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Research Article

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Investigation of the preliminary compounds; Assessing the antioxidant and *in-vitro* antibacterial activities of *Inula confertiflora* leaf extracts

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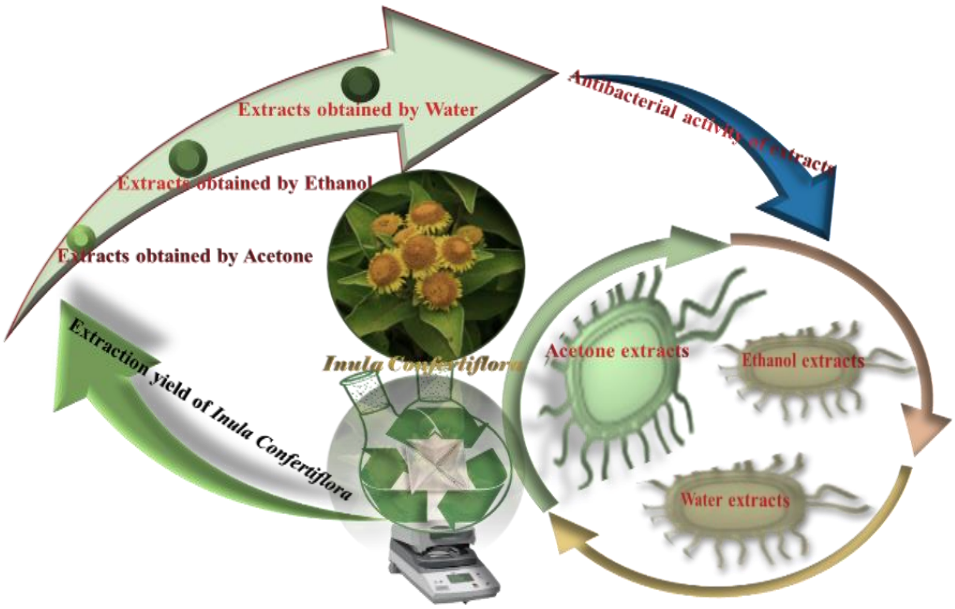
Highlights

- Efficient yields of crudes extracted by acetone, ethanol, and water organic solvents were obtained.
- Secondary metabolites found in the leaf of *Inula confertiflora* were investigated both qualitatively and quantitatively.
- The wavelength and absorbance intensity of acetone and ethanol extracts were assessed.
- Ethanol and water extracts showed higher scavenging efficiency and zones of inhibition against bacterial strains.

Graphical Abstract



Abstract

Medicinal plants, from those traditionally utilized by healers in underdeveloped nations to those employed in the manufacturing of contemporary synthetic medications worldwide, are essential in the treatment of a wide range of illnesses. Ethiopians have long utilized the leaves of *Inula confertiflora* plants to treat diseases pertaining to the skin and eyes. This encouraged us to look at the yield of crudes extracted using various polar index solvents, look at the existence of precursor compounds in the plant's leaf sections, and evaluate the ability of preliminary compounds to scavenge free radicals from DPPH and their antibacterial properties against gram-positive and gram-negative bacterial strains. Following the extraction processes of the plant soaking, filtering, and concentrating the crudes, the highest yield of crude was produced by ethanol in moderately polar solvents (3.64 mg) water with a higher polarity index (5.74 mg) and acetone in lower polar solvents (6.28 mg), which produced a relatively lower yield. The UV-Vis spectroscopic results of the ethanol extracts showed they contained a wide range of preliminary bioactive functional groups, like phenols and flavonoids, and their derivative compounds. Additionally, the ethanol extracts' inhibition zone was marginally higher than that of the water and acetone extracts', indicating that the preliminary compounds extracted in a higher polar solvent—water—and a moderate polar solvent—acetone—were obtained in the ethanol extracts. In conclusion, the presence of higher total content of the phenolic and flavonoids, higher radical scavenging efficiency in DPPH solution, and greater zone of inhibition in-vitro antibacterial activities displayed by the preliminary compounds extracted by ethanol and water extracted from the leaf of *I. confertiflora* corroborate the traditional uses by the local people against various diseases.

Keywords: Medicinal plants; secondary metabolites; *Inula confertiflora*; antioxidant and antibacterial activity.

1. Introduction

Humans have been using medicinal plants for thousands of years, not only for treating and fighting infectious diseases but also for different applications like their ecological, commercial, and cultural advantages (Awulachew, 2021; Yeshiwas et al., 2019). According to a World Health Organization (WHO) survey, between 65 and 80 percent of people in underdeveloped nations consistently utilize and believe in the efficacy of medicinal plant therapies (Robinson and Zhang, 2011). This report states that a significant portion of sick people in developing countries who cannot afford pharmaceuticals of any kind prefer to use medicinal plant products because the treatment agents in these products are less expensive and are probably the only natural remedies available in remote communities (Moges and Moges, 2019). Still today, in developed countries, the production of modern medicine through synthetic means in laboratories associated with column chromatography and spectroscopic tools is what makes it possible to isolate the active constituents from medical plants (Reeds, 1976). This suggests that medicinal plants have played a crucial role in the development of modern medicine and have served as the foundation of important bioactive compounds (Atanasov et al., 2015). Moreover, most of people who utilize aromatic and medicinal herbs have also improved as a result of the generational development in understanding of these plants from antiquity to the present (Inoue et al., 2019). However, the significance of the ingredients of aromatic and medicinal plants is not universally acknowledged. Furthermore, the essential components of medicinal plants may vanish due to plant diseases, such as climate change, or other plant attacks (Parbuntari et al., 2019). This could decrease the treatment efficiency of a variety of medicinal plants that are affected by the aforementioned effects. As a result, researchers

who looking into the components found in medicinal plants is essential for both healers and pharmaceutical companies that make contemporary medicine from these plants.

