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Research Article

Invitro Antibacterial, Antioxidant and XRF Analysis of *Commelina Diffusa Burm.F.* Plant Extracts

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Abstract

Ethiopia has a long history of using medicinal herbs for treating both human and animal illnesses. Nonetheless, not enough research has been done on the antibacterial properties and possible bioactive components of the majority of medicinal plants. Therefore, this study deals with the evaluation of phytochemical, antimicrobial, antioxidant activities, phenol content and XRF analysis of *Commelina Diffusa Burm.F.* plant extracts. Mean values of the antimicrobial activity, MIC, antioxidant activities, phenol content and XRF analysis were reported as mean ± standard deviation. The chloroform leaf extracts of the plant gave the highest yield 23.4% followed by methanol 22.27%. The presence of several metabolite components, including alkaloids, diterpenes, flavonoids, glycosides, phenol, protein, saponin, steroids, tannins, terpenoids, tri-terpenoids and amino acids, has been shown by qualitative phytochemical analysis of plant parts. Significant antibacterial activity against the test bacterial strains was demonstrated by steam extracts of *Commelina Diffusa Burm.F.* Moreover, the methanolic extract of the plant demonstrated notable antioxidant activity. The highest value of phenolic content was obtained in *Commelina Diffusa Burm.F.* steam methanol extract followed by leaf extract while *Commelina Diffusa Burm.F.* root extract shows lower phenolic content. In this study, threaten elements were determined in the *Commelina Diffusa Burm.F.* plant part by using XRF spectroscopy. Overall, this research contributes to the understanding of pharmacological potential of *Commelina Diffusa Burm.F.* and highlights the importance of further exploring its medicinal properties. The findings provide valuable insights into utilizing medicinal plants for disease treatment and support the development of natural therapeutic agents.

Keywords

Antibacterial Activity, Antioxidant Activity, XRF

1. Introduction

Since ancient times, people have used plants for medical purposes; these plants are known as medicinal plants. In another way, plants that are employed in herbology are included in the term "medicinal plant." 80% of the world's population receives their primary medical treatment from herbal remedies, according to the World Health Organization (WHO). It also declared that around 21,000 plant species have the potential of being medicinally used [1].

Because microorganisms are rapidly developing multidrug resistance to the current antimicrobial agent, the huge increase

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in the frequency of infectious diseases has been a serious worry in recent years [2]. As a result, academia, pharmaceutical corporations, and research institutes around the world are facing a difficulty in their search for novel antibiotic sources [3].

The main benefit of plants is that they continue to be the most efficient and affordable alternative source of natural products [4]. Due to their pharmacological qualities, herbal medicine the use of plants for medical purposes has been used alongside conventional medicine in Asia, Latin America, Africa and Latin America. There is also current analysis and research on the uses of plants for many diseases, both in prevention and treatment [5].

Commelina Diffusa Burma F. is a member of the *Commelinaceae* family. It's also referred to as spreading dayflower or climbing dayflower. The weeds are commonly found in many crop fileds during June to September [6]. Additionally, this herb is found in Bangladesh and other South Asian nations. It is used in traditional Chinese medicine to reduce inflammation and swelling. It is frequently used to treat urinary tract infections, coughs with sticky phlegm, diarrhea, hemorrhoids, enteritis, eye irritation, conjunctivitis and other eye conditions like ophthalmia in various Asian, African and American nations [7].

Different studies have been reported to extract plant secondary metabolite with various solvents for antimicrobial properties (ethanol, methanol, petroleum ether, chloroform, hexane, ethylacetate, dichloromethane and water) [8]. The use of natural products for antimicrobial activity screening has been the focus of numerous investigations. Therefore, the main aim of this study is to assess phytochemical, antibacterial activities, minimum inhibitory concetration (MIC), antioxidant activities, phenol content and XRF analysis of *Commelina Diffusa Burm.F.* plant extracts against selected test organisms.

2. Materials and Methods

2.1. Collection and Identification of Plant Material

Fresh whole parts of *Commelina Diffusa Burm.F.* (i.e. leaf, stem and root) used for this experiment were collected from natural habitats of Yetnebershi which is found in Arba Minch, in June 2021. Identification of plant sample was authenticated at Arba Minch University, College of Computational and Natural Sciences, Department of Biology.

2.2. Preparation of Plant for Extraction

The plant materials used to prepare the extracts were properly cleaned with distilled water to get rid of any extraneous debris and then they were dried in the shade. The dried plant material was powdered using grinder. 20 g of powdered plant materials (i.e. leaf, stem and root) were extracted successively with 100 ml of different solvents (distllid water, methanol, n-hexane and chloroform). After the extracts were carefully evaporated, the resulting pasty was placed in a sterile plastic container for storage.

2.3. Filitration, Evaporation and Yield of Extratcts

Whatman No. 1 filter paper was used to filter the extracts, which were then concentrated using a rotary evaporator. Dry weight was used to calculate the percentage yield using the fellowing formula.

$$\%\text{Yield of extract } \left(\frac{g}{100}\text{g}\right) = \frac{\text{Weight of the extract residue after solvent removal}}{\text{Weight of dried plant powder}} \text{X}100$$

Finally, the extracts were preserved and kept at 5 ^{o}C in a refrigerator until they were used.

2.4. Phytochemical Analysis

The extracts were evaluated for the presence of alkaloids, anthraquinones, diterpenes, flavonoids, glycosides, phenol, protein, saponin, steroids, tannins, terpenoids, tri-terpenoids, reducing sugar and amino acid based on the methods available in the literature [9-11].

