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Determination of the Antibacterial and Antioxidant Activity of Crude Extract of Bruceaantidysenterica Leaves

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Abstract: Bruceaantidysenterica (abalo in Amharic) is a medicinal plant widely used in traditional medicine for treatment of several diseases and ailments. This study aimed to investigate the classes of the phytochemical constituents of the leaves of Bruceaantidysenterica extracted with the solvents of petroleum ether, ethyl acetate, and methanol. The crud extract evaluated their antioxidant and antimicrobial activities. Qualitative phytochemical screening of the leaves of this plant indicated the presence of alkaloids, flavonoids, phenols, quinones, steroids, terpenoids, saponins, tannins and cumaroins. Quantitative analysis of the leaves of Bruceaantidysenterica revealed that the total phenolic content, expressed as gallic acid equivalent, ranged from 28.5 to 120.06 mg/g of dry weight of extracts. Likewise, the total flavonoid content of the extract, expressed as quercetin equivalent, varied from 26.05 to 83.663 mg/g of dry weight of extracts. All extracts showed antioxidant and antimicrobial activity. As seen from ferric reducing antioxidant power (FRAP) assay, methanol and ethyl acetate extracts of the leaves of Bruceaantidysenterica showed the highest total reducing power while petroleum ether extract exhibited the lowest total reducing power. Furthermore, all the extracts were tested against two gram positive bacteria (Staphylococcus aureus, Streptococcus pyogene) and two gram negative bacteria (Escherichia coli, k. pneumonia) bacteria by Kirby-Bauer standard disc diffusion method. Antibacterial effects of leaves extracts of bruceaantidysenterica showed different degrees of inhibition against both Gram positive and Gram negative bacteria. The methanol and ethyl acetate extracts showed higher antibacterial activity than petroleum ether extracts.

Keywords: Bruceaantidysenterica, Antioxidant Activity, Phytochemical Constituent, Antibacterial Activity

1. Introduction

Traditional medicinal herbs serve a critical role in supporting primary healthcare systems in a variety of countries, particularly in developing nations [1]. Natural goods contain biologically active drugs. There has been a growing interest in therapeutic plants as natural products in numerous parts of the world [2-7]. In rich countries, this may be due to dissatisfaction with traditional pharmaceuticals*, but* in poor countries, it is due to a paucity of medical doctors, a shortage of pharmaceutical products, and their high cost. As a result, herbal treatments are gaining popularity all over the globe [8].

The majority of medicinal plants are rich in natural chemicals that are used in the creation of pharmaceuticals. Through the use of traditional medicines and natural poisons, medicine and natural products (NPS) have been linked for thousands of years. Most early medicines, such as aspirin, digitoxin, morphine, quinine, and pilocarpine, were developed from clinical, pharmacological, and chemical studies of ancient remedies, which were mostly derived from plants. Natural products comprise more than 70% of modern

medications in India [9].

The medicinal effect of plants is determined by how bioactive phytochemical compounds function in the human body. Plant-based bioactive substances are called phytochemicals. Because the plants that create them may not require them, they are categorized as secondary metabolites. They are produced naturally in the bark, leaves, stems, roots, flowers, fruits, seeds, and other parts of the plant [10].

As reducing agents, extracts from many plants have proven to be effective. Bruceaantidysenterica extracts, which are high in alkaloids, quassinoids, and polyphenols, were used as the reducing agent in this investigation. The many OH groups in these compounds boost their antioxidant activity, making them potentially powerful reducing agents [11]. As a result, the goal of this study is to determine secondary metabolites and assess antioxidant activity in B ruceaantidysentrica leaves. In February, leaf figure 1 was photographed in Merawi (32 kilometers south of Bahir Dar).

Figure 1. *Photograph of Bruceaantidysenteric.*

Antioxidants are substances that can prevent oxidation in the human body as well as in dietary products. They are used to extend the shelf life of foods, including oil, bread, cookies, biscuits, and dairy products, by preventing lipid peroxidation and oxidative degradation. Antioxidant properties have been employed to maintain lipid food systems, preventing undesirable changes such as odor and flavor, rancidity, and bleaching of fatty food colors, and so extending the shelf life of the food [12-18]. Because oxygen and sunlight are the two main sources of oxidation, food is preserved by keeping it in the dark, sealing it in containers, or even coating it in wax, as with cucumbers.

Free radicals have been found to play both a destructive and a useful role in our systems. In low/moderate concentrations, free radicals are involved in normal physiological functions, but excessive production of free radicals is a harmful process that causes oxidative stress. Oxidative stress can damage cell components suchaslipids, proteins, RNA, and DNA, leading to a variety of diseases [19]. An antioxidant is a type of molecule that can protect against free radical damage.

