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ANALYSIS OF THE PHYTOCHEMICAL COMPOSITION AND BIOLOGICAL PROPERTIES OF *DATURA FASTUOSA SOLANACEAE (QORRAMAKKA)* ROOT EXTRACTS

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ABSTRACT

The phytochemical composition and biological properties of root extracts from Datura fastuosa (Solanaceae), also known locally as Qorramakka, are investigated in this work. The primary objectives were to identify the bioactive components of the root and evaluate its potential pharmacological effects. The most prevalent groups of phytochemicals screened and identified were flavonoids, saponins, tannins, phenolic compounds, and alkaloids. Several biological assays were used to assess the extracts' antioxidant and antimicrobial qualities. The results demonstrated strong antimicrobial action against a range of bacterial and fungal infections, as well as outstanding antioxidant qualities, as demonstrated by notable radical

scavenging activity and reducing power. Despite the fact that the extracts contained significant amounts of flavonoids and phenolics, quantitative analysis revealed that the chloroform extract had very few alkaloids. DPPH radical scavenging revealed that the range of antioxidant activity was 83.31% to 19.88%. The antibacterial activity indicated that the inhibition zones for fungi and bacteria were 9.4 to 4.17 mm and 20.30 to 11.9 mm. Methanol and acetone extracts showed stronger antifungal and antioxidant properties, while ethyl acetate and chloroform extracts showed stronger antibacterial properties. The results demonstrate Datura fastuosa's potential as a source of bioactive chemicals with therapeutic uses and support its historic use in ethnomedicine. Further research is required to determine the specific bioactive chemicals responsible for these effects, characterize them, and evaluate their safety and effectiveness in clinical settings.

KEYWORDS: Anti-microbial activity, Anti-oxidant activity, Datura fastuosa, Phytochemical Analysis.

1. INTRODUCTION

Many modern pharmaceuticals have been derived from plants, which are considered an essential source of therapeutic compounds for treating a variety of illnesses (Waliullah *et al*., 2019). Plant-derived chemicals have garnered a lot of attention recently due to their numerous applications (Aiyadurai *et al*., 2017). Indeed, plant-based products are a major component of many traditional medications (Samira *et al*., 2020). A wide range of bioactive substances with noteworthy pharmacological properties are found in medicinal plants. They are crucial for creating substitute therapies that have less side effects than synthetic medications (Andrade *et al*., 2019).

Both modern and traditional medicine are based on natural products, which are also being utilized more and more in the manufacturing of pharmaceuticals that are sold commercially (Samira *et al*., 2020). Since ancient times, plants have been used to cure a variety of illnesses, especially in communal settings (Danmusa *et al*., 2015). They are also a significant source of novel antibiotic classes (Coates *et al*., 2011).These plants are often referred to as alternative or complementary medicines (WHO, 2009).

In the study of natural antimicrobials, plants of terrestrial origin have long been a focus. Experimental evidence supports the notion that plants are a sustainable source of diverse bioactive metabolites, which can serve as lead structures for novel drug development

(Hosseinzadeh *et al*., 2015). Today, plant preparations are increasingly recognized for their antimicrobial potency, attributed to their diverse array of bioactive principles (Gemechu *et al*., 2017). Essential oils, extracts, and secondary metabolites from plants possess antimicrobial and antioxidant properties without toxic effects, making them vital for managing many diseases (Dutt *et al*., 2019). Phytochemicals, secondary metabolites with significant biological potential, are now central to drug development (Rengasamy *et al.*, 2019).

Datura species have been traditionally used to treat animal bites, such as snakebites, providing pain relief (Meenakshi *et al*., 2021). Among these, *Datura fastuosa* is particularly notable for its use in mystic and religious practices in addition to its application as herbal medicine. Belonging to the Solanaceae family, which is distributed globally, all Datura species contain biologically active tropane alkaloids. The primary alkaloids in these plants are scopolamine and hyoscyamine, which have effects such as central nervous system stimulation and peripheral nerve depression, characteristic of parasympathomimetic activity (Aiyadurai *et al*., 2017). These alkaloids are known for their medicinal properties, including spasmolytic, antiasthmatic, anticholinergic, narcotic, and anesthetic effects (Waliullah *et al*., 2019). However, previous studies have not explored the antioxidant and antimicrobial activities of *Datura fastuosa* root extracts, leaving a gap in understanding the full health benefits of this plant. Therefore, the present study aims to evaluate the chemical composition of methanol, acetone, ethyl acetate, and chloroform extracts from *Datura fastuosa* roots and investigate their antioxidant and antimicrobial activities.

2. MATERIALS AND METHODS

2,1. Description of the study area

Root samples of Datura fastuosa were collected from Orshale, a small village in the Jarso Kebele of Konso Zone, located in the South Ethiopia. Konso Zone, situated in the southwestern part of the country, is approximately 97 km from Arba Minch and 600 km from Addis Ababa, lying roughly between 5° 15′ 0″ North latitude and 37° 29′ 0″ East longitude. The majority of the Konso community, over 96%, relies on agriculture for their livelihood.

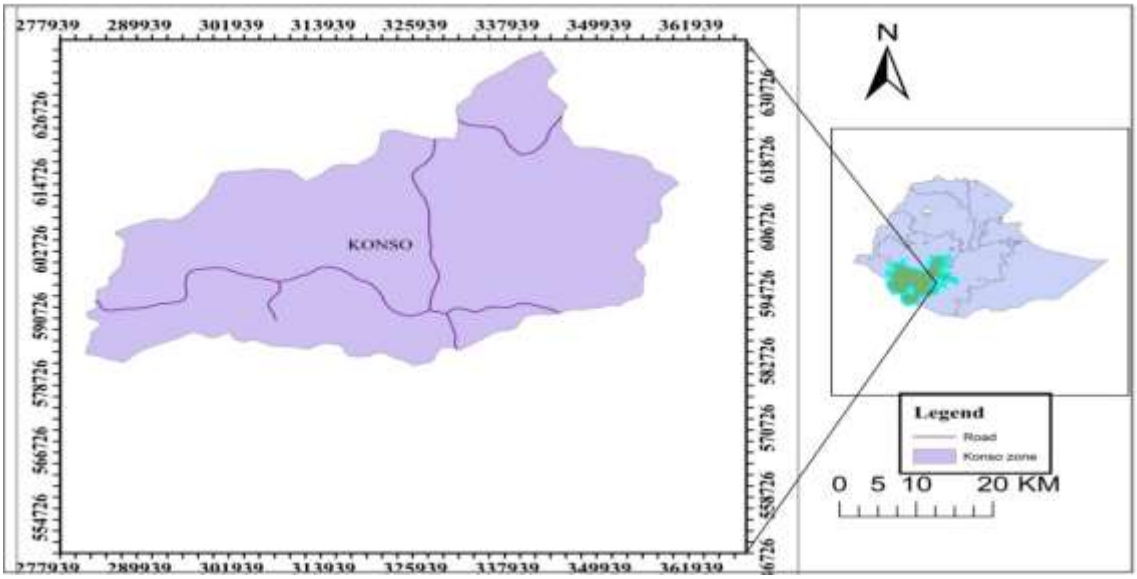


Figure 1: Geographical location map of the study area (Konso Zone).