2.4.1. Test for Alkaloids

The extract of each solvent's crude dry leaf powder was evaporated to dryness in a boiling water bath. Residues were dissolved in 2 N HCl. The mixture was filtered and the filtrate was separated into three equal portions. One portion was treated with a few drops of Mayer's reagent, one with an equivalent amount of Dragondroff's reagent while the third with an equal amount of Wagner's reagent. The appearance of creamish precipitate, orange precipitate and brown precipitate showed the existence of respective alkaloid [12].

2.4.2. Test for Anthraquinones

In a dry test tube, 0.5 g of extract was mixed with 5 ml of chloroform and agitated for 5 min. The extract was filtered, then shaken with an equal volume of 10% ammonia solution. The presence of anthraquinones is indicated by a pink, violet, or red color in the ammonical layer [13].

2.4.3. Test for Diterpenes

2 ml of extracts were mixed with 2 ml of distilled water and treated with 3-4 drops of copper acetate solution. The formation of emerald green suggests the presence of diterpenes.

2.4.4. Test for Flavonoids

1.5 ml of a 50% methanol solution was added to 2 ml of extract. The solution was heated and metal magnesium was

added. A few drops of concentrated HCl were added to this solution and flavonoids appeared red, while flavones appeared orange [13].

2.4.5. Test for Cardiac Glycosides

0.2 g of extract was mixed in 1 ml of glacial acetic acid containing one drop of ferric chloride. This was then coated with 1 ml of H_2SO_4. A brown ring at the contact suggested the presence of a deoxysugar characteristic of cardioids. [13].

2.4.6. Test for Phenol

500 mg of each extract was mixed with 5 ml of distilled water. A few drops of neutral 5% ferric chloride solution were added. A dark green colour suggested the presence of phenolic compounds.

2.4.7. Test for Protien, Amino Acids and Reducing Sugar

To 2ml of protein solution 1ml of 40% NaOH solution was mixed with 1–2 drops of 1% $CuSO_4$ solution. A violet color showed the presence of peptide linkage in the molecule [13].

2 ml of the sample was mixed with 2 ml of Ninhydrin reagent and placed in a water bath for 20 minutes. The development of the purple color showed the presence of amino acids in the sample [13].

2 drops of Molisch's reagent were added to 2 ml of extract and thoroughly shaken. 2 ml of concentrated H_2SO_4 was added to the sidewalls of the test tube. A reddish-violet ring emerged at the intersection of two layers, which quickly revealed the existence of carbohydrates [13].

2.4.8. Test for Saponins

In a test tube, 0.5 g of sample was vigorously agitated with water before being heated to boiling. Frothing was noticed, which was interpreted as preliminary proof for the existence of saponins [14].

2.4.9. Test for Steroids

2 ml of acetic anhydride was added to 2 ml of each sample's plant extract, along with 2 ml H_2SO_4. The color shifted from violet to blue or green, indicating the presence of steroids [14].

2.4.10. Test for Tannins, Terpenoids and Tri-terpenoids

In a test tube, 0.5 g of plant extract was mixed with 10 ml of water and then filtered. A few drops of 0.1% ferric chloride were applied and looked for brownish green or blue-black coloration [15].

Terpenoids were detected via mixing 3 ml of extract with 2 ml of $CHCl_3$ in a test tube. Then, 2 ml of concentrated H_2SO_4 was carefully added to create a ring layer that interfaced with a reddish-brown color, suggesting the presence of terpenoids.

5 ml of each extract was mixed with 2 ml of chloroform and 3 ml of concentrated H_2SO_4 to form a monolayer of reddish-brown coloring of the interface was demonstrated to produce positive results for the tri-terpenoids [13].

2.5. Evaluation of Antibacterial Activities

2.5.1. Inoculum Preparation and Preparation of Test Solutions

The studied bacteria were cultured individually on sterilized Muller-Hinton Agar (MHA) at 37 ℃ for 24 hrs using the streak plate method. Then, well-isolated overnight cultured colonies of the same morphological type were chosen from the culture media. The growth of each colony was transferred to a sterile test tube containing 5 ml of sterile normal saline after touching each colony with a flamed wire loop. The bacterial suspension was mixed thoroughly and evenly in the test tubes by vortexing. Next, the bacterial suspension was standardized using 0.5 McFarland turbidity standards. Adjustment and comparison of the turbidity of the inoculum tubes were done by visual observation with the naked eye against a 0.5 McFarland turbidity equivalent standard with a white background and contrasting blue lines under adequate illumination.

2.5.2. Tested Microorganisms

A total of two bacterial were used in this experiment for zone of inhibition and Minimum Inhibitory Concentration (MIC) assays. The bacteria were obtained from Ethiopian Public Health Institute (EPHI), Addis Abeba, Ethiopia. The bacterial strains under this study include *Escherichia coli* (E. *coli*) (ATCC25922) and *Staphylococcus aureus* (S. *aureus*) (ATCC25923). Bacterial isolates were maintained at 2 and 8 ℃ on nutrient broth.

2.5.3. Assay of Antibacterial Activities by Agarwell Diffusion Method

The results of the agar well diffusion method are presented as the size of the inhibition zone created by the plant extract [16]. Bacterial strains were tested against plant extracts. Using a well cutter, a 5 mm well was created in plates blotted with culture strains for the agar well diffusion method. 5 µl of plant extracts were then added to the well using a micropipette. As a control, the well was carefully filled with 5% DMSO (Dimethyl Sulfoxide). Then plates were incubated at 37 ℃ for 24 hr. After the incubation period the zone of inhibition was measured. A well was prepared in the plates with the help of a cork-borer (5mm) [17].