Raw extracts and pure components from medicinal plants have been found to be more effective antioxidants in vitro than commonly used synthetic antioxidants like BHT or vitamin E.

Flavonoids, anthocyanins, tannins, dietary glutathioline, vitamins, and endogenous metabolites all contain antioxidant properties [20]. Plant phytoconstituents also have antibacterial effects. As an alternative, the most important naturally antibacterial compounds derived from plant sources can be employed to battle infectious diseases. Tannins, terpenoids, alkaloids, and flavonoids have been shown to exhibit antibacterial properties in vitro [21-24].

2. Experimental Section

2.1. Chemicals and Reagents

Methanol (99.9%), ethyl acetate (98%), acetone (99.8%), and sodium carbonate is a product of Carlo Erba SDS. Petroleum ether (99.9%), chloroform (99.5%), hydrochloric acid (34.5%), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical, sodium hydroxide (97%), gallic acid, Folin-Ciocalteau reagent, quercetin, and sulphuric acid (98%) were the product of Trapur MIDC (Biosar, India). Ferric chloride (97%), aluminum chloride, potassium iodide, ascorbic acid, ammonia (30%), Mullar Hinton agar, trichloroacetic acid (TCA), sodium nitrite and are all product of Blulux Laboratories (P) Ltd (India).

2.2. Collection of the Plant Material

Bruceaantidysenterica leaves were obtained in Merawiworeda, which is 32 kilometers south of Bahir-Dar city. The leaves were sorted to eliminate any dead ones, then rinsed with tap water to remove any dust particles before being dried for 20 days in the shade at room temperature. The dried leaves were pounded to a fine powder with a mortar and pestle and prepared for extraction. Dr. Ali Seid, a botanist from Bahir Dar University's biology department, recognized and authenticated the plant material.

2.3. Plant Extract Preparation

Powdered plant material was extracted successively with increasing polarity organic solvents such as petroleum ether, ethyl acetate, and methanol. To extract non-polar and polar chemicals, about 250g of coarsely crushed leaves (250 g / 750 mL) were steeped for 48 hours in petroleum ether, ethyl acetate, and methanol separately in a conical flask. The extract was filtered and then concentrated by using a rotary evaporator.

2.4. Phytochemical Screening

To identify the presence or absence of phytochemical components in Brucea. antidysenterica crude methanol, ethyl acetate, and petroleum ether extracts, phytochemical screening was done using different chemical tests [25].

2.5. Flavonoid Content Determination

The flavonoid content was determined using an aluminum chloride complex-forming assay. The total flavonoid content of the extracts was determined using an aluminum chloride

complex-forming assay. As a baseline, the flavonoid content was determined using the quercetin equivalent. This was accomplished by creating a quercetin calibration curve [26]. From the standard quercetin solution, diluted (10, 20, 30, 40, and 50 ppm) concentrations of quercetin were generated in methanol. 0.25 mL of each quercetin dilution was combined with 1.25 mL of distilled water, followed by 0.075 mL of 5% sodium nitrite, and allowed to sit for 5 minutes to mix. After that, 0.15 mL of 10% aluminum chloride solution was added and left to sit for 6 minutes at room temperatureafter which 0.5 mL solution of 1M Sodium hydroxide was added sequentially. The same process was used to make 50ppm extracts from petroleum ether, ethyl acetate, and methanol extracts, and the total flavonoid concentration was estimated as quercetin equivalents (mgQE/g).

2.6. Determination of Total Phenolic Content

Total phenol content was estimated using Folin-Ciocalteau reagent-based assay with little modification [27]. 5mL of Folin-Ciocalteau reagent and 4 mL (75 g/L) of Na_2CO_3 were added to one mL of each extract (100µg/mL) in distilled water. A UV-Vis spectrophotometer was used to measure the mixture's absorbance at 765 nm after it had been allowed to stand at room temperature for 30 minutes. The calibration curve was constructed using 1 mL aliquots of 20, 40, 60, 80, and 100ppm methanol gallic acid solutions. The gallic acid calibration curve was used to calculate total phenol content, which was represented in milligrams of gallic acid equivalent (GAE) per gram of extract.

2.7. DPPH Free Radical Scavenging Activity

The 2, 2- diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method was used to determine the extracts' free radical scavenging capabilities. An antioxidant molecule gives an electron to DPPH, causing it to be reduced and changing its hue from deep violet to yellow [26]. In methanol, a fresh 0.003 percent DPPH solution was produced. Plant extracts (25, 50, 75, 100, and 125 ppm) and ascorbic acid (25, 50, 75, 100, and 125 ppm) were combined with a 1 mL DPPH solution and left to stand in darkness for 15 minutes.

