mL), and petroleum ether (62.83 ± 0.013 µg/mL). Similarly, when different extracts of *H. involuta* were evaluated for their antioxidant activity, methanol exhibited the highest % scavenging activity, followed by chloroform, distilled water, and petroleum ether. Correspondingly, the lowest $IC_{50}$ value (39.96 ± 0.16 µg/mL) was obtained with DPPH radical by methanol followed by chloroform (47.08±0.27 µg/ml), distilled water (42.02±0.22 µg/mL), and petroleum ether (57.08±0.04 µg/mL). Hence, from the above data, we can conclude that the best antioxidant activity is seen in methanolic leaf extracts of *H. annuus* (50.60±0.05 µg/mL) and *H. involuta* (39.96±0.16 µg/mL). Therefore, we can conclude that the *H. annuus* shows the lowest and more accurate $IC_{50}$ value in comparison to *H. involute* (Table 4; Figure 6). The unimpressive results were observed in other solvents, i.e., chloroform, distilled water, and petroleum ether, as they have shown less antioxidant activity and the greatest $IC_{50}$ values.

*NOSA assay*

NOSA is a useful and one of the most convenient assays to investigate the free radical scavenging activity of compounds. When different extracts of *H. annuus* were evaluated for their antioxidant activity, methanol exhibited the highest % scavenging activity, followed by chloroform, distilled water, and petroleum ether. Correspondingly, the lowest $IC_{50}$ value (44 ± 0.37 µg/mL) was obtained with NOSA radical by methanol followed by chloroform (48±0.30 µg/mL), distilled water (51.20±0.18 µg/mL), and petroleum ether (49.92±0.10 µg/mL). Similarly, when different extracts of *H. involuta* were evaluated for their antioxidant activity, methanol exhibited the highest % scavenging activity, followed by chloroform, distilled water, and petroleum ether. Correspondingly, the lowest $IC_{50}$ value (34.05 ± 0.12 µg/mL) was obtained with NOSA radical by methanol followed by chloroform (38.99 ± 0.10 µg/mL), distilled water (36.12±0.15 µg/ml), and petroleum ether (39.92±0.16 µg/mL). Hence, from the above data, we can conclude that the best antioxidant activity is seen in methanolic leaf extracts of *H. annuus* (50.60±0.05 µg/mL) and *H. involuta* (39.96±0.16 µg/mL). Therefore, we can conclude that *H. annuus* shows the lowest and more accurate $IC_{50}$ value in comparison to *H. involuta* (Table 5; Figure 7). Hence, methanolic extracts proved to show the best antioxidant activity in comparison to other solvents.

**Table 4**. $IC_{50}$ values of different extracts for antioxidant activity

| **Different extracts of *Helianthus annuus* and *Hyophila involuta*** | **DPPH ($IC_{50}$) Leaf extract of *Helianthus annuus* (µg/mL)** | **DPPH ($IC_{50}$) Leaf extract of *Hyophila involuta* (µg/mL)** |
|---|---|---|
| Methanol | 50.60±0.05[a] | 39.96±0.16[a] |
| Chloroform | 56.87±0.011[b] | 47.08±0.27[b] |
| Water | 59.86±0.09[c] | 42.02±0.22[b] |
| Petroleum ether | 62.83±0.013[d] | 57.08±0.04[a] |

Three biological means SD (n=3) illustrate each value in the table.

**Table 5**. $IC_{50}$ values of different extracts for antioxidant activity

| **Different extracts of *Helianthus annuus* and *Hyophila involuta*** | **NOSA ($IC_{50}$) Leaf extract of *Helianthus annuus* (µg/mL)** | **NOSA ($IC_{50}$) Leaf extract of *Hyophila involuta* (µg/mL)** |
|---|---|---|
| Methanol | 44±0.37[a] | 34.05±0.12[a] |
| Chloroform | 48±0.30[a] | 38.99±0.10[a] |
| Water | 51.20±0.18[a] | 36.12±0.15[b] |
| Petroleum ether | 49.92±0.10[a] | 39.92±0.16[b] |

Three biological means SD (n=3) illustrate each value in the table.

*Antimicrobial activity*

The zone of inhibition of each extract was calculated to estimate the plant's antibacterial potential. The antimicrobial study results are shown in Tables 6 and 7. The zone of inhibition was measured at different concentrations of plant extracts and methanol (control) *i.e.,* 20-100 µl but best results were observed at 100 µl. For the antibacterial assay, two bacterial strains, namely *Escherichia coli* and *Bacillus subtilis*, were used. Streptomycin and methanol were used as positive and negative controls, respectively. All the plant extracts show susceptibility to methanol. Based on the above observation, the leaf extract prepared in methanol gave the best result, and the leaf extract was processed further to test the antimicrobial efficacy. A methanol extract of *H. annuus* showed the highest zone of inhibition against both the bacterial strains [(8±0.10 mm) observed against *B. subtilis* (Figure 8) and a zone of inhibition (11.6±0.15 mm) was observed against *E. coli* (Figure 9)].