2.6. Determination of Minimum Inhibitory Concentration (MIC)

All crude extracts were tested for their minimum inhibitory concentration (MIC) against E. *coli* and S. *aureus*. Crude

plant extracts were diluted using 5% DMSO. The crude extract (2 ml) was then combined with melted MHA (18 ml) and put into sterilized Petri dishes following a series of dilutions. The plate was inoculated with the standardized (0.5 McFarland standard) bacterial inoculum and incubated at 37 ℃ for 24 hrs. The result of bacterial inhibition was assessed by comparison with the growth in positive and negative controls [18].

2.7. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

DPPH radical scavenger from *Commelina Diffusa Burm.F.* extracts were estimated using a previously published method [19]. 2 ml of DPPH solution in methanol (0.004%, 0.102 mM) was combined with 2 ml of extracts of different concentrations (200–800 mg/l). For the blank solution, the extracts were replaced with methanol and used to correct the baseline at 515 nm. The tubes were left at room temperature for 20 min. The antiradical activity was based on measuring the reducing ability of the plant extract towards DPPH radicals. Ascorbic acid was used as a standard in the range of (25–100 mg/l) and the scavenging activity of the leaf extracts was determined using a linear curve of the ascorbic acid standard.

2.8. Determination of Total Phenol Content

Total phenolic content was determined using the Folin-Ciocalteau reagent method with some modifications. 1 mg of each crude extract was dissolved in 1 ml of methanol. By mixing 10 ml of Folin-Ciocalteau reagent with 90 ml of distilled water, a total of 10% of the Folin-Ciocalteau reagent was prepared. Then, 3 g of Na_2CO_3 was dissolved in 50 ml of water to produce 5% Na_2CO_3. 200 µl of each crude extract was placed in a test tube and 1.5 ml of 10% Folin-Ciocalteau reagent was added. Afterwards, each test tube was placed in a dark place for 5 min. Finally, 1.5 ml of 5% Na_2CO_3 was added to the solutions and they were mixed thoroughly by hand. Once again, each test tube was kept in the dark for 2 hrs. Absorbance was measured for all solutions using a UV spectrophotometer at a constant wavelength of 750 nm. Gallic acid was utilized as a standard in the range of (2.5-100 mg/l), and the phenol content of the plant extracts were evaluated using a linear curve of the gallic acid standard [20].

2.9. X-ray Fluorescence Spectroscopy (XRF) Analysis

According to the study of Annalakshmi et al., the XRF analyses were performed using 100 g of the plant sample [21]. The XRF measuring system included a multichannel analyzer ORTECR, a semiconductor detector Si/Li (thickness of beryllium window = 0.25 mm, diameter of beryllium window = 5 mm) and an Amersham radionuclide source of radiation 238Pu (A = 370 MBq, E = 12 - 22 keV, T. = 86.4 years) in the form of a planar disk. Each measurement were taken in a non-coaxial geometrical arrangement of source, sample and detector, with an acquisition duration of 2000 seconds.

2.10. Data Analysis

Experiments were conducted in triplicates to reduce error and data were reported as mean ± SD (n = 3). The assay findings were statistically analyzed using Microsoft Excel.

3. Result and Discussion

3.1. Percentage Yield

The solvent n-hexane showed a higher degree of yield in root extract which is 0.59 g (2.96%) while distilled water extract of root showed less degree which is 0.22 g (1.10%) as shown in Table 1. The solvent chloroform showed a high degree of yield in stem extract which is 3.67 g (18.35%) while distilled water showed less degree which is 0.54g (2.7%) as shown in Table 1. The solvent chloroform showed a high degree of yield in leaf extract which is 4.68g (23.4%) while distilled water showed less degree which is 0.42 g (2.1%) as illustrated in Table 1.

Table 1. *Extraction yield of Commelina Diffusa Burm.F. plant extracted with different solvents.*

Plant name	Parts used	Solvent	Weight of extract obtained (g)	Yield percentage %
Commelina Diffusa Burm.F.	Root	Methanol	0.56	2.81
		Chloroform	0.49	2.46
		n-hexane	0.59	2.96
		Distill Water	0.22	1.10
Commelina Diffusa Burm.F.	Stem	Methanol	2.71	13.57
		Chloroform	3.67	18.35

Plant name	Parts used	Solvent	Weight of extract obtained (g)	Yield percentage %
Commelina Diffusa Burm.F.	Leaf	n-hexane	0.82	4.11
		Distill Water	0.54	2.7
		Methanol	4.45	22.27
		Chloroform	4.68	23.4
		n-hexane	3.85	19.26
		Distill Water	0.42	2.1

The highest yield was obtained by chloroform, while distilled water was the lowest yield value as compared with the other solvents in both plant extracts. variations in extraction yield are caused by the polarity of different compounds contained in the plant; these variations have been described in the literature concerning Vietnamese medicinal herbs [22]. The choice of solvent is critical for generating extracts with acceptable yields and high antibacterial activity. The extraction of crude antimicrobial agent for this investigation was assayed by different solvents those are methanol, chloroform, n-hexane and distilled water used.