The control was made by mixing 1 mL DPPH with 2 mL methanol and measuring the absorbance at 517nm. On the basis of the control, the percentage inhibition of the free radical DPPH was computed. At 517 nm, the absorbance was measured once more. Using the following formula, the % inhibition of DPPH by extracts was calculated:

$$\%\text{inhibition activity} = [(A_0\text{-}A_1)/A_0] \times 100$$

Where A_0, is the absorbance of pure DPPH in oxidized form while A_1, is the absorbance of a sample taken after 15 minutes of reaction with DPPH [26].

2.8. Assay for Ferric Reducing Antioxidant Power (FRAP)

The reducing power of the produced extracts was determined using a modified version of the usual procedure. For each solvent, different concentrations of B. antidysenterica leaf extracts (50, 100, 150, 200) ppm were produced (98 percent methanol, ethyl acetate, and petroleum ether). 2.5 mL extract from each sample was combined with 2.5 mL of 200 mMsodium phosphate buffer (PH = 6.6) and 2.5 mL potassium ferricyanide solution (1 percent). For 20 minutes, the combinations were incubated in a water bath at 500°C. Then, 2.5 mL of trichloroacetic acid (TCA) solution (10% w/v) was in the water bath. The water bath was then filled with 2.5 mL of trichloroacetic acid (TCA) solution (10% w/v). The process was then stopped by adding 10% trichloroacetic acid to the mixture. 5 mL distilled water and 0.5 mL $FeCl_3$ solution were added to the upper layer of the solution. The reaction mixture was incubated at room temperature for 10 minutes, and the absorbance developed a blue tint, which was measured using a UV-spectrophotometer at 700 nm against a blank solution.

$$\text{Percentage (\%) reduction power} = [(\text{A sample-A blank})/\text{A sample}] \times 100$$

Where A sample= absorbance of the sample
A blank =absorbance of blank

2.9. Determination of Antibacterial Activity

Antibacterial activities were performed in the microbiology laboratory, department of Biology, Bahir Dar University by using the agar well diffusion method. Using normal procedures, Muller Hinton agar media was made using standard protocols for culturing gram-positive (S. aureus and S. pyogens) and gram-negative bacteria (E. coli, K. pneumonia). The bacteria in question were put to the test. B. antidysenterica leaf extract (31.25, 62.5, 125, 250 g/mL) and conventional antibiotics (Gentamycin) were applied to the incubated agar using discs. After that, it was incubated for 24 hours at 37 degrees Celsius, and the experiment was repeated three times, with the average values of the zone of inhibition measured in millimeters for antibacterial activity.

2.10. Methods of Data Analysis

This study investigated and reported the antioxidant activity, total polyphenol content, and total flavonoid content of Bruceaantidysenterica leaf extracts in terms of ascorbic acid (AA), gallic acid (GA), and quercetin (QT) equivalent per gram of extraction. Origin 8 Pro was used to build the calibration curves, and the equation below was used to compute them.

T= (xppm) (volume of extract) (ml)/weight of dry sample in gram

$$T = \frac{[(xppm)(volumeofextract)(ml)]}{weightofdrysampleingram} \quad (1)$$

$$T = \frac{[(xppm)(dilutionfactor)(volumeofextract)(ml)]}{weightofdrysampleingram} \quad (2)$$

Where: T=total phenolic content or total flavonoid content

$$X= Y\text{-}C/B= \frac{Y-C}{Q}$$

Y= absorbance of the sample, C = y-intercept from calibration curve,

B= slope from the calibration curve

The percentage of DPPH radical scavenging activities of *Bruceaantidysenterica* leaves extract were calculated with the equation stated below:

$$\text{DPPH radical scavenging (\%) activity} = [(A_0- A_1)/A_0] \times 100$$

Where: A_0 = absorbance of the blank, A1 = absorbance of the sample.

3. Result and Discussion

3.1. Testing for Phytochemical Constituents

Phytochemicals detected in plant samples are linked to the biological activity of extracts. Phytochemical screening is extensively used in the study of the medicinal properties of plant materials. The presence of chemical substances such as alkaloids, tannins, flavonoids, saponins, and others that have a specific physiological impact on the human body is responsible for the medicinal benefit of traditionally important plant species [27]. In this study, phytochemical elements in crude leaf extracts of Bruceaantidysenterica were analyzed qualitatively in petroleum ether, ethyl acetate, and methanol. Phytochemical constituents are responsible for plant species' therapeutic efficacy. As a result, the existence of several secondary metabolites (phytochemicals) such as alkaloids, flavonoids, tannins, steroids, saponins, and phenols was investigated in Bruceaantidysenterica leaf extracts. Alkaloids, flavonoids, tannins, steroids, saponin, terpenoids, and phenols were found in abundance in the methanol extract, but coumarins and quinones were not. Phenols, tannins, steroids, and other compounds can be found in ethyl acetate extract. There are no coumarins, saponins, flavonoids, alkaloids, terpenoids, or quinones. Coumarins, quinones, steroids, phenol, and flavonoids were found in the petroleum ether extract, but terpenoids, tannin, alkaloids, and saponins were not. The end product is given below (Table 1).