**Table 6**. Antibacterial activity of methanolic extract of both the plants (Leaves) using disc diffusion methods

| S. No. | Name of plants | Zone of inhibition (Bacterial species) | | | | | |
|---|---|---|---|---|---|---|---|
| | | ***Bacillus subtilis*** | | | ***Escherichia coli*** | | |
| | | Plant extract | Antibiotic | Control | Plant extract | Antibiotic | Control |
| **1.** | *Helianthus annus* | 8±0.10$^{a}$ | 8±0.20$^{a}$ | 5±0.10$^{b}$ | 11.6±0.15$^{a}$ | 6±0.10$^{a}$ | 12.3±0.25$^{c}$ |
| **2.** | *Hyophila involuta* | 7.6±0.05$^{a}$ | 20±0.20$^{a}$ | 16.3±0.20$^{b}$ | 8.2±0.10$^{a}$ | 7.3±0.30$^{a}$ | 5.3±0.05$^{b}$ |

**Table 7**. Antifungal activity of methanolic leaf extract of both the plants using disc diffusion methods

| S. No. | Name of plants | Zone of inhibition (Fungal species) | | | | | |
|---|---|---|---|---|---|---|---|
| | | ***Fusarium oxysporum*** | | | ***Aspergillus niger*** | | |
| | | Plant extract | Antibiotic | Control | Plant extract | Antibiotic | Control |
| **1.** | *Helianthus annus* | 8.6±0.11$^{a}$ | 10±0.20$^{b}$ | 4.6±0.05$^{b}$ | 8±0.10$^{b}$ | 6.3±0.20$^{a}$ | 4.8±0.25$^{c}$ |
| **2.** | *Hyophila involuta* | 16.6±0.15$^{a}$ | 6±0.20$^{b}$ | 5.6±0.20$^{b}$ | 8.3±0.15$^{b}$ | 7.3±0.30$^{c}$ | 5±0.2$^{b}$ |

In this study, the methanolic extract of *H. annuus* was found to be most sensitive against *E. coli*, even though it was found to be resistant to streptomycin. When compared to control (negative) and plant extracts, the zone of inhibition obtained with antibiotics (Streptomycin) was found to be higher due to the fact that these antibiotics are broad-spectrum, chemically pure, and extremely potent, producing a larger zone of inhibition at reduced concentrations. The plant extract, on the other hand, contains a significant quantity of impurities that are static and deficient in antibacterial activities, so the larger zone of inhibition observed in antibiotics as compared to the zone of inhibition in plant extract and methanol (negative) was observed by several workers (Abubacker, 2013; Azwanida, 2015).

The highest zone of inhibition (ZOI) was again observed in *H. involuta* (16.6±0.15 mm) against *F. oxysporum* (Figure 10), followed by *A. niger* (Figure 11) but less in the case of *H. annus*. Similar to the results obtained with the antibacterial potential of plant extracts, methanol was found to be most effective against the fungal strains used in the study. However, when compared with the antibacterial activity of plant extracts, the antifungal potential was found to be less. According to D'auria *et al*. (2005), fungi are less resistant to plant extracts as compared to bacteria.