3.2. Phytochemical Studies

The phytochemical study shows a remarkable presence of alkaloids and flavonoids in the root methanol extract, while alkaloid, diterpenes, flavonoid, protein, steroids and amino acids were present in the stem methanolic extract and the leaves methanolic extract indicated the presence of glycosides, protein, steroids, tannins, triterpenoids, amino acids and reducing sugar. A photochemical analysis revealed a significant presence of triterpenoids in the n-hexane extract of the root, while diterpenes, phenol and triterpenoids were found in the n-hexane extract of the stem and the n-hexane extract of the leaves indicated the presence of diterpenes, glycosides, phenol and triterpenoids. Similarly, the photochemical study reveals a remarkable presence of alkaloid, diterpenes, glycosides and phenol in ethyl acetate extratct of root while alkaloid, diterpenes, glycosides and phenol were present in ethyl acetate extract of stem. And the ethyl acetate extract of leaves indicates the presence of alkaloid, diterpenes and tannins. The phytochemical content of each extracts are presented in Table 2.

Table 2. *Preliminary phytochemical compounds in Commelina diffusa Burm. F. plant from different extracts.*

Phytochemical Analysis	Test	Methanol extract			n-hexane extract			Ethyl acetate extract		
		Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
Alkaloids	Mayer's	+	+	_	_	_	_	+	+	+
	Dragondroff's	+	+	_	_	_	_	+	+	+
	Wagner's	+	+	_	_	_	_	+	+	+
Anthraqunies	Free and combined anthraquinones	_	_	_	_	_	_	_	_	_
Diterpenes	General test	_	+	_ _	_	+	+	+	+	+
Flavonoids	Lead acetate	+	+	_	_	_	_	_	_	_
	Sodium hydroxide	+	+	_	_	_	_	_	_	_
Glycosides	Keller-Killani	_	_	+	_	_	+	_	+	_
Phenol	General Test	_	_	_	_	+	+	_	+	_
Protein	Ninhydrin	_	+	+		_	_	_	_	_
Saponin	Frothing	_	_	_	_	_	_	_	_	_
Steroids	Salkowski's	_	+	+	_	_	_	_	_	_

Phytochemical Analysis	Test	Methanol extract			n-hexane extract			Ethyl acetate extract		
		Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
Tannins	Ferric chloride	_	_	+	_	_	_	_	_	+
Terpenoids	Salkowski	_	_	_	_	_	_	_	_	_
Tri- Terpenoids	General Test	_	_	+	+	+	+	_	_	_
amino acid	General Test	_	+	+	_	_	_	_	_	_
Reducing sugar	General Test	_	_	+	_	_	_	_	_	_

+ = Present and - = Absent

The photochemical substances identified are thought to have therapeutic properties. For example, alkaloids are known to exist as strong poisons and many alkaloids derived from medicinal plants exhibit biological activity such as anti-inflammatory [23], anti-malaria [24], anti-microbial [25], cytotoxicity, antispasmodic, and pharmaceutical effects [26]. Similarly, plant-derived steroids are known to have cardiotonic, antimicrobial, and insecticidal activities. They are frequently used in therapeutics due to their well-known biological properties. According to study, tannins exhibit bactericidal [27], anticancer and antiviral properties. Other phytochemicals, known as cardiac glycosides, have been utilized to treat congestive heart failure and cardiac arrhythmias [28]. These phytochemical substances found in root, leaf, and root extracts may be responsible for *Commelina Diffusa Burm.F.*'s biological activities and its usage as traditional medicine.

3.3. Antibacterial Activities of Plant Extracts

The antibacterial activity of *Commelina Diffusa Burm.F.* whole plant extracts were tested against pathogenic bacteria and the presence and absence of inhibition zone on the agar well plates was observed.

In this try, two strains of pathogenic bacteria *E.coli ATCC25922* and *S. aureus ATCC25923* were used to evaluate the antibacterial effects of *Commelina Diffusa Burm.F.* root, stem and leaf crude extract. As presented in Table 3, The crude extract of a medicinal plant had strong inhibitory effects on all of the harmful microorganisms examined. The majority of the extract exerted consistent antibacterial effects against *E.coli ATCC25922* and *S. aureus ATCC25923* with no zone of inhibition in root extract, 18 to 25 mm insteam extract and 15.2 to 17 mm in leaf extract Table 3. The negative control of DMSO used in the study showed no inhibitory effect.The results revealed that both stem and leaf methanol extracts followed by chloroform solvent werePotentially effective in suppressing microbial growth of the two bacteria with variable potency. Stem ethanol extract was the most effective extract retarding microbial growth on the tested bacteria. While the tested bacteria were resistant to root extract.

Table 3. *Antimicrobial screeing test of plant extract against selected bacterial strains.*

		Mean values[a] of inhibition zone (mm)	
Plant part	**Solvents**	**Inhibition zone of *S. auras***	**Inhibition zone of *E. coli***
Root	Methanol	ND[c]	-[b]
	Chloroform	ND	-
	n-hexane	-	ND
	Distill Water	-	-
Steam	Methanol	25	22
	Chloroform	23	19.5
	n-hexane	18	19
	Distill Water	-	-
Leaf	Methanol	16	15.5

Plant part	Solvents	Mean values[a] of inhibition zone (mm) Inhibition zone of *S. auras*	Inhibition zone of *E. coli*
	Chloroform	15.2	15.9
	n-hexane	17	16.5
	Distill Water	-	-

a = Mean values from triplicates, _ b= No inhibition zone and c = Not determined

The difference in antibacterial activity of a plant extract may be due to the age of the plant employed, the freshness of the plant materials, physical conditions (temperature, light-water), the time of harvesting the plant materials, and the drying method used prior to the extraction process [29]. The antibacterial assessment of the plant portion done in the study yielded varying diameters of inhibition zones with both solvents against Gram-positive and Gram-negative bacteria. For instance, methanol extracts had a larger zone of inhibition (antibacterial activity) than the other extracts. [30] reported the same observation in Ethiopia. This corresponds with the extraction efficiency of methanol in liberating the majority of biologically active phytochemical substances, which is consistent with the theories of [31, 32].