The greater knowledge of the potential therapeutic value of medicinal plant can be gained from the chemical composition of medicinal plants produced by plant cells, mostly secondary metabolites created through the metabolic pathway obtained from primary metabolites (Pisithkul et al., 2019). Following Charles Darwin's well-known theory of evolution, "natural selection" or "survival of the fittest" allows the most vulnerable individuals to persist and multiply through the use of plant-based resources, resulting in organisms that are able to adapt to their environment (Richards, 2019). More importantly, plants produce secondary metabolites that are essential to their own growth and development as well as to innate immunity (Peter et al. 2019), defensive response signaling (Isah, 2019), and the plant's ability to respond to environmental challenges (Yang et al., 2018). Therefore there is a wide range of compounds found in plant secondary metabolites, and their use in medicine is either directly or indirectly related to the parent plant's flavor, color, and aroma (Kumar et al., 2023). Moreover, the secondary metabolites present in medicinal plants are unique to that particular species of the plant. As a result of this, numerous researchers have studied various plant species in an effort to pinpoint secondary metabolites—both quantitative and qualitative—and their antibacterial properties.

The Asteraceae family, also referred to as Compositae, is home to over 100 species of *Inula*, which are found throughout the continent of Africa, Asia, and Europe (Tavares and Seca, 2019). It is believed that *Inula* genus contain around 400 distinct compounds, primarily flavonoids, alkaloids and terpenoids, many of which have intriguing pharmacological properties and are significant for scientific and medical research (Seca et al., 2014; Seca et al., 2015). Among those of the *Inula* genus, *I. confertiflora* species is widely used for medicinal purposes to treat eye and skin-related diseases such as fungal infections (tinea capitis and tinea corporis), wound infections, herpes infections, and eczematous lesions (Gebre-Mariam et al., 2006). The dried root parts of this plant have long been employed as a fumigant during childbirth and in the treatment of leprosy (Gashu, 2022). Moreover, the leaf parts of the *I. confertiflora* have commonly been used to treat ringworm infections and lumpy skin disease. However, currently there is no scientific report on the preliminary compounds found in the leaf of *I. confertiflora*, despite the fact that various researchers have reported the preliminary compounds found in *Inula* species and that *I. confertiflora* is a known medicinal plant used by traditional healers to treat animal eye-related diseases. Therefore, in this paper, we report the preliminary compounds found in the leaf parts of this plant, which were extracted in different polar index solvents under the same conditions. Furthermore, the effects of each extract on the antioxidant activities were investigated by analyzing the radical scavenging efficiency against DPPH solution and the antibacterial activities of each extract against gram-positive and gram-negative bacterial strains. Therefore, the main objectives of this study are to examine the preliminary compounds found in the leaf extract of *I. confertiflora* and assess the antibacterial and antioxidant properties of the crudes extracted using various solvents.

2. Materials and Methods

2.1. Chemicals

The organic solvents including acetone, ethanol, deionized water, and chloroform were used in this research work for soaking, extracting, and other purposes. Moreover, the chemicals used for the preparation of reagents to investigate secondary metabolites like tannins, alkaloids, saponins, flavonoids, phenols, and terpenoids are: vanillin, sulphuric acid, hydrochloric acid, potassium

hydroxide (85% Mumbai, India), acetic anhydrous (British Drug House Ltd., UK), ferric chloride (British Drug House Ltd., England), potassium iodide, and iodine. All these chemicals were of analytical grade and were directly employed in the study without any purification procedures.

2.2. Plant material collection and preparation of extracts

Samples of *I. confertiflora* were obtained from the Gettila basin, which is situated in Debre Markos, Ethiopia, for the current study. After that, a voucher specimen was carefully processed and kept in the biology lab at Debre Markos University's College of Natural and Computational Science. Following collection and vouchering, the leaf sections of the *I. confertiflora* plant were thoroughly cleaned with deionized water to remove any remaining dirt, and they were then allowed to dry for seven days under the shade in the chemistry lab. The dried leaf parts of *I. confertiflora* were then grinded into fine powder forms using a grinder and immersed in three analytical-grade solvents (acetone, ethanol, and deionized water) to extract the powdered plant material using a slightly modified maceration procedure. In detail, the dried and powdered leaf of *I. confertiflora*, about 50 g in a two-neck volumetric flask, were soaked and extracted with 200 mL of acetone, ethanol, and water through stirring using a magnetic stirrer for about three days. Taking into account the solvents' tendency to boiling point, the extracted crudes were then centrifuged once more using a rotary evaporator for sample drying. The centrifuged and dried extracts of the plant were meticulously calculated to investigate the yield of extracts with the respective solvents using the following formula:

$$\text{Yield (\%)} = \frac{(Weight\ of\ centrifuged\ and\ dried\ extracts}{Weight\ of\ dried\ leaf} x100$$

Finally, the centrifuged and dried extracts were kept in the refrigerator for further study.