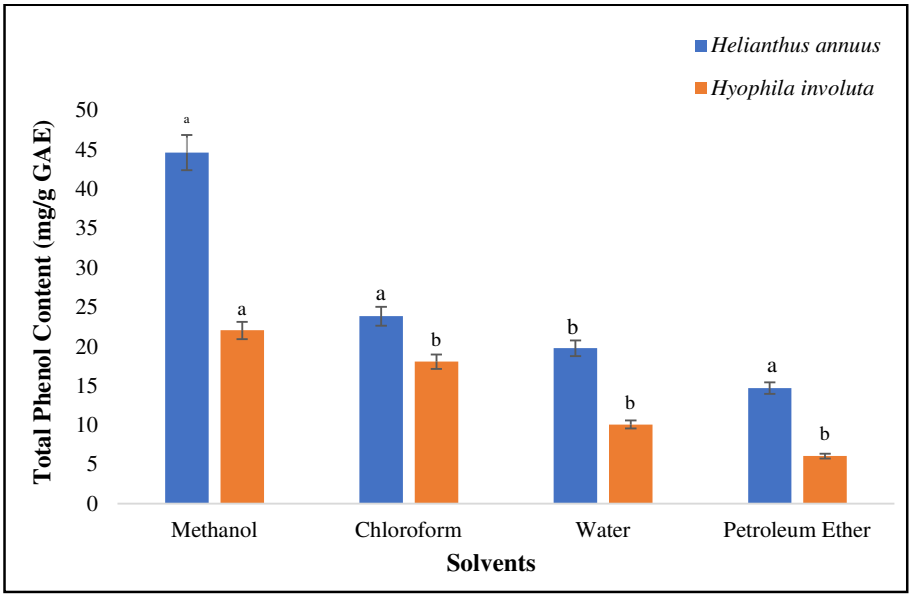


**Figure 4.** Total phenolic content in the leaves of *Helianthus annuus* and *Hyophila involute*
Data are reported as the mean ± SD of the parallel measurements.
Values with different superscripts (a and b) in the same column differ significantly (ANOVA test, $p < 0.05$).

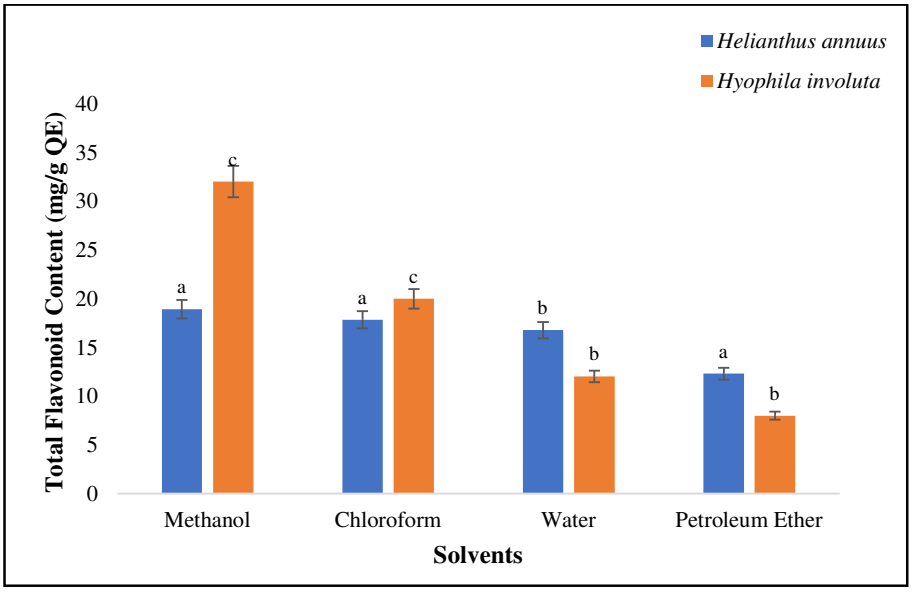


**Figure 5.** Total flavonoid content in the leaves of *Helianthus annus* and *Hyophila involute*
Data are reported as the mean ± SD of the parallel measurements.
Values with different superscripts (a, b and c) in the same column differ significantly (ANOVA test, $p< 0.05$).

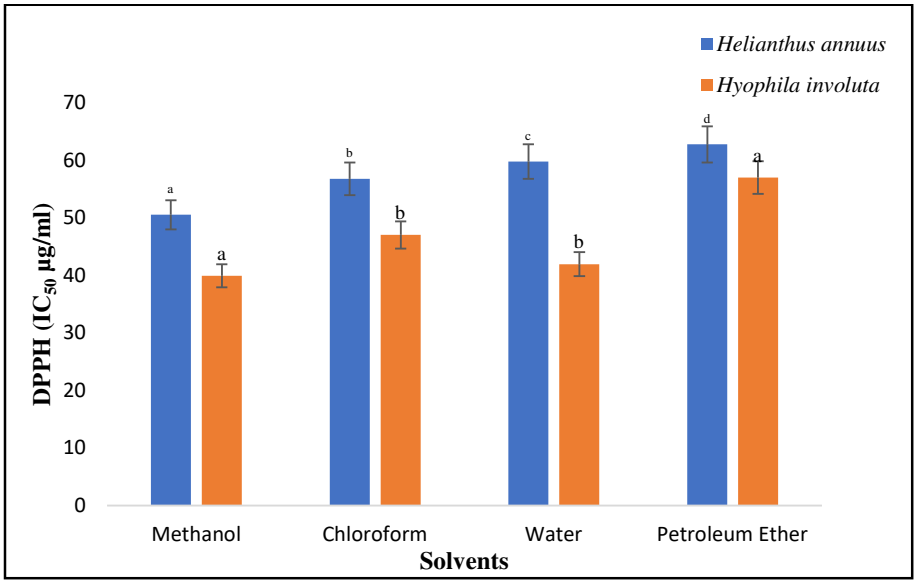


**Figure 6.** Antioxidant activity (DPPH) of *Helianthus annuus* and *Hyophila involute*
Data are reported as the mean ± SD of the parallel measurements
Values with different superscripts (a, b, c and d) in the same column differ significantly (ANOVA test, $p < 0.05$).