About 70% of the Konso Zone is classed as Kolla, which has an arid climate, while the remaining 30% is classified as Woina Dega, which has a sub-humid climate (Ethiopian National Meteorological Agency, 2008). According to the Ethiopian National Meteorological Agency (2008), the region has mostly hot and muggy weather. Because of the zone's bimodal rainfall distribution, there are two distinct wet seasons each year. Mid-February to the end of April is the main rainy season, while August to October is the secondary rainy season, according to Muhaba (2018). The zone's yearly rainfall is marked by considerable fluctuation and typically low levels, notwithstanding this periodic pattern (Tadesse, 2010). Despite this cyclical pattern, the zone's yearly rainfall is characterized by considerable variability and typically low levels (Tadesse, 2010). With reported fluctuations ranging from 280 to 880 mm^3, the average annual rainfall is around 550 mm^3 (Tadesse, 2010). The plant was authenticated by Dr. Temesgen Dingamo from the Herbarium Section of the Biological Sciences Department at Arba Minch University, Arba Minch, Ethiopia.

2.2. Sample collection, authentication and preparation

The roots of *Datura fastuosa* were excavated from the soil using a traditional backhoe, then trimmed to the desired size and stored (Waliullah *et al.*, 2019). The collected roots were immediately wrapped in aluminum foil to prevent contamination and degradation of bioactive compounds, and then covered with plastic for easier handling. After washing the roots with distilled water, they were shade-dried in a well-ventilated laboratory room for three months. Once dried, the roots were ground into a powder with a particle size of 500 mesh using a

local mortar and pestle, and an electric blender (Kiruthika and Sornaraj, 2011). The powdered sample was then packed in aluminum foil and stored at room temperature until extraction.

Figure 2: Pictures of plant sample collection from the site study, Drying and powder in the lab.

2.3. Extraction

The powdered, dried roots of *Datura fastuosa* (140 g) were soaked in 750 mL of methanol for 72 hours. The mixture was occasionally agitated on an orbital shaker set at 250 rpm, and then filtered through Whatman #1 filter paper (320 mm diameter). A rotating vacuum evaporator set at 40°C was used to concentrate the resultant solution under lower pressure. Before undergoing additional examination, the concentrated extract was moved to a glass vial with a label and kept in a refrigerator at 4°C (Yaschilal *et al*., 2019). Using acetone, ethyl acetate, and chloroform, the same process was used to create extracts. Additionally, these extracts were refrigerated in vials until they were required for additional examination.

Figure 3: Pictures of concentrated crude extract.

2.4. Qualitative Phytochemical Analysis

The crude extracts of *Datura fastuosa* roots were subjected for the detection of phytochemical compounds by employing the procedures of Evans (Evans, 2006). Qualitative analysis of crude extracts were done for the identification of various classes of

phytoconstituents like alkaloids, Phenolics, tannins, flavonoids, triterpenoids, terpenoids, Saponins, and steroides in the plant.

2.4.1. Test for Alkaloids

Mayer's reagent was used to determine whether the plant extracts contained alkaloids. This reagent was made from scratch by dissolving potassium iodide and mercuric chloride in water. Four drops of iodine solution were added to 0.5 grams of each extract (chloroform, ethyl acetate, acetone, and methanol) after 1 milliliter of Mayer's reagent had been added to test for alkaloids. A yellow precipitate formed, indicating the presence of alkaloids (Kuri *et al*., 2014).

2.4.2. Test for Flavonoids

The presence of flavonoids was determined using the alkaline reagent test. To 3 mL of each extract, sodium hydroxide solution was added, and the mixture was observed for an intense yellow color. The addition of 10 drops of dilute H_2SO_4 or HCl to the solution, which turned colorless, indicated the presence of flavonoids (Edori *et al*., 2019). Alternatively, for each extract (chloroform, ethyl acetate, acetone, and methanol), 0.5 grams were mixed with small fragments of magnesium ribbon and 10 drops of concentrated HCl. The appearance of a pink scarlet color confirmed the presence of flavonoids (Ajuru *et al*., 2017).

2.4.3. Test for Phenols

To 1 mL of the plant extract, one drop of 5 % $FeCl_3$ (w/v) was added. The formation of violet precipitate indicated the presence of phenolics (Kumar *et al*., 2013).

2.4.4. Test for Saponins

A foaming test was used to find out whether saponins were present. In this test, 5 mL of distilled water and 3 mL of each extract were combined in a test tube, and the mixture was violently shaken for two minutes. Saponins were present because stable froth formed (Kumar *et al*., 2013). A 1 cm layer of foam formed when 0.5 grams of each extract—chloroform, ethyl acetate, acetone, and methanol were dissolved in 2 milliliters of distilled water and vigorously shaken to confirm the presence of saponins (Dyana *et al*., 2012).

2.4.5. Test for Tannins

To test for the presence of tannins, 3 mL of the extract was mixed with 10% newly made potassium hydroxide (KOH), and the development of a filthy white precipitate a sign of

tannin content—was watched for (Lightfoot *et al*., 2015). Furthermore, 1 milliliter of ferric chloride ($FeCl_3$) was combined with 0.5 grams of each extract (methanol, acetone, ethyl acetate, and chloroform). The presence of tannins was verified by the formation of a black or blue-green precipitate (Ayoola *et al*., 2008).

2.4.6. Test for Steroids

Three milliliters of acetic anhydride, two drops of concentrated sulfuric acid (H_2SO_4), and one milliliter of each of the extracts of methanol, acetone, ethyl acetate, and chloroform were mixed together to test for steroids. When three drops of concentrated H_2SO_4 were added to three milliliters of the extract, a red coloring was seen, indicating the presence of steroids (Lightfoot *et al*., 2015).