3.4. Minimum Inhibitory Concentration of Plant Extract

The MIC test was performed by serial dilution of crude plant extract with 5% DMSO. The MIC was estimated for extracts with a growth inhibition zone diameter of 6 mm or more at 25 mg/ml. The crude extracts of methanol, chloroform, n-hexane and distilled water were applied to reference bacterial strains at concentrations ranging from 1.101-6.251 mg/ml to 12.5 mg/ml and the findings are presented in Table 4. In comparison to the bacterial strains, *S.aureus* demonstrated better antibacterial activity at lower doses, while *E. coli* were inhibited at higher concentrations in both crude plant extracts.

Table 4. *MIC of crude plant extracts against selected bacterial strains (mg/ml).*

Plant parts	Solvents	MIC of crude extracts (mg/ml) a S. *auras*	E. *coli*
Root	Methanol	ND[c]	-[b]
	Chloroform	ND	-
	n-hexane	-	ND
	Distill Water	-	-
Steam	Methanol	2.109	2.234
	Chloroform	1.101	1.401
	n-hexane	1.098	1.539
	Distill Water	-	-
Leaf	Methanol	4.562	6.251
	Chloroform	2.914	3.125
	n-hexane	1.243	1.625
	Distill Water	-	-

a= Mean values from triplicates, _b= No MIC and c= Not determined

The MIC assessment demonstrated that different minimum concentrations of the crude extract were obtained in the plant portions, which may inhibit the growth of the reference bacteria. The n-hexane leaf extract of *S. aureus* has the lowest

MIC value against *E. coli*. In contrast, the higher concentration of crude extracts inhibited the growth of *S. aureus*, a G-positive bacteria. The MIC value is consistent with this plant's antibacterial activity against the test bacterium.

3.5. Antioxidant Activity of Plant Extracts

The plant extracts' DPPH radical scavenging activities were assessed by comparing the DPPH's percentage scavenging activity to a standard, ascorbic acid (Figure 1). In this investigation, DPPH, a stable free radical with a distinctive absorption at 515nm, was used to investigate the radical-scavenging effects. Ascorbic acid has been used as a standard since it is known to be a powerful antioxidant due to its ability to scavenge free radicals and bind transition metal ions.

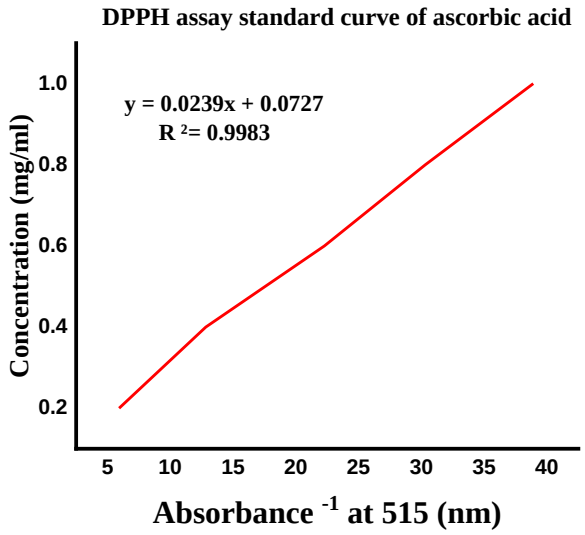

Figure 1. *Standard curve of ascorbic acid.*

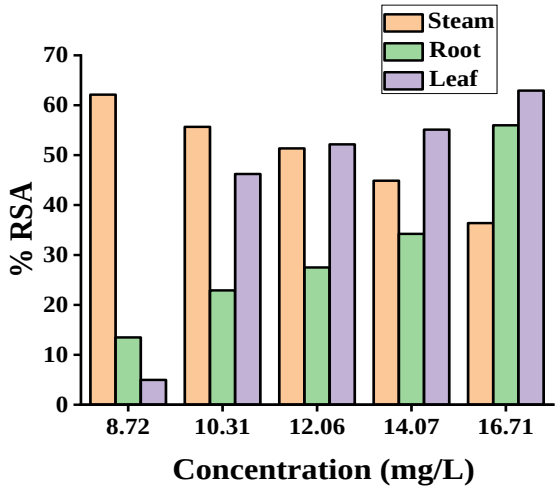

Figure 2. *Antioxidant activities of Commelina Diffusa Burm.F. plant.*

The findings of DPPH free radical scavenging were shown in Figure 2. The reduction in absorbance was used to determine the degree of radical scavenging. The radical-scavenging activity values were calculated as the percentage of sample absorbance decrease versus the absorbance of DPPH solution in the absence of extract at 515 nm. *Commelina Diffusa Burm.F.* plant parts were shown to inhibit the DPPH free radical scavenging activity with an IC_{50} value as indicated in Figure 2. This suggests it has a high level of antioxidant activity in terms of DPPH free radical scavenging.

In general, among all the plant extracts, the crude extracts obtained from *Commelina Diffusa Burm.F.* steam extracts showed the best antioxidant activity on the other hand, *Commelina Diffusa Burm.F.* leaf and root extract shows lower antioxidant activities respectively. The inhibition effect of different concentration of the plant extract and ascorbic acid in scavenging of DPPH was evaluated. The IC_{50} value of the plant extracts were presented in Figure 3.