2.3. Phytochemical Screening-Qualitative test

The preliminary qualitative phytochemical analysis test was carried out to investigate the presence or absence of secondary metabolites in acetone, ethanol, and water leaf extracts of *I. confertiflora*. To study the phytochemical screening test qualitatively, around 400 mg of the freshly extracted *I. confertiflora* leaf by each solvent was diluted in 100 mL of each solvent, respectively, to make a stock concentrated solution of 4 mg/ml. Accordingly, the following methods (Cho et al., 2003; Clarke, 1975) were applied to the conventional phytochemical screening tests in order to perform the preliminary phytochemical screening test of *I. confertiflora* leaf extracts:

2.3.1. Tannins-using Ferric Chloride Test

Two milliliters of 5% ferric chloride were added to one milliliter of *I. confertiflora* leaf extract. The development of a greenish-black or dark blue hue suggests the presence of tannins.

2.3.2. Alkaloid-using Mayer's Test

2 mL of strong hydrochloric acid were added to 2 mL of *I. confertiflora* extract. Next, a couple of drops of Mayer's reagent were introduced. Alkaloids are present when a precipitate that is either green or white is present.

2.3.3. Saponins-Foam Test

In a graduated cylinder, 2 ml of *I. confertiflora* extract and 2 ml of distilled water were combined and shaken for 15 minutes lengthwise. Saponins are present when a layer of foam forms that is almost one centimeter thick.

2.3.4. Flavonoids-Sodium Hydroxide Test

1 mL of 2N sodium hydroxide was added to two milliliters of *I. confertiflora* extract. The appearance of a yellow color indicates the presence of flavonoids.

2.3.5. Phenols-Ferric Chloride Test

Three drops containing the mixture of 10% ferric chloride and 10% ferrocyanide were added to three milliliters of *I. confertiflora* extract. The presence of phenolics is indicated by the formation of an orange-brown precipitate.

2.3.6. Terpenoids-Salkowski's Test

After adding about 5 ml of *I. confertiflora* extract to the test tube, 2 ml of chloroform and 2 ml of concentrated H_2SO_4 were added. The presence of terpenoids is indicated by the formation of a reddish-brown ring.

2.4. Phytochemical Screening-Quantitative Test

The total contents of phenolic, and flavonoids in leaf extracts of *I. confertiflora w*ere determined using the following standard procedures (Chatatikun and Chiabchalard, 2013; Sandip et al., 2014):

2.4.1. Total phenolic content of leaf extracts of I. Confertflora

The modified calorimetric Folin-Ciocalteu technique was utilized to assess the total phenolic content of *I. confertiflora* leaf extracts extracted by each solvent. In this experimental methodology, Gallic acid acts as a positive control. To be more precise, 50 mL of 50% methanol (v/v) was used to dissolve 50 mg of tannic acid to prepare 1 mg/mL of the positive control's stock solution. After serially diluting the stock solution with a concentration of 1 mg/mL to the following concentrations: 0, 20, 40, 80, 160, and 320 µg/mL, a solution containing 10 mL of *I. confertiflora* leaf extracts in 50% methanol at a concentration of 1 mg/mL was prepared. After combining an aliquot of 1 mL of *I. confertiflora* extracts or a positive control separately with 2.5 mL of Folin-Ciocalteu (10% v/v in water) solution and adding 5 mL of 7.5% sodium carbonate (w/v) solution, the mixture was vortexed and left to stand at room temperature for two hours. The UV-Vis absorbance of the reaction mixtures was measured at 760 nm with respect to a blank solution consisting of 50% methanol. Gallic acid equivalents (GAE) were calculated as the total phenolic content (TPC) of the *I. confertiflora* extract based on the calibration curve obtained from the tannic acid solution. The results were represented as TPC per milligram of dry weight of *I. confertiflora* extracts.

2.4.2. Total Flavonoid Content of Leaf Extracts of I. Confertiflora

The total flavonoid contents found in *I. confertiflora* leaf extracts were determined by applying a slight modification to the calorimetric aluminum chloride method. The positive control in this experiment was quercetin, which was dissolved in 5 mL of 50% methanol to produce a 1 mg/mL concentration for the stock solution. From the quercetin stock solution, serial dilutions of 0, 20, 40, 80, 160, and 320 µg/mL were prepared, and a 10 mL solution containing a concentration of 1 mg/mL of *I. confertiflora* extracts was prepared. After mixing 1 mL of 5% sodium nitrite with

around 1 mL of standard solution, 5 minutes later, 1 mL of 10% aluminum chloride was added. Following a two-minute waiting period, 2 mL of 1.0 M sodium hydroxide was added to the solution. After completely mixing and diluting the mixture with 4 mL of distilled water, the UV-Vis absorbance was measured at 510 nm. Based on the quercetin calibration curve, the total flavonoid content of the each-extracted *I. confertiflora* extracts was calculated, and the results are given in milligrams of quercetin equivalent (QE) per dry weight of the leaf extracts.

2.5. Antioxidant activity using the DPPH method

With a few minor adjustments to the protocol used by Roberta et al. and Blois (Blois, 1958; Roesler, Malta, Carrasco and Pastore, 2006), the antioxidant activity of *I. confertiflora* leaf extracts against radical scavenging activity was examined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay methods. In particular, 2 mL of *I. confertiflora* extracted using acetone, ethanol, and water extracts was dissolved in 0.2 mM of DPPH solution, which was the equivalent concentration. Once each extract had been incubated for 20 minutes at physiological temperature, the scavenging efficiency was evaluated against the DPPH solution using UV-Vis spectrophotometer at an absorbance of 517 nm. The ascorbic acid standard solution (2 mM) exhibited a maximum absorbance intensity of 1.37 a.u and served as the reference solution for this DPPH antioxidant experiment.