2.4.7. Test for Terpenoids

In a test tube, 3 mL of the extract and 2 mL of chloroform ($CHCl_3$) were combined to check for terpenoids. The presence of terpenoids was then shown by the careful addition of 2 mL of concentrated sulfuric acid (H_2SO_4), which produced a noticeable ring layer at the interface with a reddish-brown hue (Edori *et al*., 2019). Furthermore, 1 mL of concentrated H_2SO_4 was added to 0.5 g of each extract (methanol, acetone, ethyl acetate, and chloroform) and heated for two minutes. Terpenoids were also detected by the combination taking on a grayish hue (Birhanu *et al*., 2019).

2.4.8. Test for Triterpenoids

One gram of each extract: chloroform, ethyl acetate, acetone, and methanol was combined with two milliliters of acetic anhydride to conduct the triterpenoid test. After that, the mixture was cooked and allowed to cool. Then, with caution, 2 mL of concentrated sulfuric acid (H_2SO_4) was introduced to the test tube's side. Triterpenoids were present when a reddish-colored solution appeared (Sheel *et al*., 2014).

2.5. Quantitative determination of phytochemical compounds in *Datura fastuosa*

2.5.1. Estimation of alkaloid contents

The total alkaloid content was determined using the method outlined by Unuofin *et al*. (2017). To each 10 mg of extract, 7.5 mL of 10% acetic acid in ethanol was added. The mixture was allowed to stand at room temperature for 4 hours, and then filtered. The filtrate was concentrated to a quarter of its original volume using a water bath set at 55°C. To precipitate the alkaloids, concentrated ammonium hydroxide (NH_4OH) was added dropwise

to the concentrated filtrate. The solution was left to settle, and the precipitate was collected, washed with dilute NH_4OH, and then filtered again. The resulting residue was dried thoroughly and weighed to determine the alkaloid percentage in the samples. All procedures were performed in triplicate (Ogochukwu et al., 2019). The alkaloid content was calculated using the following equation:

$$\% \text{ Alkaloid} = \frac{weight\ of\ the\ precipitate}{weight\ of\ the\ orginal\ sample} x\ 100\%$$

2.5.2. Total phenolic contents

Total phenolic content was measured using a colorimetric method with the Folin-Ciocalteu reagent (FCR) (Singleton *et al*., 2000). The Folin-Ciocalteu reagent is employed for the colorimetric in vitro assay of phenolic and polyphenolic antioxidants (Mahinder *et al*., 2019). For instrument calibration, an intermediate Gallic acid standard solution at 100 ppm was prepared from a stock solution. From this intermediate solution, five working standard solutions (2, 4, 6, 8, and 10 mg/mL) were made in 100 mL volumetric flasks using 95% methanol.

A calibration curve was constructed to determine phenolic concentrations. A Folin-Ciocalteu reagent solution (1:10 dilution with distilled water) was prepared. Each standard Gallic acid solution was placed in a 25 mL flask, and 5 mL of Folin-Ciocalteu reagent was added. Similarly, 300 µL of each sample extract and a blank solution (without extract) were prepared. After 10 minutes, 4 mL of 2.5% (w/v) sodium carbonate solution was added, and the mixture was allowed to stand at room temperature for 1 hour. The absorbance was then measured at 765 nm using a UV-Visible spectrophotometer.

One gram of each extract: chloroform, ethyl acetate, acetone, and methanol was combined with two milliliters of acetic anhydride to conduct the triterpenoid test. After that, the mixture was cooked and allowed to cool. Then, with caution, 2 mL of concentrated sulfuric acid (H_2SO_4) was introduced to the test tube's side. Triterpenoids were present when a reddish-colored solution appeared (Sheel *et al*., 2014).

$$\text{Total phenol Content } \left(\frac{\text{mg}}{\text{100g}}\ \text{GAE}\right) = \frac{\text{Conc.}\left(\frac{\text{mg}}{\text{L}}\right) x\ Volume\ of\ flask\ (L)\ x\ Df}{Sample\ mass\ (g)} x\ 100\%$$

Where; Df – is Dilution Factor, GAE- Gallic acid equivalents.
All samples were analyzed in triplicates (Altemimi *et al*., 2015).

2.5.3. Total flavonoid contents

Total flavonoid content was assessed using the $AlCl_3$ colorimetric assay (Wang *et al*., 2013). To perform the assay, 100 µL of the extract was mixed with 150 µL of 0.5 M $NaNO_2$ and 150 µL of 0.3 M $AlCl_3$. After 10 minutes, the solution was neutralized by adding 1.5 mL of 1 M NaOH. The mixture was thoroughly mixed, and the absorbance was measured at 510 nm after 15 minutes, using a water blank as the reference. The quantification was carried out using a calibration curve, with quercetin as the standard.

A calibration curve was prepared using quercetin solutions at concentrations of 2, 4, 6, 8, and 10 mg/L. The flavonoid content was calculated using a dilution factor (Df) of 10 and expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE/g DW), based on the quercetin calibration curve. Total flavonoid content was determined according to this calibration.

$$\text{Total Flavonoid Content } \left(\frac{\text{mg}}{\text{100gQE}}\right) = \frac{\text{Conc.} \left(\frac{\text{mg}}{\text{L}}\right) x\, Volumme\, of\, flask\, x\, Df}{Mass\, of\, sample\ (g)} \text{ X } 100\%$$

Where; Df - dilution factor, QE/g DW - quercetin equivalents per gram of dry weight
Triplicate measurements were performed for all samples (Lightfoot *et al*., 2015).

2.6. Total antioxidant capacity (TAC) by DPPH assay

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging experiment was used to evaluate the root extracts of *Datura fastuosa* for its capacity to scavenge free radicals (Macdonald-Wicks *et al*., 2006). According to Molyneux (2004), the process entailed dissolving 4 mg of DPPH in 100 mL of methanol when it was dark. Next, 100 µL of methanol solutions containing different concentrations of the root extract (12.5, 25, 50, 100, 200, and 400 µg/mL) were combined with a 4 mL aliquot of a 0.1 mM methanol solution of DPPH. Ascorbic acid, prepared at the same concentrations, served as a positive control, while a blank solution without the extract was also prepared.

Without any tested samples, a negative control was created using DPPH solution. A UV-Vis spectrometer was used to test the mixture's and the control's absorbance at 517 nm after 30 minutes. Three tests of each concentration were conducted, and the average result was

utilized. The following formula was used to determine the root extracts' radical scavenging efficacy as a percentage of inhibition (Lightfoot *et al*., 2015).

$$\%\ \text{Inhibition}(I\%) = \frac{\text{A control (A0)} - \text{A extract (A1)}}{\text{A control (A0)}} x\ 100\%$$

Where $\mathbf{A_0}$ is the absorbance of the control and $\mathbf{A_1}$ is the absorbance of the root extract (sample).