Table 1. *Phytochemical constituent of different crude leaf extracts of Bruceaantidysenterica.*

Phytochemicals	Methanol	Ethyl acetate	Petroleum ether	Observed color	Reagents
Saponin	+	-	-	white foam	foam test
Flavonoids	++	+	+	Intense yellow	wagner
Alkaloids	+	-	-	Brown precipitate	wagner
Phenols	++	+	+	Black color	ferric chloride test
Tannin	+	+	-	Dark green	ferric chloride test
Steroids	++	+	+	Red color	Salkowski's Test
Terpenoids	+	-	-	Reddish-brown	Salkowski's Test
Quinones	-	-	+	Yellow precipitate	HCl test
Coumarins	-	+	++	Yellow color	NaOH test

Table 2. *Percentage (%) reduction power (FRAP).*

№	Concentration (ppm)	AA	ME	EA	PE
1	50	92.5	84.5	79.0	79.68
2	100	94.7	87.73	82.3	80.16
3	150	95.265	90.2	83.06	81.7
4	200	96.1	92.5	84.76	82.87

Key: AA= ascorbic acid, *ME*= methanol extrat, *EA*= ethyl acetate extract, *PE*= petroleum ether extract

Table 3. *Absorbance and concentration of the standard and the extracts with the absorbance of the control (0.62).*

Absorbance					
№	Concentration in mg/L	AA	ME	EA	PE
1	25	0.1236	0.1534	0.2634	0.5893
2	50	0.1136	0.1274	0.2364	0.4638
3	75	0.0767	0.0928	0.1958	0.3736
4	100	0.0517	0.07351	0.1751	0.2416
5	175	0.0363	0.0552	0.1452	0.14716

Table 4. *The %inhibition Vs concentration of ascorbic acid, and extracts with the absorption of the control (0.62).*

№	Concentration in mg/L	AA	ME	EA	PE
1	25	80.0	75.2	58	4.8
2	50	82.0	79.4	61.7	25.2
3	75	87.6	85.0	68.4	39.7
4	100	91.7	88.1	71.8	61.0
5	125	94.1	91.1	78	75.0

3.2. Antioxidant Potential Determination of Extracts of B. Antidysenterica Leaves

3.2.1. Antioxidant Activity by Ferric Reducing Power, FRAP

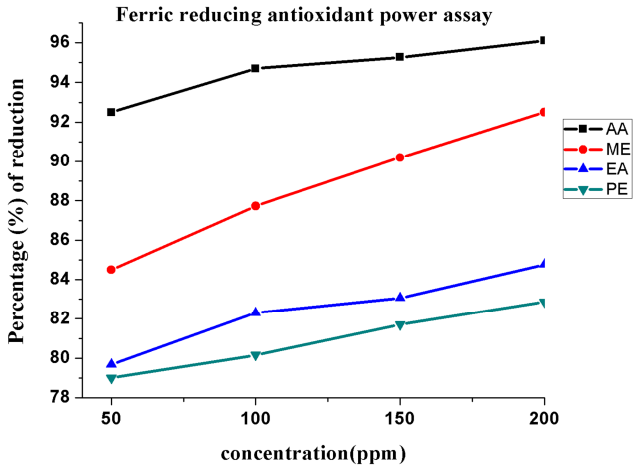

Figure 2. *Percentage (%) reduction power (FRAP).*

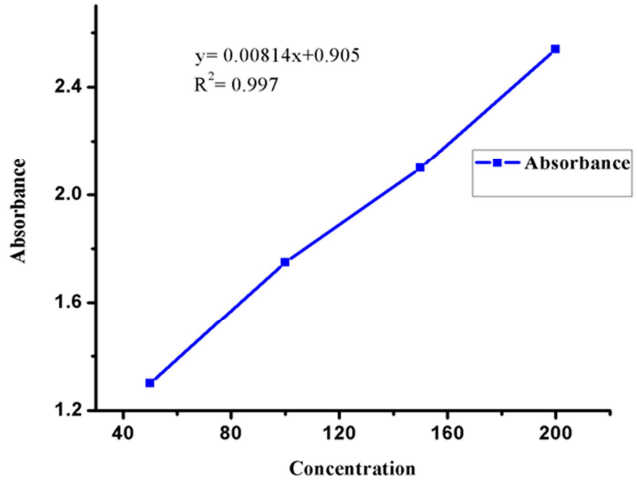

Figure 3. *Calibration curve that shows the absorbance of ascorbic acid at the different concentration at 700 nm.*