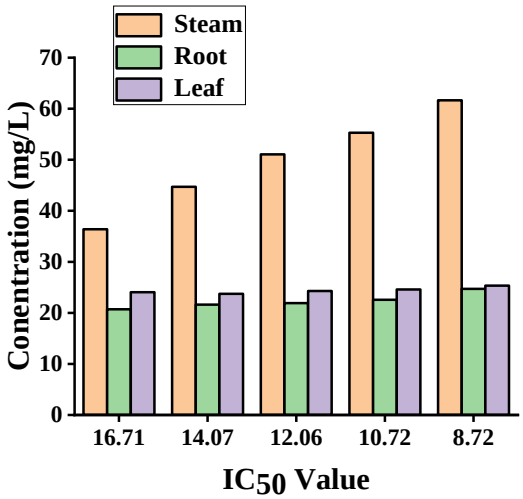

Figure 3. *IC_{50} values of Commelina Diffusa Burm.F. plant extracts.*

In general, among all the plant extracts, the crude extracts obtained from *Commelina Diffusa Burm.F.* steam extracts showed the highest IC_{50} on the other hand *Commelina Diffusa Burm.F.* leaf and root crude extract shows lower IC_{50} respectively.

3.6. Total Phenolic Content of Plant Extracts

The phenolic content of *Commelina Diffusa Burm.F.* plant extracts has been determined using the regression equation of the gallic acid calibration curve (Figure 4) and reported as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g).

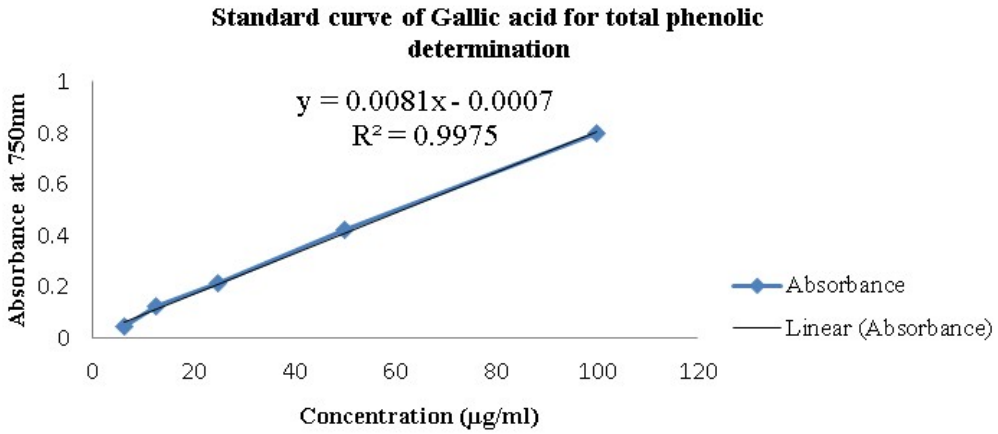

Figure 4. *Total phenolic content for standard of gallic acid, values expressed in tems of gallic acid.*

The total phenolic content calculated in this study is illustrated in Table 5. The highest value of phenolic content was obtained in *Commelina Diffusa Burm.F.* steam methanol extract followed by leaf extract while *Commelina Diffusa Burm.F.* root methanol extract shows lower phenolic content Table 5.

Table 5. *Total phenolic content (mg GAE/g) of methanol extract of Commelina Diffusa Burm.F.*

Extracts	Parts used	Total phenolic content (mg GvE/g)
Methanol	Root	53.6
Methanol	Stem	61.9
Methanol	Leaf	53.6

Phenolic compounds are renowned as potent chain-breaking antioxidants, which may directly contribute to antioxidative activity [33, 34]. These phenolic compounds contribute to antioxidant action because the position of functional groups (hydroxyl) in their nuclear structure allows for hydrogen donation to stabilize radical molecules [35].

3.7. X-ray Fluorescence Spectroscopy (XRF) Analysis of *Commelina Diffusa Burm.F.* Plant Part

In this study, the concentration of thirteen element were determined in the *Commelina Diffusa Burm.F.* plant by using XRF spectroscopy. The result showed various concentration and available percentages of one macro element: Ca.five microelements (trace elements): Sr, W, Zr, Mo and Rb, six heavy elements: Fe, Zn, Cu, V and Uin the root of *Commelina Diffusa Burm.F.* given in Table 1. Similarly two macro elements: K and Ca. five microelements (trace elements): Sr, W, Zr, Mo and Rb, six heavy elements: Fe, Zn, Cu, V and U in steam and leaf of *Commelina Diffusa Burm.F.* Table 6.

Table 6. *Evaluation of elemental content of Commelina Diffusa Burm.F. plant by XRF.*

S. No	Root		Steam		Leaf	
	Element	Con (ppm)	Element	Con (ppm)	Element	Con (ppm)
1	Fe	544.59	Fe	4767.55	Ca	419.13
2	Ca	218.35	Ca	383.87	Fe	273.41
3	Cr	147.63	K	216.95	K	168.58
4	Zn	84.75	Cr	177.54	Cr	155.53
5	Cu	42.52	Zn	121.32	Zn	82.09
6	Sr	31.68	Zr	52.47	Sr	38.59
7	V	29.83	Cu	49.46	Cu	37.64
8	W	28.39	W	44.39	V	32.38

S. No	Root		Steam		Leaf	
	Element	Con (ppm)	Element	Con (ppm)	Element	Con (ppm)
9	Zr	15.23	Sr	39.07	W	27.81
10	Mo	7.69	V	31.26	Zr	14.3
11	Rb	7.13	Rb	9.99	Mo	9.08
12	U	4.74	Mo	8.23	Rb	6.96
13	K	<LOD	U	7.84	U	4.57

The concentration of these elements was high in the stem than leaf and root. The mineral composition of the *Commelina Diffusa Burm.F.* plant demonstrated the presence of all mineral elements; the interaction of trace mineral compositions found in medicinal plants is critical to understanding their actions in the human body.