IC50 refers to the concentration of a sample that produces 50% inhibition and is derived from a dose-response curve. This curve plots the percentage of inhibition against varying extract concentrations (ranging from 12.5 to 400 µg/mL). The IC50 value is calculated using the fitted line from the equation:

Y= mx + b where: $IC_{50} = \frac{(0.5 - b)}{m}$

1

There is no absolute measure for a compound's antioxidant capacity; results are typically compared to a standard, such as ascorbic acid (Alemayehu *et al*., 2018). Tests were conducted in triplicate and analyzed graphically using linear regression.

2.7. Antimicrobial Activity of Crude Extract of the Roots of *Datura fastuosa*

The paper disc diffusion technique was used to evaluate the bactericidal activity of *Datura fastuosa* root extracts in methanol, acetone, ethyl acetate, and chloroform in vitro (Birhanu *et al*., 2019). These extracts were tested for their antimicrobial efficacy against four bacterial species on nutrient agar (NA) medium incubated at 37°C: two Gram-positive bacteria, Staphylococcus aureus (S. aureus) and Bacillus subtilis (B. subtilis), and two Gram-negative bacteria, Salmonella enterica (S. enterica) and Escherichia coli (E. coli). Additionally, using potato dextrose agar (PDA) medium, the antifungal activity was assessed against two fungus species: Fusarium oxysporum (F. oxysporum) and Aspergillus Niger (A. Niger) (Laport *et al*., 2012).

The typical antifungal medication was fluconazole, while the standard antibiotic for bacterial tests was amoxicillin. As a negative control, dimethyl sulfoxide (DMSO) was employed. The Microbiology Laboratory in the Biology Department at Arbaminch College of Teachers Education (AMCTE) was the site of all antimicrobial testing.

(1) Antibacterial Activity

The agar disc diffusion technique was used to evaluate the extracts' antibacterial properties (Kokate, 2000). In a hot air oven, petri dishes were completely cleaned, dried, and sterilized for one hour at 180°C (Adibe and Eze, 2004). Every piece of equipment was autoclaved for 15 minutes at 121°C to disinfect it. Each petri dish was then covered with 20 mL of nutrient-rich overlay gel, inverted, and incubated for 48 hours at 37°C (Alemayehu *et al*., 2018). One milligram of each extract was dissolved in dimethyl sulfoxide (DMSO) to produce a 0.5 mg/mL solution. Amoxicillin was used at a normal dose of 0.5 mg/mL (Ammar *et al*., 2017). The disc diffusion method was used to assess the extracts' antibacterial activity against two Gram-positive bacteria, Bacillus subtilis (B. subtilis) and Staphylococcus aureus (S. aureus), as well as two Gram-negative bacteria, Salmonella enterica (S. enterica) and Escherichia coli (E. coli), on nutrient agar plates (Birhanu et al., 2019). After being impregnated with the extracts, Whatman #1 filter paper discs (6 mm in diameter) were put on the agar plates that had been infected. For 48 hours, the plates were incubated at 37°C.

Antibacterial activity was evaluated by measuring the diameter of the inhibition zones around the discs (Ludwiczuk *et al*., 2017). The inhibitory zones and those of amoxicillin, a common antibiotic, were contrasted. According to Lightfoot *et al*. (2015), each test was carried out in triplicate, and the mean ± standard deviation (S.D.) was used to record the results (Shikha *et al*., 2019). The positive control was amoxicillin, whereas the negative control and solvent was DMSO (Solomon, 2015).

(2) Antifungal activity

Filter paper discs with a 6 mm diameter were sterilized in an oven at 180°C for 1 hour. After sterilization, 50 µL of each extract concentration was pipetted onto the sterile discs in triplicate. Using sterile forceps, the impregnated discs were then transferred to potato dextrose agar (PDA) plates that had been inoculated with a spore suspension of the test fungi, as described in the inoculum preparation section. The Petri dishes were incubated at 27°C for 5 days. All experiments were carried out in triplicate, and the diameter of the inhibition zones surrounding the discs was used to measure the antifungal activity (Birhanu *et al*., 2019).

2.8. Analysis of Statistics

The mean ± standard deviation was used to express the experimental data, which included the total phenolic content, total flavonoid content, microbial activity, and antioxidant activity. One-way analysis of variance (ANOVA), multiple comparisons of means, and Bonferroni

correction were used in the statistical study. At a $p < 0.05$ level of confidence, the results were considered statistically significant. IBM SPSS Statistics 21 software was used to compute the correlation coefficient for antioxidant characteristics (Ammar *et al*., 2017).

3. RESULTS

3.1. Extraction yield

The crude chemical constituents of *Datura fastuosa* root were extracted using methanol, acetone, ethyl acetate, and chloroform. The extraction yields for each solvent were determined using the maceration method, and the results are presented in Table 1.

Table 1: Extraction yield using different solvent.

No.	Solvent	Dry mass of sample in g	Mass of Dish in g	Mass of dish & extract in g	Mass of extract in g	%Yield
1.	**Methanol**	**140**	**28.08**	**34.94**	**6.06**	**4.90**
2.	Acetone	140	31.85	32.71	0.85	0.61
3.	Ethyyl acetate	140	30.58	31.12	0.54	0.39
4.	Chloroform	140	27.57	28.33	0.758	0.54

The extractable compound percentages in the roots of *Datura fastuosa* for methanol, acetone, ethyl acetate, and chloroform were found to be 4.9%, 0.61%, 0.39%, and 0.54% (w/w), respectively.

3.2. Phytochemical screening of root extracts of *Datura fastuosa*

Table 2: Phytochemical screening of *Datura fastousa* root

#No	Phytochemicals	Solvent Extract			
		Me Ext	Ac. Ext	Ea. Ext	Ch. Ext
1.	Alkaloids	++	++	+	+
2.	Flavonoids	++	++	+	+
3.	Phenols	++	++	+	+
4.	Saponins	++	++	+	-
5.	Tannins	+	+	++	+
6.	Steroids	+	+	++	++
7.	Terpenoids	+	+	-	-
8.	Triterpenoids	+	+	-	-

Where: (+) present, (++) present in high percentage, (-) absent, Ext = Extract, Me = methanol, Ac =Acetone, Ea = Ethyl acetate, Ch = Chloroform.

Analysis of bioactive compounds in the root extracts (in different solvents) of *Datura fastuosa* are given in above Table 2. Phytochemical screening of the different extracts showed that saponins were absent in the chloroform extract but were present in low levels in

the ethyl acetate extract. Terpenoids and triterpenoids were not found in the ethyl acetate or chloroform extracts. Alkaloids, flavonoids, phenols, tannins, saponins, and steroids were detected in all other extracts, but in varying amounts.

