# PHYTOCHEMICAL SCREENING, INVESTIGATION OF ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF ROOT EXTRACTS OF *WORQ BEMEDA (Dorstenia foeteda)* FROM AWI ZONE, AMHARA REGION, ETHIOPIA

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***ABSTRACT***

*Dorstenia foeteda which is a succulent plant in the genus Dorstenia is a typical traditional medicinal plant in Amhara and Benishangul regions, Ethiopia used for the treatment of most of fungal and protozoan diseases.. The phytochemical screening result showed the presence of the most secondary metabolites in the extracts. The total antioxidant capacity (TAC), total flavonoid content (TFC), and antioxidant analysis result also showed a strong positive correlation between total phenolic content (TPC), TFC, and antioxidant power of the extracts. The DPPH, FRAP, and Phosphomolybdate assays revealed significant antioxidant activity of the plant extracts. DPPH scavenging activities extracts lied from, 23.67 ± 0.71 (in chloroform) to 70.87 ± 1.32 µg AAE /mL (in acetone,) for extracts. Phosphomolybdate total reducing the power of extracts varied from 29 ± 1.00 (in hexane) to 32.6 ± 0.15 g AAE / kg (in acetone extract) dried powder of extract. Similarly, the TFC of extracts of Dorstenia varied from 33.4± 0.028 (in hexane) to 56.3 ± 0.013 mg QE/50 g(in methanol extract dry powder of extracts. Moreover, TPC of the extract varied between 500 ± 0.037 (in hexane) to 662.8 ± 0.035 mg GAE/50g (in acetone) for the root extract of dried powder. The cumulative investigation of the study leads us to the conclusion that the plant has metabolites that contribute medicinal value. Therefore, it is recommended strongly that to isolate metabolites and antioxidant components.*

*Key-words: antioxidant, DPPH assay, Dorstenia foeteda, total antioxidant capacity, total flavonoid content, total phenolic content.*

## *1.1.* INTRODUCTION

More than 80% of the world's population relies on traditional medicine to meet their daily health requirements [1].This is partly due to accessibility, efficacy in treatment, and afford cost compared to Western medication [2]. Medicinal plants were regularly used by people in prehistoric times for biomedically curative and psychotherapeutic purposes [3]. Knowledge of medicinal plants has resulted from trial and error methods, and often based on speculation and superstition [4]. Nearly 50,000 species of higher plants have been used for medicinal purposes, and they are also used in food, cleaning, personal care, and perfumery [3].

Traditional medicine [TM] is regarded as a combination of knowledge, skill and practice originated from theories, experiences and attitudes native to various cultures that is utilized to treat and diagnose enormous types of physical and mental complications and maintain health [5]. Traditional knowledge of medicinal plants is important for modern medicine development [6]. Major pharmaceutical drugs have been derived from biological diversity [7]. For example, Aspirin was discovered independently by residents of both the New and Old worlds as a remedy for aches and fevers [8]. Despite the wide utilization of medicinal plants for healthcare, medicinal plants across Africa are poorly documented [9].



Dorstenia is a predominately Old and New World plant genus within the mulberry family, Moraceae. Depending on the author, there are said to be 100 to 170 species within this genus, second only in number to the genus Ficus within Moraceae. Dorstenia species are mainly known for their unusual inflorescences and growth habits. [10]. Dorstenia L. holds a fascinating position within the Moraceae (mulberry family) With 105 species, Dorstenia is the second largest genus within the family, second only to Ficus L., and is the only Moraceae genus with herbaceous, succulent and woody species [11]. The Dorstenia Moraceae are comprised of 37 genera and 1100 species. The family exhibits cosmopolitan distribution, with the majority of extant species found in the Old World tropics [12]. Molecular studies have shown that Moraceae is a well-supported monophyletic group, although taxon sampling for Dorstenia within the study was very poor, including only two out of 105 species [13].

The vast majority of Ethiopia's population lives in rural areas where the health care coverage is low and where existing public sector resources are being stretched to the limits. One of the greatest challenges facing the country is determining how best to narrow the gap between the existing services and the population whose access to them is very limited [12]. Hence, TM is broad and diverse. Consequently, different societies have evolved different forms of

indigenous healing methods that are captured under the broad concept of TM, e.g. Chinese, Indian, and African traditional medicines. This explains the reason why there is no single universally accepted definition of the term. This notwithstanding, one of the most accepted definitions of TM has been provided by the World Health Organization (WHO).

A total of 122 biologically active compounds have been identified, derived only from 94 species of plants. Consistent findings should be carried out to discover a probable abundance of medicinal extracts in these plants [17].