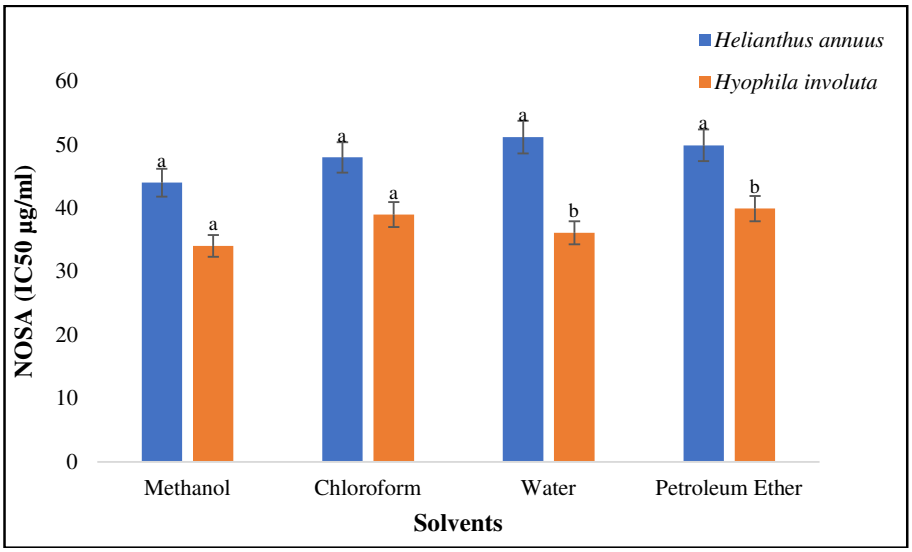


**Figure 7.** Antioxidant activity (NOSA) of *Helianthus annus* and *Hyophila involute*
Data are reported as the mean ± SD of the parallel measurements.
Values with different superscripts (a and b) in the same column differ significantly (ANOVA test, $p < 0.05$).

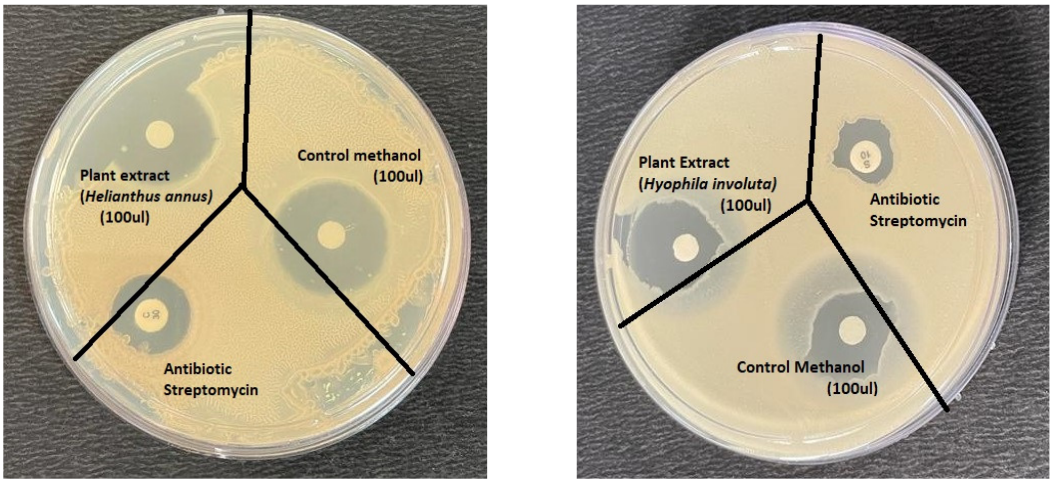


**Figure 8.** Comparative antibacterial activity of both the plants against *Bacillus subtilis*
[100 µl- concentration of plant extract and control (methanol)]