3.3. Quantitative phytochemical evaluation

(1)Total Alkaloid Contents (Tal C)

The percentage of alkaloid content in the four extracts followed this trend: ethyl acetate extract > chloroform extract > methanol extract > acetone extract (Table 3). The results indicate that the ethyl acetate extract contained the highest concentration of alkaloids compared to the other solvents.

Table 3: Total alkaloid content of *Datura fastuosa* root

No	Mass of original sample (10mg)	% Tal C
1.	**Methanol**	**0.53%**
2.	Acetone	0.49%
3.	Ethyl acetate	0.41%
4.	Chloroform	0.36%

Where µppt = mean of precipitate, SD = standard deviation and Tal C % = total alkaloid content

(2)Total Phenolic Contents (TPC)

Four different solvent extracts were tested for total phenolic content using the Folin-Ciocalteu technique, with gallic acid serving as the standard (Singleton *et al.*, 2000). The methanol extract contained the greatest phenolic concentration of all the extracts, with 99.7 mg GAE/100 g of dry extract.

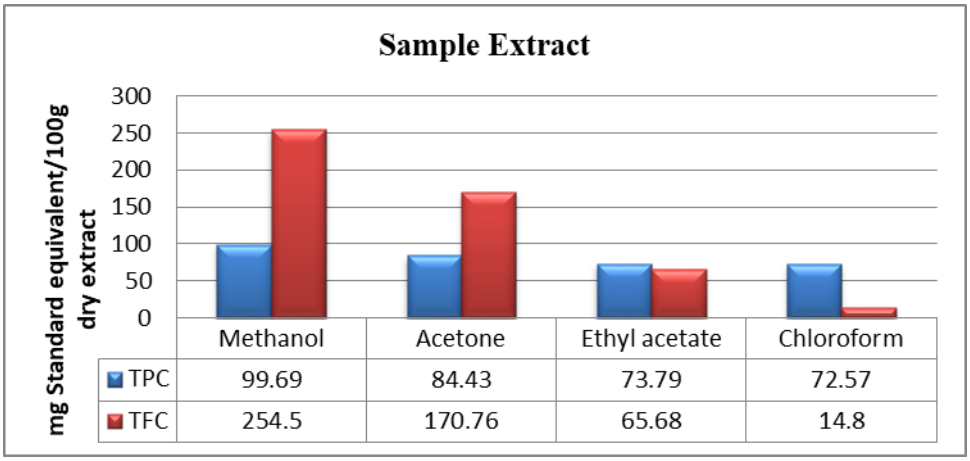
Acetone, ethyl acetate, and chloroform had lower levels, at 84.5 mg GAE/100 g, 73.8 mg GAE/100 g, and 72.5 mg GAE/100 g, respectively.

(3) Total flavonoid contents (TFC)

Flavonoids, often known as vitamin P, are a class of secondary plant metabolites that are primarily responsible for producing the many colors that give a plant its appearance (Ahlem *et al.*, 2015). Raheel *et al.* (2015) state that polyphenols, which include anthocyanins, condensed tannins, isoflavones, flavones, and flavanols, are potent antioxidants. Many studies have been conducted on the identification and quantification of flavonoids in plants in the Datura family. This study emphasizes the substantial flavonoid content of *Datura*

fastuosa root extract. It was shown that the more polar the solvents employed, the higher the flavonoid content. Additionally, Table 4 shows that the plant extracts had greater total flavonoid content (TFC) than other species in the Datura genus (Zia-UL-Haq *et al*., 2012). Table 4 shows that the total flavonoid concentration of the various root extracts varied from 254.50 to 14.8 mg quercetin/g of dry weight.

Table 4: Total phenolics and total flavonoid contents of Datura fastuosa root.



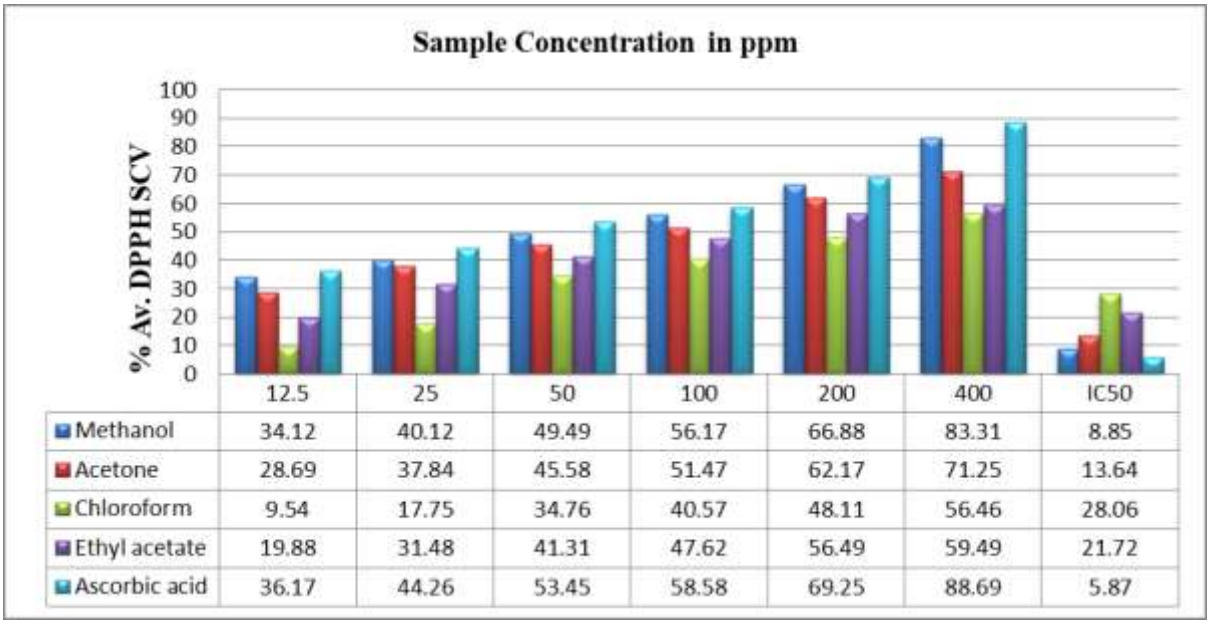
Among the four extracts, the methanol extract contained the highest amount of flavonoid compounds, with 254.50 ± 1.6 mg quercetin/g of dry weight, followed by the acetone extract with 170.76 ± 4.65 mg quercetin/g of dry weight, the ethyl acetate extract with 65.68 ± 6.14 mg quercetin/g of dry weight, and the chloroform extract with 14.80 ± 4.02 mg quercetin/g of dry weight. Both methanol and acetone extracts yielded significantly higher amounts of flavonoids compared to the ethyl acetate and chloroform extracts ($P < 0.05$). Additionally, the ethyl acetate extract also had a significantly higher yield than the chloroform extract ($P < 0.05$).