The reducing power (antioxidant capacity) of Bruceaantidysenterica extracts in methanol, ethyl acetate, and petroleum ether was determined, and the findings are given in Figure 2. The reducing power of the methanol extract was the highest, while the reducing power of the ethyl acetate and petroleum ether was the lowest [28]. This reveals that polar molecules are more responsible for antioxidant actions and that high polar solvents are better for extracting them. With increasing extract concentration, all three extracts showed a nearly identical increasing trend in lowering power. The presence of reducers (i.e. antioxidants) leads the Fe^{3+} ferricyanide complex to be reduced to the ferrous form in this test. As a result, the blue hue formed indicated the existence of Fe^{2+} concentration and was detected at 700nm. A calibration curve (Figure 3) was created to assess the antioxidant activity of Bruceaantidysenterica leaf extracts in terms of ascorbic acid equivalent, a calibration curve was created. The calibration curve was plotted as UV-response (absorbance) vs. ascorbic acid concentration (50, 100, 150, 200) ppm, with the value of the absorbance obtained corresponding to the concentration supplied in ppm.

3.2.2. DPPH Radical Scavenging Activity

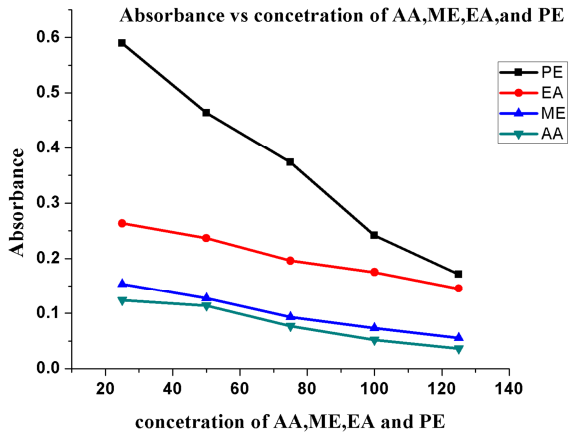

Figure 4. *Absorbance Vs concentration of ascorbic acid, petroleum ether extract, ethyl acetate extract, and methanol extract.*

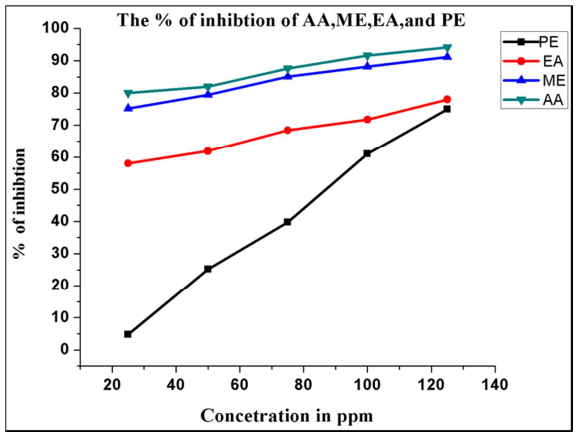

Figure 5. *% of inhibition of ascorbic acid (AA), petroleum ether extract (PE), ethyl acetate (EA), and methanol extract (Me).*

Using the stable radical, DPPH, the antioxidant activity of each extract of Bruceaantidysenterica was assessed in terms of radical scavenging ability. In the assay (Figure 4), Methanol extract of Bruceaantidysenterica leaves at a concentration of 125mg/L had the maximum radical scavenging activity (90.1%), while at a concentration of 25mg/L, it had the lowest (75.25%). The strong polarity of the solvent could explain the high % inhibition for methanolic extract is shown in Figure 5. This suggests that phytochemicals that are soluble in methanol have a greater ability to scavenge DPPH free radicals. The antioxidant capacity of Bruceaantidysenterica leaf extracts was closely related to the increase in DPPH scavenging activity. That is why, when compared to other extracts, methanol extraction of leaves of Bruceaantidysenterica, which has the highest DPPH radical scavenging activity, demonstrated the highest

antioxidant activity. The stronger free radical scavenging activity of the methanol extract than the ethyl acetate and petroleum ether extracts was apparently due to the presence of more highly polar flavonoids and phenols in the methanol extract, allowing hydrogen atom transfer [15]. When the extracts were introduced to the DPPH solution, the color change (purple to yellow) qualitatively indicated DPPH radical scavenging action. The different intensities of yellow color production suggested that the samples had different levels of DPPH radical scavenging activity. DPPH. (Purple) + *Bruceaantidysenterica* extracts DPPH (yellow). The % scavenging activity of each extract vs concentration in Bruceaantidysenterica leaves was plotted graphically below using the DPPH assay.