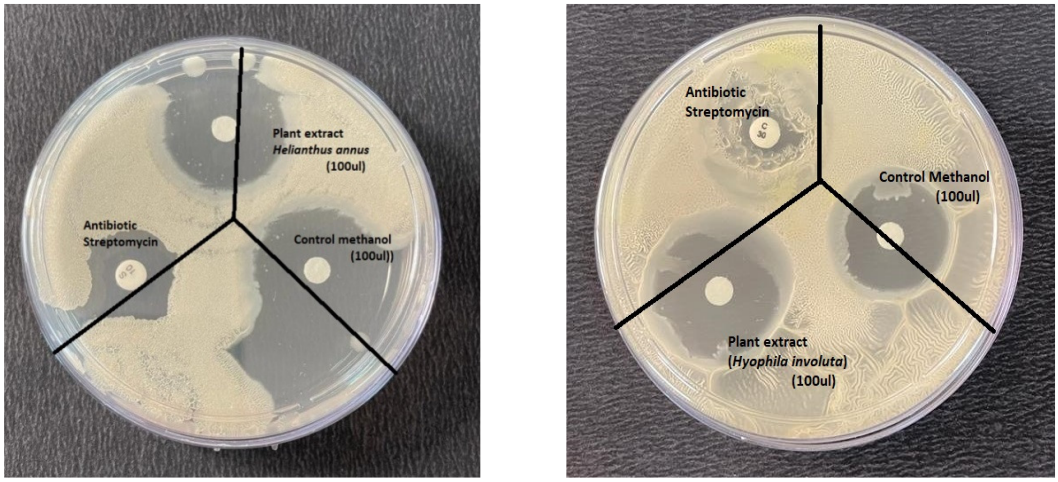


**Figure 9.** Comparative antibacterial activity of both the plants against *Escherichia coli*
[100 µl- concentration of plant extract and control (methanol)]

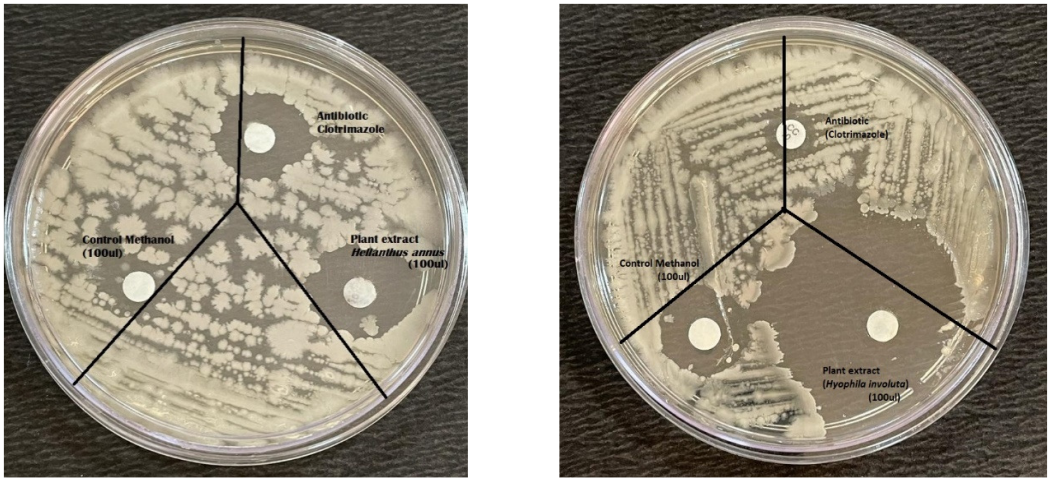


**Figure 10.** Comparative antifungal activity of both the plants against *Fusarium oxysporum*
[100 µl- concentration of plant extract and control (methanol)]

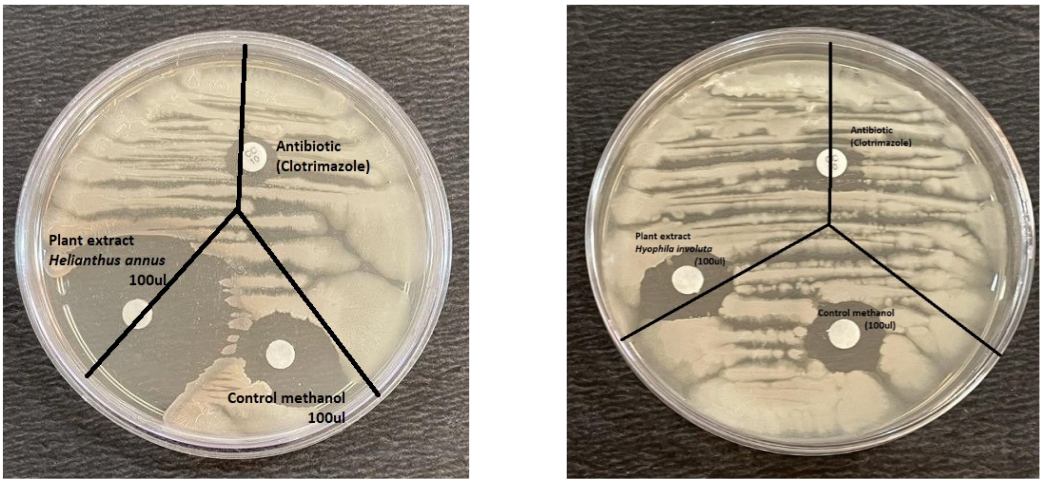


**Figure 11.** Comparative antifungal activity of both the plants against *Aspergillus niger*
[100 µl- concentration of plant extract and control (methanol)]