3.4. Total antioxidant capacity (TAC) by DPPH

The results of the DPPH radical scavenging assay indicate that all extracts exhibited significant scavenging activity against DPPH radicals. This was evidenced by a color change from dark greenish-blue (or violet/purple) to orange, yellow, or colorless, as described by Pérez-Jiménez *et al*. (2008). The extent of this color change is directly proportional to the antioxidant activity present in the extracts, as antioxidants react with the stable DPPH free radicals (Modinah *et al*., 2018).

The percentage of inhibition (% I or % SCV) or the radical scavenging activity of the extracts increased in a dose-dependent manner across various concentrations (12.5, 25, 50, 100, 200, 400 mg/mL). Specifically, the methanol extract showed inhibition ranging from 34.12% to 83.31%, the acetone extract ranged from 28.69% to 71.25%, the ethyl acetate extract ranged from 19.88% to 59.49%, the chloroform extract ranged from 9.54% to 56.46%, and ascorbic acid, used as a standard, ranged from 26.17% to 68.69%. The antioxidant properties were evaluated using the effective concentration (IC50), which is the sample concentration required to reduce the initial DPPH radical absorbance by 50% (Priyanka *et al*., 2012). The IC50 values, which were determined by evaluating the extracts at various concentrations (12.5, 25, 50, 100, 200, and 400 ppm), inversely represented their antioxidant activity.

Table 5: Percentage of scavenging activity and IC_{50} value in *Datura fastuosa* root extracts.



	12.5	25	50	100	200	400	IC50
Methanol	34.12	40.12	49.49	56.17	66.88	83.31	8.85
Acetone	28.69	37.84	45.58	51.47	62.17	71.25	13.64
Chloroform	9.54	17.75	34.76	40.57	48.11	56.46	28.06
Ethyl acetate	19.88	31.48	41.31	47.62	56.49	59.49	21.72
Ascorbic acid	36.17	44.26	53.45	58.58	69.25	88.69	5.87

Conc. ppm = concentration in parts per million, % Av.SCV = % average of scavenging activity, IC_{50} = the concentration of an inhibitor where the response is reduced by half or IC_{50} is the effective concentration of the extract that scavenges 50% of the DPPH radical.

3.5. Antimicrobial activities

(1) Antibacterial activities

The antibacterial activities of the extracts of roots of *Datura fastousa* were evaluated the agar disc diffusion method on for separate bacterial isolates (2 Gram-positive and 2 Gram-negative bacteria).

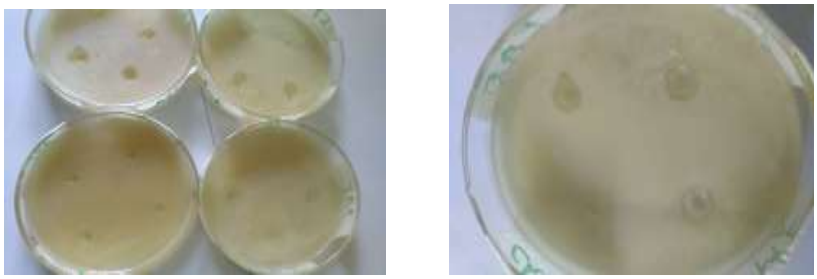
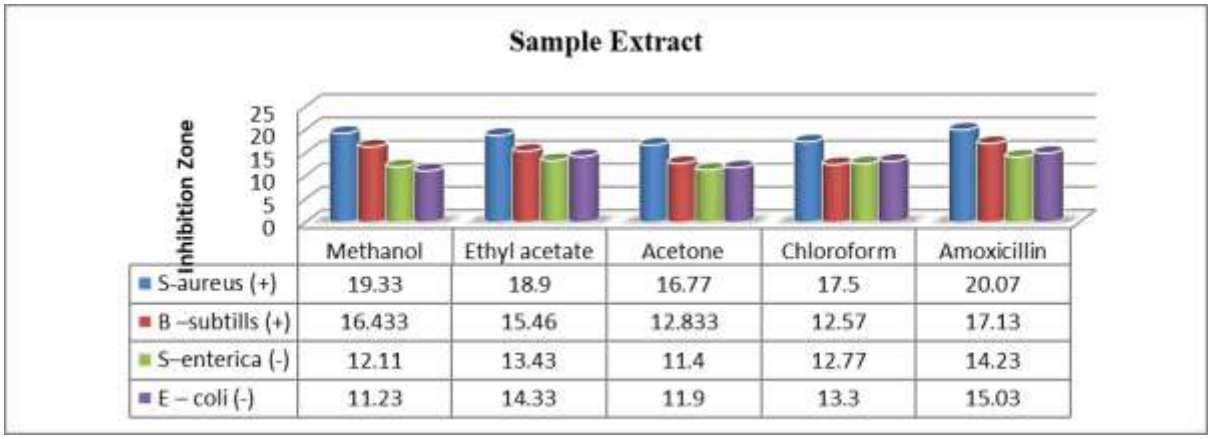


Figure 1: Inhibition Zone of both gram (+) and gram (-) bacteria.

The methanol root extract exhibited the largest zone of inhibition against S. aureus (+) with a diameter of 20.30 ± 0.16 mm, followed by B. subtilis (+) with 17.43 ± 0.63 mm. The smallest zone of inhibition was observed against S. enterica (-) at 12.11 ± 0.16 mm, and E. coli (-) at 11.23 ± 0.04 mm. The ethyl acetate extract demonstrated the second-largest zone of inhibition after the methanol extract, with 18.90 ± 0.33 mm against S. aureus (+) and 15.47 ± 0.21 mm against B. subtilis (+). It also showed the largest inhibition zone against E. coli (-) at 14.33 ± 0.12 mm and S. enterica (-) at 13.43 ± 0.09 mm among all the extracts. However, the zones of inhibition for E. coli (-) and S. enterica (-) were still minimal for the ethyl acetate extract.

Chloroform and acetone extracts displayed minimal zones of inhibition across all bacterial strains: S. aureus (17.50 ± 0.24 and 16.77 ± 0.32 mm), B. subtilis (12.57 ± 0.25 and 12.83 ± 0.21 mm), S. enterica (12.77 ± 0.12 and 11.4 ± 0.08 mm), and E. coli (13.3 ± 0.21 and 11.9 ± 0.38 mm) at a concentration of 50 mg/mL of the root extract. Overall, methanol and ethyl acetate extracts exhibited greater antibacterial activity compared to acetone and chloroform extracts. The inhibition effect generally decreased as the volume of the solvents used increased.