4. Conclusion

The extraction process and solvent used have a significant impact on extract yield and bioactivity. A comparison research was done to evaluate the percentage yield of *Commelina Diffusa Burm.F.* plant extract with four different solvents among those solvents methanol yields high compared to other solvent in root and stem of *Commelina Diffusa Burm.F.* plant part and chloroform was better for *Commelina Diffusa Burm.F.* steam part than other solvents. *Commelina Diffusa Burm.F.* is used in traditional medicine to combact and cure diseases and it is found to be rich in secondary metabolites. The presence of secondary metabolites may be attributed to their curative properties. Based on the antibacterial assay used in this work, *S. aureus* was found to be more susceptible to the plant extracts used than *E. coli*. The MICs of all plant extracts were determined against *E. coli* and *S. aureus*. Methanol extracts shown higher antibacterial and MIC activity with antibiotics compared to other extracts. The study's findings revealed that methanol extracts prepared using maceration techniques of *Commelina Diffusa Burm.F.* stem plant parts exhibited high antioxidant activity and phenolic content, as well as a number of macro elements, micro elements (trace elements) and heavy metals.

Abbreviations

DMSO	Dimethyl Sulfoxide
DPPH	2,2-Diphenyl-1-picrylhydrazyl
MIC	Minimum Inhibitory Concentration
XRF	X-ray Fluorescence Spectroscopy

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Author Contributions

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Data Availability Statement

All data and materials are mentioned in the paper.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Akerele, O. Nature's medicine bounty: don't throw it away. World Health Forum. 14(4) (1993) 390-5.

[2] Nirosha, N. & Mangalanayi, R. Antibacterial activity of leaves and stem extract of *Caricapapaya* L. *International Journal of Advances in Pharmacy Biology and Chemistry,* 2(3) (2013) 473-476.

[3] Doughari, J. Studies on the antibacterial activity of root extracts of *Carica papaya* Linn. *Africa Journal of Microbial Research*, 14(2007) 037-041.

[4] Pretorius, C. & Watt, E. "Purification and identification of active components of *Carpobrotusedulis* L. *Journal of Ethropharm*, 76 2001) 87-91.

[5] Odoo, A. Phytochemical characterization and comparative Efficacies of crude extracts of *Carica papaya*. *International Journal of Drug Research Tech*nology, 2(5) (2012) 399-406.

[6] Karthikeyan, A, Sudan, I. & Satheeshkumar, R. In vitro regeneration of *Commelina* diffusa Burm. F. Using nodal explants. *World Journal of Pharmaceutical Research*, 7(3) (2018) 978-986.

[7] Kumari, P., Kumari, C. & Singh, P. Phytochemical Screening of Selected Medicinal Plants for Secondary Metabolites. *International Journal of Life Sciences Scientific Research*, 3(4) (2017) 1151-1157. https://doi.org/10.21276/ijlssr.2017.3.4.9

[8] Eloff, J. A Sensitive and Quick Microplate Method to Determine the Minimal Inhibitory Concentration of Plant Extracts for Bacteria. *PlantaMedica*, 64(1998) 711-713. https://doi.org/10.1055/s-2006-957563

[9] Adetuyi, A. & Popoola, A. *Journal of Science, Engineering and Technology*, 8(2) (2001) 3291-3299.

[10] Trease, G. & Evans, W. Pharmacognosy 11thEdn. Brailliar Tiridacanb Macmillian Publishers. (1989).

[11] Sofowora, A. Medicinal Plants and Traditional Medicine in West Arica, John Wily and Sons. New York. (1982) 256.

[12] Salehi-Surmaghi, M., Aynehchi, Y., Amin, GH. & Mahhmoodi, Z. DARU, 2(1992) 1-11.

[13] Siddiqui, A. & Ali, M. Practical pharmaceutical chemistry.1stedition. C B S Publishers and Distributors, New Delhi. (1997) 126-131.

[14] Sathish, M., Selvakumar, S., Rao, M. & Anbuselv, S. Preliminary phytochemical analysis of Dodonaeaviscosaleaves. *Asian Journal of Plant Science and Research*, 3(1) (2013) 43-46.

[15] Segelman, AB, Fransworth, NR & Quimbi, MD. Lloydia, 32 (1969) 52-58.

[16] Eloff, J. Which extractant should be used for the screening and isolation of antimicrobial components from plants? *Ethanopharmacol*, 60 (1998) 1-8. https://doi.org/10.1016/s0378-8741(97)00123-2

[17] Perez, C., Pauli, M. & Bazerque, P. An antibiotic assay by agar well-diffusion method. *Acta Biologia et Medicine Experimentaalis*, 15(1990) 113-115.