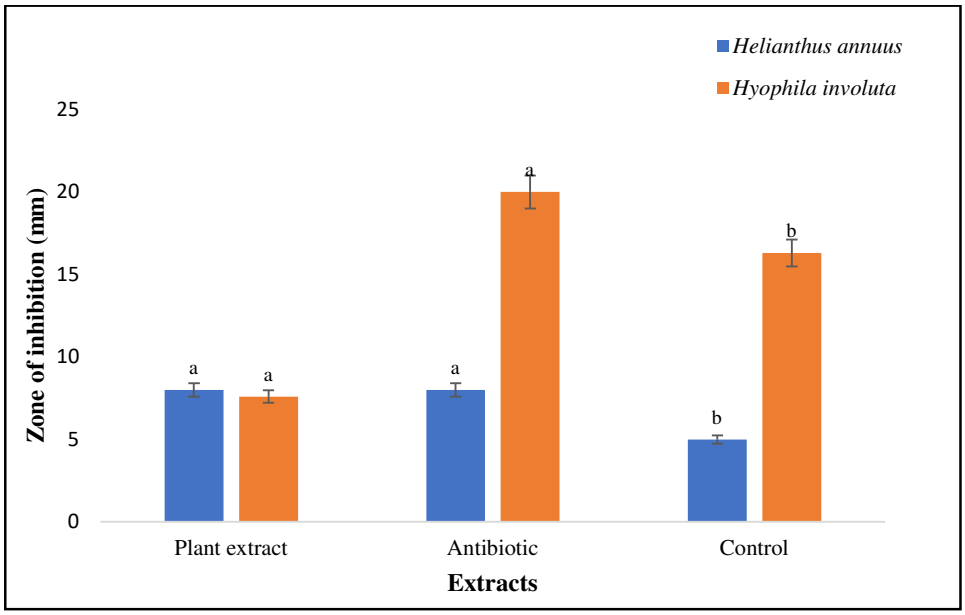


**Figure 12.** Antibacterial activity of extracts of *Helianthus annuus* and *Hyophila involuta* against *Bacillus subtilis*
Data are reported as the mean ± SD of the parallel measurements.
Values with different superscripts (a and b) in the same column differ significantly (ANOVA test, $p < 0.05$).

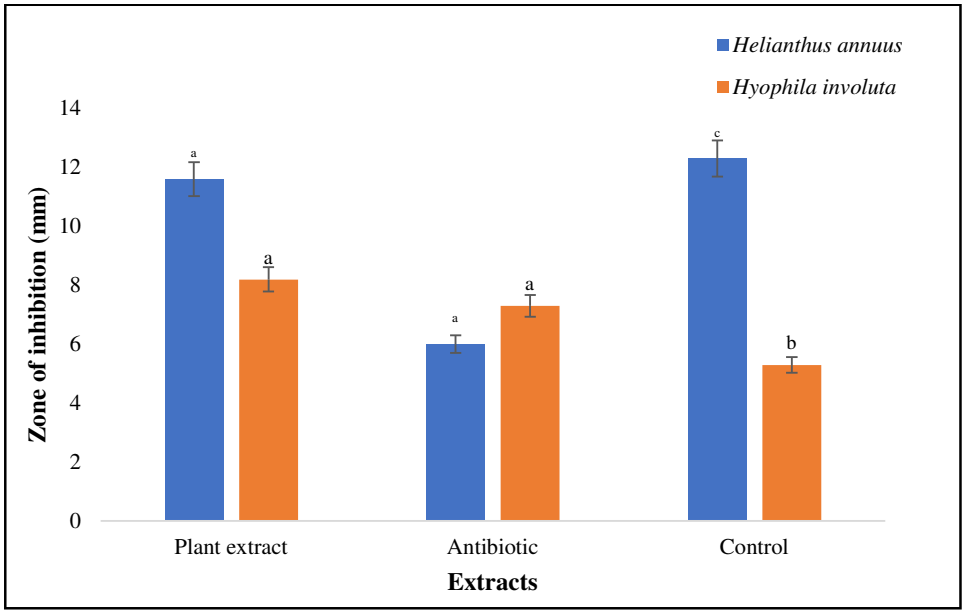


**Figure 13.** Antibacterial activity of extracts of *Helianthus annuus* and *Hyophila involuta* against *Escherichia coli*
Data are reported as the mean ± SD of the parallel measurements.
Values with different superscripts (a, b and c) in the same column differ significantly (ANOVA test, $p < 0.05$).