Table 6: Mean inhibition zone of four solvent extracts of *Datura fastuosa* root at concentrations of 0.5 mg/mL, on different test bacteria in comparison with Amoxicillin.

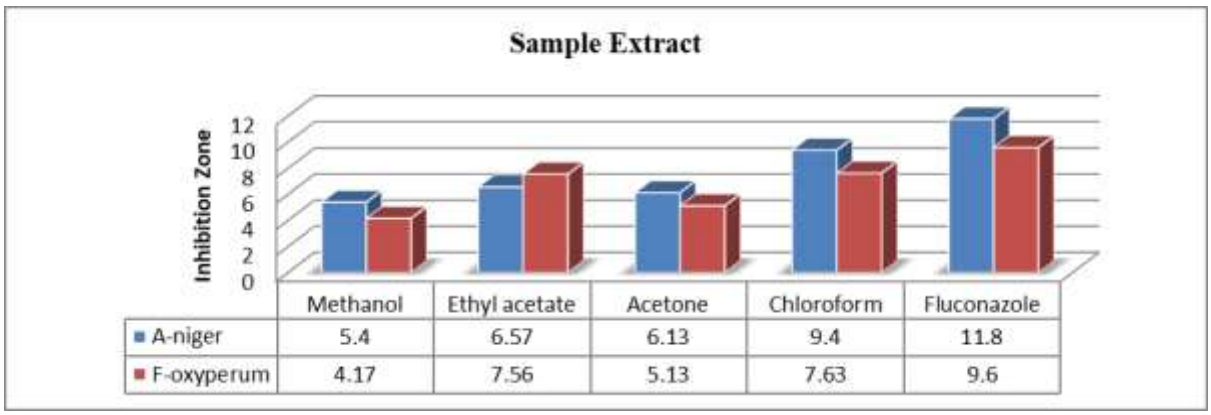


	Methanol	Ethyl acetate	Acetone	Chloroform	Amoxicillin
S-aureus (+)	19.33	18.9	16.77	17.5	20.07
B –subtills (+)	16.433	15.46	12.833	12.57	17.13
S–enterica (-)	12.11	13.43	11.4	12.77	14.23
E – coli (-)	11.23	14.33	11.9	13.3	15.03

(1)Antifungal activities

The extracts' antifungal qualities were assessed on potato dextrose agar (PDA) medium using the paper disc diffusion method (Laport *et al*., 2012).

Table 7: Mean inhibition zone of extracts of root of *Datura fastuosa* at concentrations of 0.5 mg/mL on fungi in comparison with Fluconazole.



	Methanol	Ethyl acetate	Acetone	Chloroform	Fluconazole
A-niger	5.4	6.57	6.13	9.4	11.8
F-oxyperum	4.17	7.56	5.13	7.63	9.6

The methanol and acetone extracts displayed the smallest zones of inhibition: 5.4 ± 0.21 mm and 6.13 ± 0.17 mm against A. Niger, and 4.17 ± 0.63 mm and 5.13 ± 0.09 mm against F. oxysporum, respectively, at a concentration of 50 mg/mL of the root extract. Among these, the methanol extract showed the least inhibition against both fungal species.

4. DISCUSSION

4.1. Extraction yield

The highest yield was achieved with methanol at 4.9% w/w, while ethyl acetate produced the lowest yield at 0.39% w/w. This variation is likely due to methanol's superior solubility for bioactive components such as polyphenols, phenolics, steroids, and other bioactive molecules

compared to acetone, ethyl acetate, and chloroform (Silva *et al*., 2014). The differences in extraction yields are attributed to the varying polarities of the solvents, which influence the solubility of phytochemical compounds (Naima *et al*., 2015). The solubility of these compounds is also affected by their structural properties and the polarity of the solvents used (Felhi *et al*., 2016a). The solvents can be ranked by polarity as follows: chloroform (4.81/4.1), ethyl acetate (6.02/4.4), acetone (20.7/5.1), and methanol (32.7/5.1) (Naima *et al*., 2015).

4.2. Phytochemical screening of root extracts of *Datura fastuosa*

The yields obtained from the extraction procedures employing methanol, acetone, ethyl acetate, and chloroform were utilized to screen for secondary metabolites, such as phenols, alkaloids, flavonoids, steroids, tannins, saponins, terpenoids, and triterpenoids. Table 2 presents the findings of the phytochemical screening of *Datura fastuosa*, which show a wide variety of unique phytochemical substances. Phytochemical screening is necessary to identify the chemical components of plant extracts and to identify bioactive chemicals that can be utilized as building blocks for the manufacturing of valuable pharmaceuticals (Yakubu *et al*., 1986). Compounds with pharmacological properties, such as antiviral, antioxidative, hepatoprotective, antibacterial, anti-inflammatory, and anticancer activities, include flavonoids, phenolics, steroids, and terpenes (Kumar *et al*., 2013; Saxena *et al*., 2013).

The methanol extract had substantial concentrations of phenols, alkaloids, flavonoids, and saponins, and the acetone extract did as well. The chloroform extract exhibited substantial amounts of steroids, while the ethyl acetate extract was noteworthy for having high levels of both tannins and steroids (Table 2). These findings highlight the diverse range of natural chemicals included in the extracts, which are significant from a medicinal perspective. According to Ambasta *et al*. (2005), phytochemical research is crucial to identifying new sources of medicinal plant compounds with both therapeutic and commercial applications.

4.3. Quantitative phytochemical evaluation

The quantitative phytochemical evaluation of *Datura fastuosa* root extracts across various solvents reveals that flavonoids and phenols are present in relatively high concentrations, while alkaloids are also found in notable amounts, as detailed in Tables 3 and 4. In terms of phenolic content, the methanol extract performed much better than the acetone, ethyl acetate, and chloroform extracts ($P < 0.05$). Additionally, the acetone extract had a significantly higher phenolic content than the ethyl acetate and chloroform extracts, whereas ethyl acetate

also had a slightly higher phenolic content than chloroform ($P < 0.05$). As shown in below table 4, methanol was the most effective solvent for extracting phenolic compounds, followed by acetone, ethyl acetate, and chloroform.

4.4. Antioxidant Potential

These extracts' antioxidant activity was also assessed, and the methanol extract showed the strongest antioxidant activity. According to research by Fereidoon and Ying, total phenolic content (TPC) is a commonly used metric to evaluate antioxidant extracts and is a crucial indicator of total antioxidant capacity (TAC) (Fereidoon and Ying, 2015). Since these substances can successfully impede free radical reactions, the current investigation validates a strong link between total phenolic content and antioxidant activity (Raheel *et al*., 2015). TPC and antioxidant activity are thus directly correlated.