3.3. Quantitative Analysis for Total Phenolic and Total Flavonoid Contents

3.3.1. Total Phenolic Content

The Folin-Ciocalteu assay uses an oxidation/reduction (redox) process to quantify total phenolic chemicals. The technique is based on the transfer of single electrons (SET) from phenolic compounds to molybdenum in an alkaline solution to generate a blue complex that can be spectrophotometrically measured at 750-765 nm [15]. Using the Folin-Ciocalteu's reagents, the total phenolic content in the investigated plant extracts is represented in terms of gallic acid equivalent (the standard curve equation: (y = 0.00214x + 0.02715 and R2 = 0.99568). The total phenolic content of Bruceaantidysenterica extracts was determined, with the results shown in Table 5 and Figure 7. The total phenolic content of the extracts ranged from 28.5 to 121.0546 mg/g. Highly polar solvent extracts had the highest total phenolic concentration, while the least polar solvent extracts had the lowest total phenolic content. Methanols (121.0654 0.066793 mg GA/g), ethyl acetate (93.7380.18526 mg GA/g), and petroleum ether (28.5 0.035355 mg GA/g) have the lowest phenolic concentration of B. antidysenterica leaf extracts. These findings suggested that the polarity of the solvent utilized affects the extraction of phenolic chemicals. The color intensity is determined by the polarity of the solvent and represents the amount of polyphenol in the sample. In comparison to ethyl acetate and petroleum ether extracts, which have very little color intensity, methanol extract of B. antidysenterica leaves exhibits a very deep blue color.

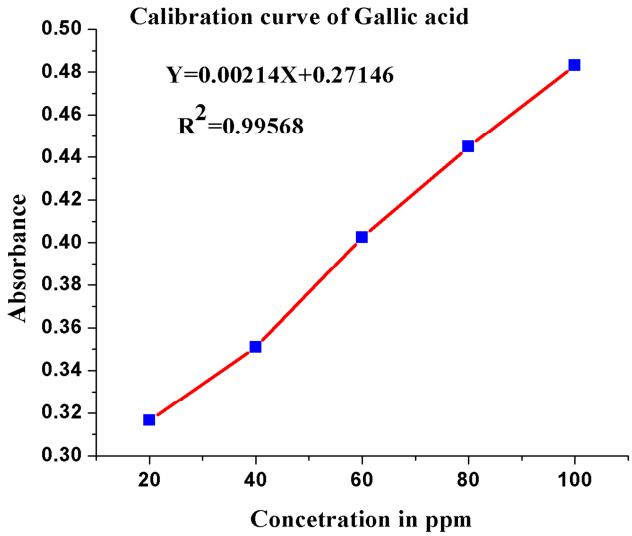

Figure 6. *Total phenolic content calibration curve.*

Table 5. *Total phenolic content.*

Solvent system	Total Phenolic Content (mg GA/g) ±STD
Petroleum ether	28.5 ±0.035355
Ethyl acetate	93.738±0.18526
Methanol	121.0654 ±0.066793

Table 6. *Total flavonoid content.*

Solvent System	Total Flavonoid Content (mg QE/g)±STD
Petroleum ether	26.05±0.035
Ethyl acetate	52.746±0.527
Methanol	83.663±0.0445

Table 7. *Zone inhibition of the antibacterial activity of different extracts of Bruceaantidysentericaat the concentration of 250mg/mL.*

zone of inhibition + S. D (mm)					
Test bacteria	**Conc (mg/mL)**	**Methanol**	**Ethyl acetate**	**Petroleum ether**	**Gentamicin**
Escherichia coli	250	30.0±0.626	17.4±0.39	15±0.41	25.25±0.35
	125	31.3±0.8	16.9±0.14	16.5±0.71	
	62.5	28.±0.4	14.75±0.35	16.8±0.01	
	31.25	25.4±0.56	13.85±0.20	15.5±0.70	
K. pneumonia	250	29.75±0.76	19.8±0.06	18.5±0.12	23.4±0.56
	125	31.8±0.69	18±0.80	17.8±0.06	
	62.5	30.85±0.2	15.05±0.48	15.5±0.12	
	31.25	28.9±0.98	13±0.40	14.8±0.06	
Staphylococcus aureus	250	32.35±0.33	18.75±0.35	19.8±0.283	24.1±0.14
	125	31.35±0.92	18.05±0.76	16.8±0.47	
	62.5	30.6±0.979	15.6±0.85	15.6±0.97	
	31.25	16.4±0.41	13.5±0.707	13.2±0.76	
Streptococcus Pyogenes	250	31.5±0.70	28.7±0.31	19.3±0.83	28.8±0.28
	125	30.06±0.92	24.25±0.80	16.2±0.06	
	62.5	29.15±0.20	18.75±0.52	15.8±0.62	
	31.25	19.0±0.82	16.3±0.91	14.2±0.13	