The percent scavenging efficiency of extracts against DPPH was calculated using the following formula:

$$(\%)\text{Inhibition of DPPH} = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} x100$$

Where, $A_{control}$ = Absorbance of DPPH; and A_{sample} = Absorbance of acetone, ethanol and water extracts.

2.6. Bacterial strains

The antibacterial potency of the leaf extracts of the *I. confertiflora* was investigated using two Gram-positive (*Staphylococcus aureus* and *Bacillus cereus)* and Gram-negative (*Escherichia coli* and *Pseudomonas)* bacterial strains. All the bacterial strains were prepared in Bahir-Dar university, College of natural science at the department of Biology.

2.7. *In-vitro* antibacterial activity of extracts

All the extracts of *I. confertiflora* were assessed using disc-diffusion methods with little modification that was assayed with the following methodology. In detail, the dried crude extracts of leaf *I. confertiflora* using acetone, ethanol, and water were dissolved in distilled water to obtain a yield 1 mg/mL concentrated stalk solution. Furthermore, each extracted crude was inoculated at the required concentrations of 200 µg/ml on inoculums of bacteria with disc diameters of about 6 mm and placed on plates after being incubated at 37 ºC for 24 hours. Based on the above concentrations, the antibacterial activity of each extracts was investigated by measuring the zone of inhibition in millimeter against gram-positive and gram-negative bacterial strains. In this activity to the common drug gentamycin, which was used as a positive control antibiotic agent and DMSO, which was used as a negative control. Finally, the diameters of the inhibition zones discovered around the discs represent antibacterial efficiency of the leaf extracts of the *I. confertiflora*.

3. Results and Discussion

3.1. Extraction yield and phytochemical screening test of the plant

The leaf parts of the *I. confertiflora* was successfully soaked and extracted with acetone, ethanol, and water solvents successively at shaded area. The crude solutions, which were soaked for 72 hours before being filtered, were clearly noted to exhibit distinct rates of settling at the bottom of two neck conical flasks; however, the rate of reimbursement in acetone was excessively rapid. In addition, the yield of extracts made with ethanol was higher than that of the filtrated solutions that were obtained in acetone after centrifugation. These findings would suggest that fewer preliminary chemicals were extracted from *I. confertiflora* leaves and that the internal components in this plant have a poorer solubility efficiency in lower polarity index of acetone solvents. Additionally, this conclusion was further supported by calculating the extracted yield of the *I. confertiflora* leaf after it had dried. As shown in Table 1, the yield obtained by ethanol (12.56%) was greater than that of acetone (7.28 %) and water (11.48 %), further suggesting that the *I. confertiflora* leaf soaked in a moderate-polar solvent resulted in a high concentration of secondary metabolites in the plant's leaf parts. Several studies show that high-polar organic solvents have a higher tendency to percolate into well-grinded powder parts of plants and extract organic constituents like flavonoids, phenolic compounds, and their derivatives. Further, the nature of the crudes identified in the greater polarity index of water may be similar to those found in the moderate polarity index of ethanol, which has a strong potential to extract higher yields of crudes. Thus, from the same amounts of *I. confertiflora* leaf with the same rate of soaking time, the yield of the extracts with moderate polar organic solvents of ethanol is higher than the acetone and water extracts. This suggests that the polarity nature of ethanol and maceration methods extract higher biologically active compounds from this plant.

Table 1. The net weight and yield of the leaf extracts of the *Inula confertiflora* after 3 days.

Solvents	Weight of extracts (mg)	Yield (%) of extracts
Acetone	3.64	7.28
Ethanol	6.28	12.56
Water	5.74	11.48

Consequently, after we obtained the required amount of the yield of extracts, we access the preliminary phytochemical screening test to examine the presence of secondary metabolites like tannin, alkaloids, saponins, flavonoids, phenols and terpenoids using standard methods. Table 2 shows that while alkaloids, saponins, flavonoids, phenols, and terpenoids are present in plant parts of *I. confertiflora* soaked in moderate and high organic polar solvents, tannin compounds are absent from leaf parts of *I. confertiflora* extracted with relatively lower-polar solvents. This suggests that major secondary metabolites are active in the leaf of *I. confertiflora* after being soaked for roughly 72 hours. The presence of secondary metabolites, usually referred to as the primary active components of the plant, is obviously a crucial part of the plant's medical application system for society. Therefore, this finding shown in Table 2 provides additional evidence that the leaf parts of *I. confertiflora* can be utilized medicinally to cure illnesses, contingent upon the quantitative study of the secondary metabolites. Additionally, the leaf extracts of *I. confertiflora* showed greater quantitative values for secondary metabolites such as alkaloids, flavonoids, phenol, and terpenoid chemicals in ethanol and water extracts. The presence of these

metabolites in leaf *I. confertiflora* extracted by moderate and higher organic polar solvents may further confirm that the efficacy treatment efficiency of the leaf parts of this plant has better treatment potential against different ailments since most of the traditional medicinal plant healers prepare the drug using water as a common solvent.

Table 2. Qualitative phytochemical screening test to leaf extracts of the *Inula confertiflora.*

Extracted Solvents				
Secondary metabolites	**Reagents**	**Acetone**	**Ethanol**	**Water**
Tannin	Ferric Chloride Test	+	-	-
Alkaloids	Mayer's Test	+	+	++
Saponnins	Foam Test	-	+	+
Flavonoids	Sodium Hydroxide Test	+	+	++
Phenols	Ferric Chloride Test	+	++	++
Terpenoids	Salkowski's Test	-	++	+

Where, + is positive (presence), - is negative (absence) and ++ is deep positive result.