Plant remedies are the most important source of therapeutics for nearly 80% of the population in Ethiopia and most of the knowledge is still in the hands of traditional healers [7]. However: the great role of traditional medicine and medicinal plants in primary health care, little work has so far been done in the country to properly document and promote the associated knowledge covering only a few out of the estimated 80 or more socio-cultural (language) groups in Ethiopia [13]. Dorstenia foeteda is known for its medicinal uses in Africa, including Ethiopia. People living in west Gojjam, Awi in Amhara region, and Shinasha people from Benishangul Gumuz use the plant as a traditional medicine to treat different types of diseases. While the Awi people are among those who use Dorstenia foeteda as traditional medicine.

# 2 METHDOLOGY

## 2.1 Chemicals and reagents

The analytical grade chemicals and reagents used in this study were acetone (99%, Blulux India), HPLC methanol (99.9%, sigmalderic Israel), chloroform (99.8%, Lobal chemia, India), hexane (99.9%, Lobal chemia India), ferric chloride (99%, BDH), Iodine in potassium iodide (Lobal chemia, India), aluminum chloride (99%, BDH), potassium acetate (99%, BDH) , hydrochloric acid (35.5%, Lobal chemia India), sulfuric acid (98%, Lobal chemia, India), sodium hydroxide (98%, Blulux, India), nitric acid (70%, Blulux, India), sodium carbonate (99.5%, Blulux India), iodine (95%, Blulux India) sodium di hydrogen phosphate (99%, Blulux India), di sodiumhydrogenphosphate (99%, Blulux, India) phosphoric acid(85%, Blulux India) sodium molybdate (99.5%, Oxoid UK), sodium tungstate (98%, BDH), trichloroaceticacid (98%,sigmalderic, Israel), potassium hexacyanoferrate (99%, BDH), iron chloride (99%, Oxoid CM, UK), ascorbic acid (99%, Blulux, India), gallic acid (99%, Oxoid CM, UK), DPPH (99%, bio chemical's and reagents, quercetin (85%, Blulux, India), ammonia solution (85%, Blulux India), a Standard antibiotic disc 'Tetracycline disc and gentamycin' (HI media laboratories, India) and Mueller Hinton agar (Oxoid CM,UK) were used for this experiment.



## 2.2 Instruments and equipment

The necessary apparatus and instruments used in this study were top-loading electronic balance (India), vacuum rotary evaporator (RE-2S-VD, Germany), UV-vis spectrophotometer (Cary 60, Agilent technologies, China) digital pH meter(China), Watt man No.1 filter paper (cam lab UK), Freeze dryer (Turkish), juice maker(Hungary), electrical grinder (India) were among the equipment used.

## 2.3 Sample Collection

The plant known by its local name Worq bemeda (Amharic) was authenticated by a botanist in the department of biology, Bahir Dar University to be Dorstenia Foeteda. After authentication 5 Kg of Dorstenia Foeteda was collected from the selected Zigem (wingi) and Jawi (work bemeda) sites.

## 2.4 Description of the study area

Agew Awi zone, which consists of 7 city administrations and nine woredas' is one of the 10 Zones in the Amhara Region of Ethiopia. Awi zone covers the area of 9148.43 km2. It is bordered on the west by Benishangul-Gumuz Region, on the north by North Gondar Zone, and on the east by west Gojjam.. Most people use traditional medicine for the treatment of different diseases. by the help of Agew Kunfel and sorcerers (debtera's).Worq bemeda is a typical traditional drug in this zone. The samples were collected from the two woreda's in the Agew Awi zone, Zigem, and Jawi woredas'.

The samples were collected and kept in the icebox in the laboratory until treatment. The samples were washed with tap water to avoid soil and other impurities followed by distilled water, sliced into pieces, and put in to freeze dryer for 96 hours. Finally, the dried samples were ground separately by the electrical grinder

## 2.5 Extraction and sample preparation procedures

The extraction procedure was carried out by modifying the method used in [14].



50 g of separate dry powder of dorstenia foeteda was weighed into four 250 mL Erlenmeyer flasks and filled up to the mark with methanol, acetone, chloroform, and hexane, respectively. The flasks completely covered with aluminum foil were kept at room temperature for 48 hours with occasional shaking. While the filtrate for each was collected in a separate flask, the residue was further extracted for two rounds following the same procedure. Phytochemical screening

Phytochemical screening refers to the extraction, screening and identification of the medicinally active substances found in plants [15]. Some of the bioactive substances that can be derived from plants are flavonoids, alkaloids, carotenoids, tannin, Coumarins, terpenoids saponins glycosides, antioxidants and phenolic compounds. Phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of new drugs for curing of various diseases. This study was focused on screening of the phytochemicals in the crude extracts extracted using the four solvents (methanol, acetone, hexane and chloroform).

**i)** Detection of alkaloids (Wagner's Test)

Filtrates are treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids [16].

**ii)** Detection of phenols (Ferric Chloride Test)

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish- black color indicates the presence of phenols [17].