DMSO - - -

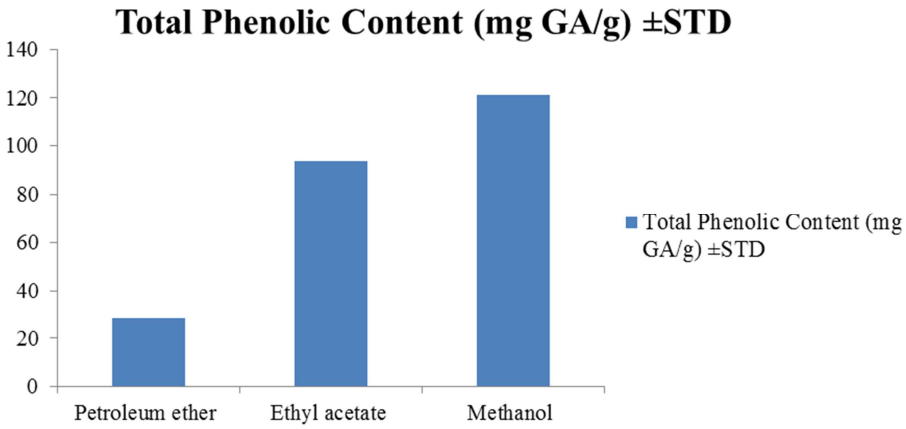

Figure 7. *Total phenolic content chart.*

3.3.2. Total Flavonoid Content

An aluminum chloride complex-forming assay was used to evaluate the total flavonoid content of the extracts. Quercetin was used as a control. Figure 8 shows the total flavonoid concentration (TFC) of numerous Bruceaantidysenterica extracts in terms of quercetin equivalents (mgQE/g). The amount of flavonoids in Bruceaantidysenterica extracts was determined using the spectrophotometric method with aluminum chloride. y = 0.0143x-0.05303, R2 = 0.998, was the standard calibration curve equation utilized in the calculations.

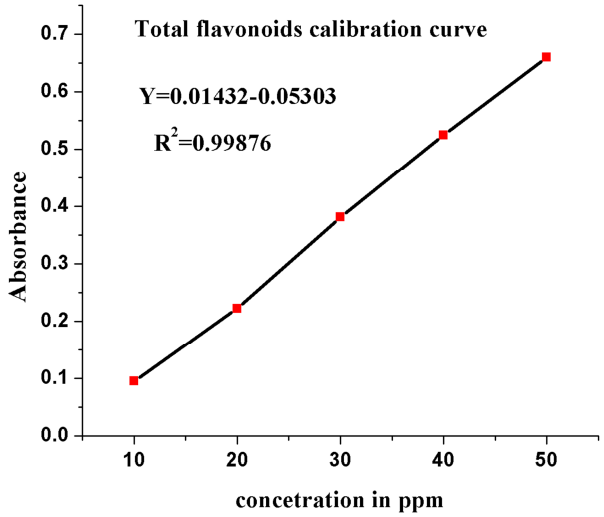

Figure 8. *Total flavonoid content calibration curve.*

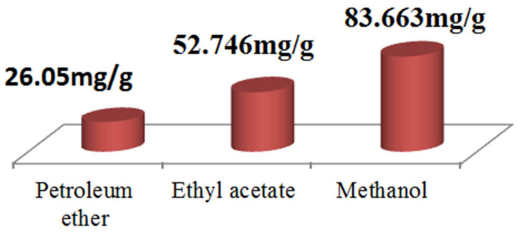

Figure 9. *Total flavonoid content chart.*

The flavonoid content of Bruceaantidysenterica extracts ranged from 26.5 to 83.663 mg QE/g. The highest flavonoid content was observed in methanol extract, whereas the lowest flavonoid concentration was found in petroleum ether extract and it is tabulated in Table 6. The polarity of the solvents used in extract preparation affects the concentration of flavonoids in plant extracts. The presence of a large amount of polyphenol in the plant's leaf extract may account for the greatest content of total flavonoid in the highly polar solvent methanol extract of Brucea antidysenterica leaves [26]. The calibration curve was made by mapping known quercetin concentrations against absorbance (UV response).