The total content of phenols (TCP), and flavonoids (TCF) from dried and centrifuged *I. confertiflora* that had been extracted using acetone, ethanol, and deionized water solvents was then examined. The total content of phenols extracted by ethanol (276.71 ± 9.7 mg GAE/g) was greater than that of water extracts (183.69 ± 11.5 mg GAE/g), and acetone extracts (102.48 ± 6.3 mg GAE/g), as Table 3 illustrates. This suggests that ethanol, with its higher relative polarity index of 0.654, has a greater capacity to extract phenolic chemicals than acetone, with its lower relative polarity index of 0.355. Moreover, the total content of phenols found in leaf extracts *of I. confertiflora* in the water extracts (with a higher polarity index of 1.00) was lower than in the ethanol extracts, which may suggest that most of the phenols that dissolve in polar solvents were probably extracted earlier in the ethanol within 72 hours. Moreover, the total content of the flavonoids found in water extracts was lower than in acetone and ethanol extracts, which may imply that many of the lipophilic flavonoids and flavonoids are extracted by acetone and ethanol accordingly.

Table 3. The total content of phenols and flavonoids in leaf extracts of *I. confertiflora*

Extracts	**TPC (mg GAE/g)**	**TFC (mg QE/g)**
Acetone	102.48 ± 6.3	116.51 ± 10.2
Ethanol	276.71 ± 9.7	189.63 ± 8.4
Water	183.69 ± 11.5	93.85 ± 5.9

The absorbance characteristics of the acetone and ethanol extracts were then determined using UV-Vis spectrophotometers with the same concentration in aqueous solutions, and it was investigated how the leaf extracts of *I. confertiflora* differed in wavelength and absorbance intensity in aqueous solutions. Figure 1 illustrates that the acetone extracts of *I. confertiflora* leaf parts displayed two absorbance peaks at approximately 245 and 290 nm. In the presence of two or more absorbance peaks observable between 200 and 400 nm, it is possible that the *I. confertiflora* leaf extracted with moderately polar solvents includes unsaturated groups and heteroatoms (carbon, oxygen, and nitrogen (Njokua et al., 2013). Furthermore, the UV-Vis absorbance wavelengths of

the ethanol extracts showed more than three absorbance at 241, 260, 295, and 317 nm, respectively. This shows that, compared to leaves extracted using moderately polar solvents of acetone, *I. confertiflora* leaves extracted with higher polar solvents of ethanol contain a different form of functional chemical, such as aromatic compounds and phenols and their derivatives. More significantly, as shown in Figure 1, the ethanol extracts' absorbance intensity was found to be lower than that of the acetone extracts, providing additional evidence that the ethanol extracts had a higher internal concentration of secondary metabolites within the extracts.

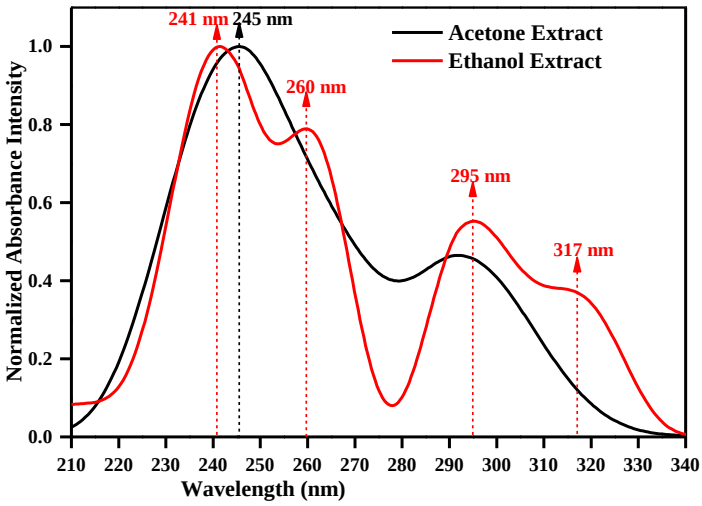


Figure 1. The UV-Vis absorbance of acetone and ethanol extracts at a concentration of 0.8 mg/ml in an aqueous solution.

We then evaluate each extract's antioxidant activity efficiency against the DPPH utilizing the DPPH assay, with ascorbic acid serving as a positive control while accounting for the presence of secondary metabolites in the *I. confertiflora* leaf extracts. The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) compound is a commonly used free radical scavengers for evaluating the phytochemical preliminary antioxidant activity of various plant extracts in various solvents. The antioxidant activity of the extracts in different concentrations is directly proportional to the decrease in UV-Vis absorbance intensity of the DPPH solution (Brand-Williams et al., 1995). As shown in Figure 2a, the absorbance intensity of the acetone extracts in the DPPH solution decreased slightly with increasing concentration, which implies that the leaf of *I. confertiflora* extracted by moderate polar solvents of acetone has a good tendency to accept hydrogen from the DPPH solution. Moreover, after 30 minutes of incubation, the absorbance intensity of *the I. confertiflora* leaf extracted with ethanol and water, as shown in Figures 2a and b, significantly decreases with increasing concentration. This suggests that the scavenging efficiency of the DPPH solution from the ethanol and water extracts has a higher capacity than that of the acetone extracts, which may indicate that the ethanol and water extracts contain a higher amount of phenols. Next, we calculate the percentage radical efficiency of the DPPH solution against each extract, and as shown in Figure 2d, the radical scavenging efficiency of acetone extracts was found to be lower than that of ethanol and water extracts of *I. confertiflora* leaf. This suggests that the presence of high-content phenols in ethanol and water extracts contributes to the increased free radical scavenging efficiency of DPPH solutions. The most frequent plant extracts that possess antioxidant properties are those that contain phenolic chemicals and their derivatives, which shield human body tissues from the

detrimental effects of oxidative stress (Rahman et al., 2021). This provides more evidence that *I. confertiflora* leaves extracted in organic solvents with high polarity possess strong antioxidant characteristics.