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According to the results of the DPPH experiment, longer reaction durations and greater scavenging activity are correlated with lower IC50 values (Solomon, 2015). Strong antioxidant activity is shown by the IC50 values for the standard and all extracts.

All extracts demonstrated significant antioxidant potential when compared to the standard ascorbic acid, with notable variations in DPPH scavenging activity values ($P < 0.05$), ranging from 12.5 to 400 mg/mL AAE/g of the extract. The methanol extract exhibited the highest

antioxidant potential among the tested solvents, followed by acetone, ethyl acetate, and chloroform, which was the least effective.

When comparing the antioxidant activities of the extracts to ascorbic acid, the polar solvents (methanol and acetone) showed the greatest scavenging potential, as indicated by the smallest IC50 values. In contrast, the less polar solvents (ethyl acetate and chloroform) were less efficient. Specifically, the antioxidant capacity of the root extracts, as measured by DPPH scavenging activity, revealed that the methanol extract had an IC50 of 8.85 µg/mL, which was slightly lower than the standard ascorbic acid (IC50 of 5.87 µg/mL), but higher than the acetone (IC50 of 13.64 µg/mL), ethyl acetate (IC50 of 21.72 µg/mL), and chloroform (IC50 of 28.06 µg/mL) extracts. All extracts and the standard demonstrated significant inhibitory activity in a concentration-dependent manner.

Compounds such as phenolics, flavonoids, steroids, and terpenoids, which contain hydroxyl groups, are responsible for the radical scavenging activity observed in the plant (Sabine et al., 2019). The percentage of inhibition and IC50 values for methanol, acetone, ethyl acetate, and chloroform extracts were compared to ascorbic acid (Modinah et al., 2018). The results indicate that the root extracts of Datura fastuosa have considerable antioxidant capacity and can trap DPPH radicals similarly to the reference antioxidant ascorbic acid. Antioxidant molecules such as ascorbic acid, tocopherol, flavonoids, and tannins induce DPPH decolorization due to their hydrogen-donating ability (Jan *et al*., 2013) and may be useful in treating oxidative stress-related pathologies (Modinah *et al.*, 2018). Overall, the methanol, acetone, ethyl acetate, and chloroform extracts of the plant roots exhibited substantial antioxidant capacity. The diverse compounds present in these extracts demonstrate significant potential for radical scavenging.

4.5. Antimicrobial Potentials

Antimicrobial agents are valuable in treating infections because they target and kill harmful microorganisms without damaging the host's normal cells (Muhammad *et al*., 2018). Datura species are known for their antimicrobial properties, attributed to phytochemical compounds such as steroids, terpenoids, polyphenols (including flavonoids), phenolics, and alkaloids (Kuang *et al*., 2009). Additionally, β-carboline alkaloids, flavonoids, and phenolic acids contribute to their antimicrobial activity (Okwu and Igara, 2009). This study provides an overview of the antimicrobial activities of Datura fastuosa root extracts against four bacterial

species (two Gram-positive and two Gram-negative) and two fungal species, as detailed in Tables 6 and 7.

The results of the qualitative phytochemical screening showed that saponins were found in negligible amounts in the ethyl acetate extract of the root samples but absent from the chloroform extract. Due to of this low saponin content, the antibacterial activity has a lower inhibition zone. Because of their well-known antibacterial qualities, saponins help shield plants from insect infestations and the growth of mildew (Godwin *et al*., 2017). These substances, which are a component of plant defense mechanisms, have been shown to have a hypocholesterolemic effect by decreasing the absorption of nutrients like glucose and cholesterol in insects' guts through intraluminal physicochemical interactions (Aberoumand, 2012).

All things considered, extracts of *Datura fastuosa* roots in methanol, acetone, ethyl acetate, and chloroform showed antibacterial action. By interacting with and disrupting the proteins in the cell membranes, the phenolic chemicals in the extracts most likely prevent the growth of bacteria and ultimately cause their death (Mangunwardoyo *et al*., 2012). Gram-positive bacteria were shown to be more vulnerable to the plant extracts than Gram-negative bacteria. According to Ibrahim *et al.* (2017), this implies that the plant may have antibacterial properties to fight diseases that cause wounds. Additional investigation is required to examine the root extract's potential as a model for creating new medications to treat wound infections and other conditions, including extraction using various solvents and toxicity assessments of the extract.

Regarding the fungal species Aspergillus niger (A. niger) and Fusarium oxysporum (F. oxysporum), both highly polar and low-polar solvent extracts demonstrated significant antifungal activity The four unrefined extracts of *Datura fastuosa* roots were methanol, acetone, ethyl acetate, and chloroform. Of them, the chloroform extract showed the largest inhibitory zones, measuring 9.4 ± 0.08 mm against A. niger and 7.63 ± 0.28 mm against F. oxysporum. In comparison to other solvent extracts, the chloroform extract exhibited greater antifungal effectiveness against both fungi, as seen by the broader inhibition zones. After chloroform, ethyl acetate was the second most effective extract for both fungal species. It demonstrated the second-largest inhibition zone (7.56 ± 0.9 mm) against F. oxysporum.

Overall, the chloroform and ethyl acetate extracts demonstrated superior antifungal activity compared to the acetone and methanol extracts. Notably, the chloroform extract exhibited the most pronounced antifungal effect.

5. CONCLUSION

Lastly, the study found that *Datura fastuosa* root extracts have strong antioxidant, antifungal, and antibacterial properties in addition to noteworthy phytochemical components. Due to its high content of polyphenolic components, including flavonoids and phenolics, the methanol extract showed the highest level of antioxidant activity. This suggests that the plant might contain novel antibacterial and antioxidant chemicals. Further research is necessary to identify and isolate the active compounds responsible for these antioxidant and antibacterial properties.

6. Author contributions

- Girma Mengesha Melese performed the experimental work, conducted data analysis, interpreted the results, and authored the initial manuscript.
- Kefita Kaba Deykanto contributed to the project in terms of design, reviewing, as well as the analysis and interpretation of the results.
- Belachew Boranto Borale played a significant role in providing guidance for result interpretation, contributing to the reviewing process, and offering overall research support.
- Birhanu Zeleke Tilinti; Conceptualization, Revising and finalizing of the full Manuscript paper
- Amanuel Dubale Kussia, Writing - review & editing, and Data curation of the full Manuscript paper.

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9. Conflict of interest

The authors declare no conflict of interest.

10. Ethics committee approval

This article does not involve any studies conducted on human participants or animals by any of the authors.

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