3.4. Antibacterial Activities of Bruceaantidysenterica Extracts

The antibacterial activity was determined by measuring the diameter of the zone of inhibition. The antibacterial properties of three crude leaf extracts of Bruceaantidysenterica were studied. Table 7 displays the results of the antibacterial activity test of the extracts. The positive control (gentamycin) had a good inhibitory effect on the microorganisms tested, while the negative control (DMSO) had no effect on the inhibiting microbial test. Most of the bacteria examined showed that petroleum ether, ethyl acetate, and methanol extract had good antibacterial action. The methanol extract was found to have good antibacterial activity against gram-negative bacteria Escherichia coli (31.3±0.8) and Klebsiella pneumonia (31.8±0.69) as well as high activity against gram-positive bacteria (Staphylococcus aureus and Streptococcus pyogene).

4. Conclusion

Bruceaantidysenterica is a well-known medicinal plant that has long been used as a traditional medicine in many parts of the world. Alkaloids, phenols, flavonoids, tannins, terpenes, saponin, and coumarin were found in various amounts in each extract of the plant, according to the results of phytochemical research. Based on the findings, it can be concluded that all of the extracts (petroleum ether, ethyl acetate, and methanol) of Bruceaantidysenterica's leaves have antioxidant activity, as evidenced by the free radical scavenging and ferric reducing power properties, which

could be due to the presence of phenolic and flavonoid components in the extract. The largest concentrations of phenolic and flavonoid chemicals in the extracts were obtained using solvents with high polarity (methanol and ethyl acetate extract), indicating that the leaves of Bruceaantidysenterica have more phenolic and flavonoid compounds. The high concentrations of phenolic and flavonoid chemicals, as well as the considerable linear association between the concentrations of these compounds and antioxidant activity, indicate that these compounds contribute to the strong antioxidant activity. The extracts were also tested against two gram-positive bacteria (Staphylococcus aureus and Streptococcus pyogene) as well as two gram-negative bacteria (Staphylococcus aureus and Streptococcus pyogene) (Escherichia coli, Klebsiella pneumonia).

The antibacterial activities of bruceaantidysenterica leaf extracts inhibit Gram-positive and Gram-negative bacteria to varying degrees. The bactericidal activity of methanol and ethyl acetate extracts was higher than that of petroleum ether extracts. As a result, more research is needed to extract bioactive components from this medicinal plant that have stronger antioxidant and antibacterial properties.

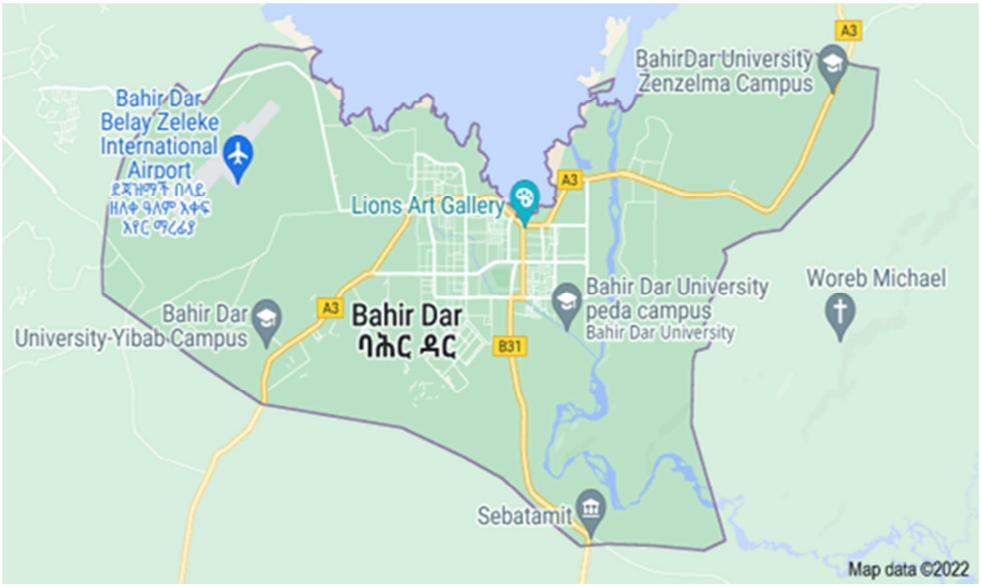

Figure 10. *Geolocation information.*

Conflict of Interests

The authors declare that they have no competing interests.

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