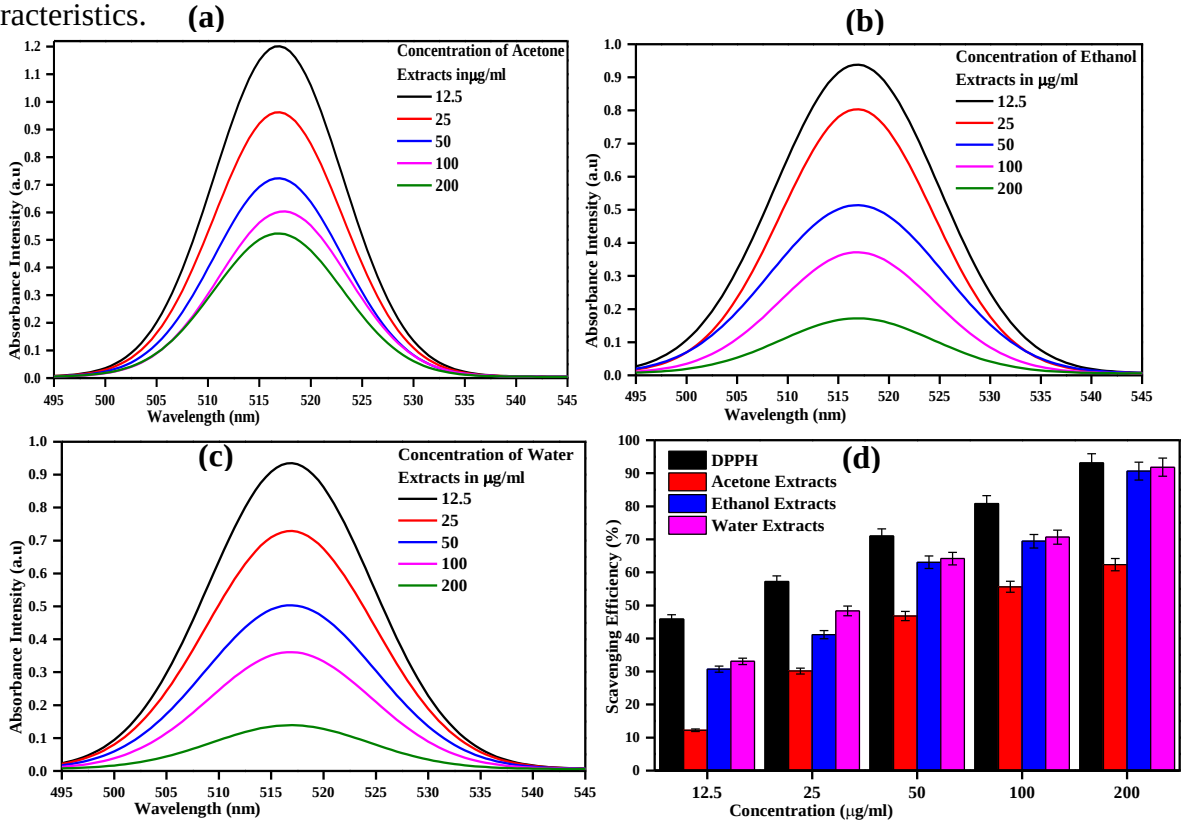


Figure 2. The UV-Vis absorbance intensity of the (a) acetone extracts, (b) ethanol extracts, (c) water extracts in DPPH solution after 30 minutes of sample preparation; and (d) the percentage scavenging efficiency of DPPH and each extract.

In addition to phenolic and their derivatives' strong antioxidant capacity, a variety of phenolic and flavonoid compounds have the potential to have potent antibacterial effects on both gram-positive and gram-negative bacteria (Bouarab-Chibane et al., 2019). Even though *I. confertiflora* leaf extracts have antioxidant properties, this study also looks into the antibacterial properties of each extract against gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) and gram-negative (*E. coli* and *Pseudomonas*) bacteria. The presence of distinct functional groups and a higher concentration of precursor compounds in the crudes likely contribute to the increased antibacterial activity of ethanol and water extracts, as evidenced by the lower inhibition efficiency of gram-positive bacterial strains in the extracts compared to acetone extracts (Figure 3a). Several researches have confirmed that gram-positive bacteria are more likely to cause the numerous ocular infections that are associated with them (Teweldemedhin et al., 2017). While the antibacterial efficiency of each extracts against gram-negative bacteria was lower than that of the gram-positive bacteria, this may imply that the nature of bacterial membrane needs high concentration and retention time to segregate into the bacterial cell and induce the growth of the gram-negative bacteria against leaf extracts of *I. confertiflora*. Thus far, *I. confertiflora* has been utilized medicinally to treat a variety of illnesses due to the plant's preliminary chemicals, effective radical scavenging ability, and antibacterial activity in leaf extract.