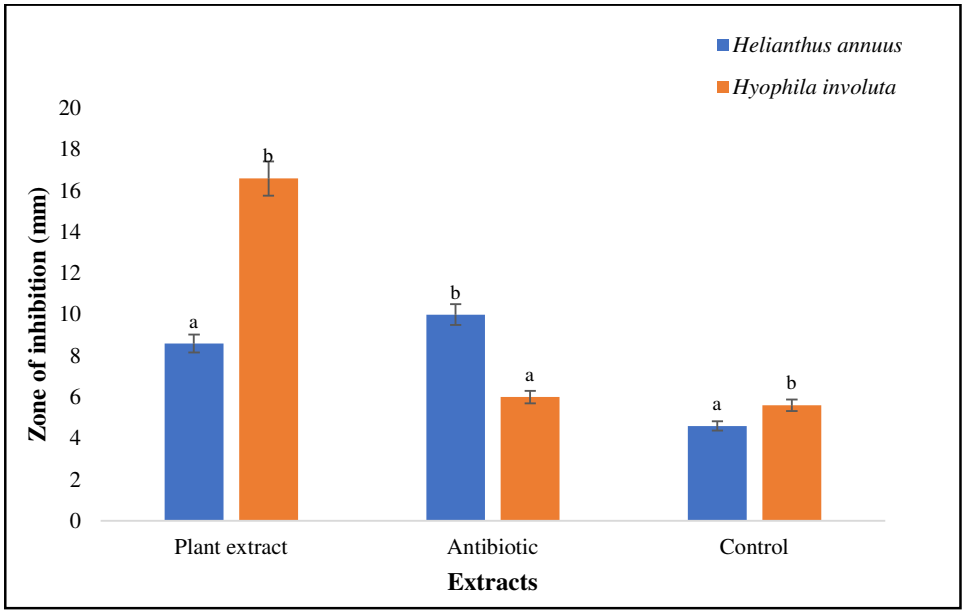


**Figure 14.** Antifungal activity of extracts of *Helianthus annuus* and *Hyophila involuta* against *Fusarium oxysporum*
Data are reported as the mean ± SD of the parallel measurements.
Values with different superscripts (a and b) in the same column differ significantly (ANOVA test, $p < 0.05$).

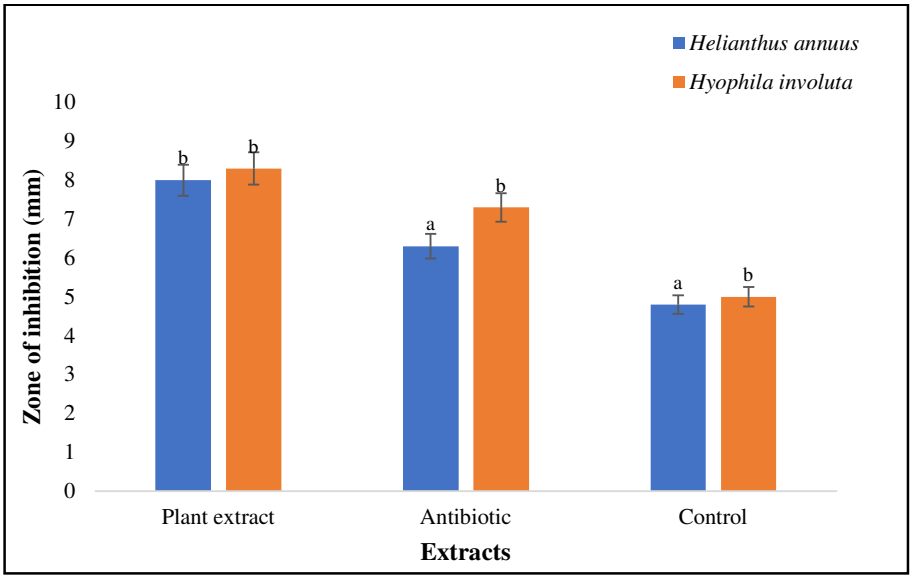


**Figure 15.** Antifungal activity of extracts of *Helianthus annuus* and *Hyophila involuta* against *Aspergillus niger*

Data are reported as the mean ± SD of the parallel measurements.
Values with different superscripts (a and b) in the same column differ significantly (ANOVA test, $p < 0.05$).