[18] Wayne, P. Performance standards for antimicrobial susceptibility testing. (2001). *Clinical Microbiology Newsletter*, 23(6), 49. https://doi.org/10.1016/s0196-4399(01)88009-0

[19] Irshad, M., Zafaryab, M., Singh, M., Moshahid, M. & Rizvi, A. Comparative analysis of the antioxidant activity of Cassia stula extracts. *International Journal of Medicinal Chemistry*, (2012) 1-6. https://doi.org/10.1155/2012/157125

[20] Kaur, C. & Kapoor H. Anti-oxidant activity and total phenolic content of some Asian vegetables. *International Journal of Food Science & Technology*, 37(2002), 153-161. https://doi.org/10.1046/j.1365-2621.2002.00552.x

[21] Annalakshmi, R., Uma, R., Subash, G., Savariraj, C. & Charles, A. Evaluation of elemental content of leaves of Madhucalongifolia by X-ray fluorescence spectroscopy (XRF). *Journal of Natural Product and Plant Resources*, 2(4) (2012) 490-493.

[22] Quang-Vinh, N. and Jong-Bang, E. Antioxidant activity of solvent extracts from Vietnamese medicinal plants. *Journal of Medicinal Plants Research*, 5(13) (2011) 2798.

[23] Augusto, L., Josean, F., Marcelo, S., Margareth, F., Petronio, F. & Jose, M. Anti-inflammatory activity of alkaloids. *Molecule*s, 16(2011) 8515-8534.

[24] Dua, V. K., Verma, G., Singh, B., Rajan, A., Bagai, U., Agarwal, D., … Rastogi, A. (2013). Anti-malarial property of steroidal alkaloid conessine isolated from the bark of Holarrhena antidysenterica. *Malaria journal*, 12(2013) 1-6. https://doi.org/10.1186/1475-2875-12-194

[25] Benbott, A., Yahyia, A. & Belaıdi, A. Assessment of the antibacterial activity of crude alkaloids extracted from seeds and roots of the plant PeganumharmalaL. *Journal of Medicinal Plants Research*, 2(2012) 568-573.

[26] Ameyaw, Y. & Duker-Eshun, G. The alkaloid contents of theethno-plant organs of three antimalarial medicinal plant species inthe eastern region of Ghana. *International journal of chemical science*, 7(2009) 48-58.

[27] Akiyama, H. (2001). Antibacterial action of several tannins against *Staphylococcusaureus*. *Journal of Antimicrobia Chemotherapy*, 48(4), 487–491. https://doi.org/0.1093/jac/48.4.487

[28] Kren, V., & Martinkova, L. (2001). Glycosides in Medicine: "The Role of Glycosidic Residue in Biological Activity" *Curent Medicinal Chemistry*, 8(11) (2001), 1303–1328. https://doi.org/10.2174/0929867013372193

[29] Parekh, J. & Chanda, S. In-vitro Antimicrobial Activities of Extracts of *Launaea procumbens*Roxb. (Labiateae), *Vitisvinifera*L. (Vitaceae) and *Cyperusrotundus*L.(Cyperaceae). *African Journal of Biomedical Research*, 9 (2006) 89-93. https://doi.org/10.4314/ajbr.v9i2.48780

[30] Seid, M. & Ayisha, A. Extraction and phytochemical determination of some selected traditional medicinal plants for antimicrobial susceptibility test, in Adama, Ethiopia. *International Journal of Engineering, Science and Technology*, 3(5) (2015) 1290-1297.

[31] Bakari, S., Daoud, A., Felhi, S., Smaoui, S., Gharsallah, N. & Kadri A. Proximate analysis, mineral composition, phytochemical contents, antioxidant and antimicrobial activities and GC-MS investigation of various solvent extracts of cactus cladode. *Journal of Food Science and Technology*, 37(2) (2017) 286–293. https://doi.org/10.1590/1678-457x.20116

[32] Felhi, S., Daoud, A., Hajlaoui, H., Mnafgui, K., Gharsallah, N. & Kadri, A. Solvent extraction effects on phytochemical constituents profiles, antioxidant and antimicrobial activities and functional group analysiSs of Ecballium elaterium seeds and peels fruits. *Journal of Food Science and Technology*, 37(3) (2017) 483-492. https://doi.org/10.1590/1678-457x.23516

[33] Aghraz, A., Gonçalves, S., Rodríguez-Solana, R., Dra, L. A., Di Stefano, V., Dugo, G., Cicero, N., Larhsini, M., Markouk, M., & Romano, A. Antioxidant activity and enzymes inhibitory properties of several extracts from two Moroccan Asteraceae species. *South African Journal of Botany*, 118(2018) 58-64. https://doi.org/10.1016/j.sajb.2018.06.017

[34] Alam, M. A., Syazwanie, N. F., Mahmod, N. H., Badaluddin, N. A., Mustafa, K. A., Alias, N., Aslani, F., & Prodhan, M. A. Evaluation of antioxidant compounds, antioxidant activities and capsaicinoid compounds of Chili (Capsicum sp.) germplasms available in Malaysia. *Journal of Applied Research on Medicinal and Aromatic Plants*, 9(2018) 46-54. https://doi.org/10.1016/j.jarmap.2018.02.001

[35] Soobrattee, M., Bahorun, T., Neergheen, V., Googoolye, K., & Aruoma, O. Assessment of the content of phenolics and antioxidant actions of the Rubiaceae, Ebenaceae, Celastraceae, Erythroxylaceae and Sterculaceae families of Mauritian endemic plants. *Toxicology in Vitro*, 22(1) (2008) 45-56. https://doi.org/10.1016/j.tiv.2007.07.012