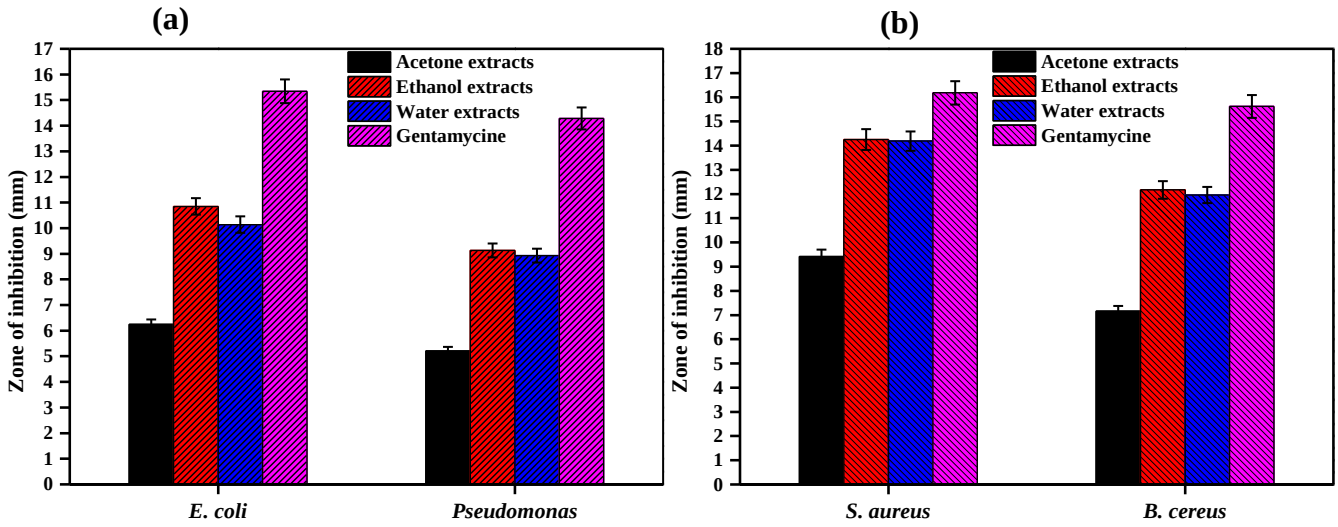


Figure 3. The antibacterial activity of each extract and gentamycin against (a) gram-negative and (b) gram-positive bacteria at a concentration of 200 µg/ml.

Conclusion

Acetone, ethanol, and water are three distinct polarity-index organic solvents that we have effectively soaked and extracted from the *I. confertiflora* leaf in order to acquire the necessary crude yields. Alkaloids, flavonoids, and phenolic compounds—preliminary compounds—that were extracted and soaked in acetone, ethanol, and water solvents are the most often detected ingredients in *I. confertiflora* leaves. Furthermore, the total flavonoid content of leaf extracted with ethanol was 1.63 times higher than the flavonoids extracted with acetone and 2.02 times higher than the flavonoid levels obtained with water extracts. This shows that the intermediate polar solvent of ethanol has a stronger potential to segregate into the powdered leaves of *I. confertiflora* over the course of 72 hours and extract higher phenols and their derivatives of flavonoids than do the lower polar solvent of acetone and the universal organic solvent of water. The DPPH assay revealed that ethanol extracts with higher phenolic and flavonoid contents demonstrated comparable percentage scavenging efficiency to extracts with higher polar organic solvents in water; this implies that the extracts contained precursor compounds that were probably going to donate hydrogen or free radicals to the DPPH solution. Furthermore, for the microbial activity against *Staphylococcus aureus* gram-positive bacterial strains and *E. coli* gram-negative bacterial strains, respectively, the antibacterial zone inhibiting efficiency (mm) of ethanol extracts was 1.41 times longer than that of acetone extracts and more than 1.47 times longer than that of acetone extracts. Over all, the leaf of *I. confertiflora* can be used medicinally to treat many bacterial and fungal-related disorders in animals since it contains precursor chemicals, has a higher phenolic content, has good antioxidant properties, and can limit the proliferation of bacterial strains.

Author Contribution

Abere Habtamu: created the project, designed the study, analyze and organized the obtained experimental data, conceptualization and edited the manuscript. **Esubalew Meku**: Wrote the first draft of the manuscript. **Sintayehu Leshe**: provided critical suggestions and evaluation on antioxidant activity results.

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Conflict of Interest
The authors declare no competing conflict of interest.

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References
Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E. M., Linder, T., Wawrosch, C., Uhrin, P., & Heiss, E. H. (2015). Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology advances*, *33*(8), 1582-1614.

Awulachew, M. T. (2021). Hand Book of Common Ethiopian Traditional Medicinal Plants: Their Parts and Uses for Human and Animal Treatments. *Journal of Diseases and Medicinal Plants*, *7*(3), 48-60.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature, 181*(4617), 1199-1200.

Bouarab-Chibane, L., Forquet, V., Lantéri, P., Clément, Y., Léonard-Akkari, L., Oulahal, N., . . . & Bordes, C. (2019). Antibacterial properties of polyphenols: characterization and QSAR (Quantitative structure–activity relationship) models. *Frontiers in Microbiology*, *10*, 440698.

Brand-Williams, W., Cuvelier, M.-E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, *28*(1), 25-30.

Chatatikun, M., & Chiabchalard, A. (2013). Phytochemical screening and free radical scavenging activities of orange baby carrot and carrot (Daucus carota Linn.) root crude extracts. *Journal of Chemical and Pharmaceutical Research, 5*(4), 97-102.