## Discussion

*Total phenol and flavonoid content*

Total phenolics and flavonoids were identified as gallic acid and quercetin, respectively (Tables 2 and 3; Figures 1 and 2). The maximum total phenol content was recorded in *the H. annus* methanol leaf extract as compared to the *H. involuta*, followed by chloroform, distilled water, and petroleum ether. However, the total phenol content of the petroleum ether and chloroform extracts was significantly lower than that of the methanol extract. Surprisingly, *H. involuta* methanol leaf extracts had a higher flavonoid concentration than *H. annus* extracts (Angiosperm-Asteraceae). The *H. annuus* methanol leaf extracts showed more significant results in comparison to *H. involuta*. Therefore, it was proven that plant extracts in methanol were the most effective extracts to use in the future. Due to the polarity of the solvents, petroleum ether and distilled water produced unimpressive results.

*Antioxidant activities*

The higher total phenolic content shows that it has good antioxidant potential. The redox characteristics of plant phenolic compounds, which allow them to function as antioxidants, are assumed to be connected to their significant free radical scavenging action. Free radical DPPH's absorbance decreases when the color shifts from purple to yellow as a result of the antioxidant's ability to scavenge free radicals. In the present study, the methanolic leaf extract of *H. annuus* showed greater potency, followed by the chloroform extracts as depicted in Table 4. The methanolic extract of *H. involuta* does not produce impressive results. The other solvents i.e., distilled water and petroleum ether have shown insignificant results. The NOSA assay also resembled the results similar to DPPH assay *i.e.,* the methanolic leaf extract of *H. annuus* showed greater potency in comparison to *H. involuta*, followed by the chloroform extracts as depicted in Table 5 and other solvents does not produce appreciable results.

*Antimicrobial activities*

The plant extracts were evaluated for their antimicrobial efficacy against two bacterial strains and two fungal strains. All the strains show susceptibility to methanol. In the current study, the methanol leaf extract of *H. annus* was found most sensitive against *Escherichia coli* even though it was found resistant to streptomycin. The methanol extract was shown to have maximum antimicrobial efficacy for all the plant extracts except for *B. subtilis* (Figures 12, 13). When compared to control (negative) and plant extracts, the zone of inhibition obtained with antibiotics (streptomycin) was found to be higher because these antibiotics are broad-spectrum, chemically pure, and extremely potent, producing a larger zone of inhibition at reduced concentrations.

In this study, the methanol extract of the selected plants was found to exhibit the highest antibacterial activity, which may be due to many phytoconstituents, TPC, and TFC. A zone of inhibition was obtained with the positive control, *i.e*., streptomycin was much higher as compared to plant extracts as both are pure chemical compounds and broad-spectrum antibiotics. On the other hand, plant extracts are heterogeneous mixtures of chemical compounds and contain several phyto-compounds that may be inert and devoid of antibacterial efficacy. It is interesting to note that plants presented maximum sensitivity towards methanol even though they were found to be resistant to the standard used, *i.e.,* streptomycin This clearly shows the presence of novel bioactive compounds in the extract, which can be of great value to mankind.

The antifungal efficacy of plant extracts was investigated against two fungal species*: A. niger* and *F. oxysporum.* Clotrimazole was used as a positive control, and methanol was used as a negative control. Similar to the results obtained with the antibacterial potential of plant extracts, methanol was found to be most effective against the fungal strains used in the study (Figures 14, 15). However, when compared with the antibacterial activity of plant extracts, the antifungal potential was found to be less.

## Conclusions

*Hyophila involuta* is a bryophyte, the first land plant, while *Helianthus annuus* is a member of the most advanced dicotyledonous family (Asteraceae). The goal of this comparative study was to identify similarities and variations in their phytochemical profiles, phenol and flavonoid contents as well as their antioxidant and antimicrobial properties, in order to assess evolutionary changes.

The study's findings revealed the presence of phytochemicals of similar nature such as alkaloids, flavonoids, phenols, and other compounds in both distantly related species, indicating that, like Angiosperms, Bryophytes are valuable sources of these phytochemicals with medicinal properties that can be used in various medicinal preparations and as drug additives in traditional medical practice and pharmaceutical manufacturing. However, the angiosperm *H. annuus* produced better findings in terms of research parameters, demonstrating that Angiosperms are more suited and dispersed throughout evolution due to their superior phytochemical profiles. Overall, the findings of this study show that both Angiosperms and Bryophytes have the potential to provide bioactive chemicals with therapeutic effects, and Bryophytes should be accorded similar importance in future pharmaceutical applications.

## Authors' Contributions

Both authors conceptualized and designed the document. Sharma SK carried out the research under the direct supervision of Alam A. Sharma SK carried out all statistical analyses and drafted the paper. Both authors read and approved the final manuscript.

**Ethical approval** (for researches involving animals or humans)

Not applicable.

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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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