Cho, E., Yokozawa, T., Rhyu, D., Kim, S., Shibahara, N., & Park, J. (2003). Study on the inhibitory effects of Korean medicinal plants and their main compounds on the 1, 1-diphenyl-2-picrylhydrazyl radical. *Phytomedicine*, *10*(6-7), 544-551.

Clarke, E. G. C. (1975). *Isolation and Identification of Drugs in Pharmaceuticals, Body Fluids, and Post-mortem Material: Volume 2*: Pharmaceutical Press.

Gashu, M. (2022). Epifriedelanol and Dammara-20, 24-dien-3-yl Acetate from Ethiopian *Inula confertiflora* Root Extract. *Journal of Chemistry, 2022*(1), 2114595.

Gebre-Mariam, T., Neubert, R., Schmidt, P., Wutzler, P., & Schmidtke, M. (2006). Antiviral activities of some Ethiopian medicinal plants used for the treatment of dermatological disorders. *Journal of Ethnopharmacology, 104*(1-2), 182-187.

Inoue, M., Hayashi, S., & Craker, L. E. (2019). Role of medicinal and aromatic plants: Past, present, and future. *Pharmacognosy-medicinal plants*, 1-13.

Isah, T. (2019). Stress and defense responses in plant secondary metabolites production. *Biological research, 52*.

Kumar, S., Korra, T., Thakur, R., Arutselvan, R., Kashyap, A. S., Nehela, Y., Keswani, C. (2023). Role of Plant Secondary Metabolites in Defence and Transcriptional Regulation in Response to Biotic Stress. *Plant Stress*, 100154.

Moges, A., & Moges, Y. (2019). Ethiopian common medicinal plants: their parts and uses in traditional medicine-ecology and quality control. *Plant science-structure, anatomy and physiology in plants cultured in vivo and in vitro, 21*.

Njokua, D., Chidiebere, M., Oguzie, K., Ogukwe, C., & Oguzie, E. E. (2013). Corrosion inhibition of mild steel in hydrochloric acid solution by the leaf extract of Nicotiana tabacum. *Advances in Materials and Corrosion, 2*(1), 54-61.

Parbuntari, H., Etika, S. B., Mulia, M., & Delvia, E. (2019). A Preliminary Screening of the Different of Secondary Metabolites Ruku-Ruku Leaves (Ocimum tenuiflorum Linnen) in West Sumatera. *Eksakta: Berkala Ilmiah Bidang MIPA, 20*(2), 17-24.

Peter, A. E., Sudhakar, P., Sandeep, B., & Rao, B. G. (2019). Antimicrobial and anti-quorum sensing activities of medicinal plants. *Implication of Quorum Sensing and Biofilm Formation in Medicine, Agriculture and Food Industry*, 189-217.

Pisithkul, T., Schroeder, J. W., Trujillo, E. A., Yeesin, P., Stevenson, D. M., Chaiamarit, T., Amador-Noguez, D. (2019). Metabolic remodeling during biofilm development of *Bacillus subtilis*. *MBio, 10*(3), 10.1128/mbio. 00623-00619.

Rahman, M. M., Rahaman, M. S., Islam, M. R., Rahman, F., Mithi, F. M., Alqahtani, T., Hossain, M. S. (2021). Role of phenolic compounds in human disease: current knowledge and future prospects. *Molecules, 27*(1), 233.

Reeds, K. M. (1976). Renaissance humanism and botany. *Annals of science, 33*(6), 519-542.

Richards, R. J. (2019). *Was Hitler a Darwinian? Disputed questions in the history of evolutionary theory*: University of Chicago Press.

Robinson, M. M., & Zhang, X. (2011). Traditional medicines: global situation, issues and challenges. *The world medicines situation, 3*.

Roesler, R., Malta, L. G., Carrasco, L. C., & Pastore, G. (2006). Evaluation of the antioxidant properties of the Brazilian Cerrado fruit Annona crassiflora (Araticum). *Journal of Food Science, 71*(2), C102-C107.

Sandip, P., Shreewardhan, R., Sandeephan, M., Abhay, C., & Debjani, D. (2014). Phytochemical evaluation and free radical scavenging potential of Hugonia Mystax (L.) leaf extract. *Bionano Front, 7*, 20.

Seca, A. M., Grigore, A., Pinto, D. C., & Silva, A. M. (2014). The genus *Inula* and their metabolites: From ethnopharmacological to medicinal uses. *Journal of Ethnopharmacology, 154*(2), 286-310.

Seca, A. M., Pinto, D. C., & Silva, A. M. (2015). Metabolomic profile of the genus *Inula*. *Chemistry & biodiversity, 12*(6), 859-906.

Tavares, W. R., & Seca, A. M. (2019). *Inula* L. secondary metabolites against oxidative stress-related human diseases. *Antioxidants, 8*(5), 122.

Teweldemedhin, M., Gebreyesus, H., Atsbaha, A. H., Asgedom, S. W., & Saravanan, M. (2017). Bacterial profile of ocular infections: a systematic review. *BMC ophthalmology, 17*, 1-9.

Yang, L., Wen, K.-S., Ruan, X., Zhao, Y.-X., Wei, F., & Wang, Q. (2018). Response of plant secondary metabolites to environmental factors. *Molecules, 23*(4), 762.

Yeshiwas, Y., Tadele, E., & Tiruneh, W. (2019). The dynamics of medicinal plants utilization practice nexus its health and economic role in Ethiopia: a review paper. *International Journal of biodiversity and conservation, 11*(1), 31-47.