**iii)** Detection of flavonoids

2 mL of the extract was treated with 2 mL of dilute $NH_3$ solution and a few drops of concentrated sulphuric acid. Appearance of yellow color formed which indicates the presence of flavonoid [16].

**iv)**Detection of terpenoids (Salkowski's Test)

2 mL of chloroform was added to plant 0.5 g of extract in a test tube four droplets of concentrated sulfuric acid was added. Formation of a reddish brown interface confirms the presence of terpenoids [17].

**v)** Detection of steroids (Salkowski's test)

2 mL of chloroform extract, 1 mL of concentrated $H_2SO_4$ acid was added carefully along the sides of the test tubes. A **red color** was produced in the chloroform layer and confirms the presence of steroids [18].

**vi)**Detection of glycosides

A small amount of extract was dissolved in 1 mL of water and the aqueous NaOH solution was added. Formation of **yellow** color indicates the presence of glycosides [19].

**vii)** Detection of saponins

To a little amount of each of the sample in a test tube, 2 mL of distilled water was added and vigorously shaken for 15 minutes. Formation of 1 cm foam confirms a positive result [16].

**viii)** Detection of tannins (ferric chloride test)

2 mL of the aqueous extract was added to 2 mL of water, a 1 to 2 drops of diluted ferric chloride solution was added. **A dark green or blue -green** color indicates the presence of tannins [18].

**ix)**Detection of Coumarins

3 mL of the extract was treated with 3Ml of 10% of NaOH the formation of **yellow** color indicates the presence of Coumarins [20].

## 2.7 Analytical methods

### 2.7.1 Determination of antioxidant activity

#### 2.7.1.1 DPPH radical scavenging assay

DPPH in oxidized form gives a deep violet color in methanol to yellow. The free radical scavenging activities of the extracts were determined by using 2,2- Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. DPPH is a very stable nitrogen-centered free radical which produces purple color in methanol solution [21]. Unlike in vitro generated free radicals such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions. A freshly prepared DPPH solution exhibits a deep purple color with an absorption maximum at 517 nm. This purple color generally fades when antioxidant molecules quench DPPH free radicals yellow (i.e. by providing atoms or by electron donation, conceivably via a free-radical attack on the DPPH molecule) and convert them into a colorless /bleached product (i.e. 2, 2-diphenyl-1-hydrazine, or substituted analogous hydrazine), resulting in a decrease in absorbance at 517 nm band. DPPH in oxidized form gives a deep violet color in methanol to yellow.



(nonradical) An antioxidant compound donates the electron to DPPH thus causing its reduction and in reduced from its color changes from deep violet to yellow [35]. A 100 mL fresh 0.002% solution of DPPH was prepared in methanol. 20, 40, 60, 80, and 100 ppm ascorbic acid and20,40,60 ppm(µg/mL) of plant extracts were prepared and ascorbic acid was used as reference (standard). The absorbance was again recorded at 517 nm. The percentage inhibition of DPPH by extracts was calculated by using the following formula.

% inhibition=(Acontrol-Asample)/Acontrol×100

Where A control is the absorbance of pure DPPH in oxidized form and A sample is the absorbance of a sample taken after 15 minutes of reaction with DPPH96 [21].

Preparation of standard solutions:

DPPH standard: It was prepared by dissolving 0.002 g 0f DPPH powder in 100 mL methanol and stored in the brown bottle until use at -4 0C [21].

Ascorbic acid standard: Ascorbic acid stock solution was prepared by dissolving 250 mg ascorbic acid powder in 1000 mL of distilled water. Working standard solutions of AA in the range 20-100 ppm were prepared by serial dilution from the stock solution.

Ferric reducing antioxidant power (frap) assay

The reducing power of the crude extracts was determined according to the standard method [21] with minor modification.

Percentage (%) reduction power = ((A-B))/B×100

Where A = Absorbance of sample and B = Absorbance of blank

**Determination of Total antioxidant activity by Phosphomolybdate assay**

To carry out a Phosphomolybdate assay, the procedure reported elsewhere was followed [22]. Briefly: the Phosphomolybdate reagent was prepared by mixing equal volumes (100 mL) of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. Test samples were prepared by dissolving 1 mg of plant methanol extract or any of its sub-fractions in 1 mL of methanol. Then, 0.1 ml of the sample was dissolved in 1 mL of reagent solution in a test tube which was capped with silver foil and incubated in a water bath at 95 °C for 90 min. After cooling the sample to room temperature, the absorbance was observed at 695 nm against a blank. Ascorbic acid was used as a standard.



### 2.7.2 Estimation of total flavonoid content (TFC)

An aluminum chloride complex-forming assay was used to determine the total flavonoid content of the extracts. Quercetin was used as standard and flavonoid content was estimated as Quercetin equivalent. A calibration curve for quercetin was drawn for the calculation of unknown concentration [23].

### 2.7.3 Estimation of total phenolic content (TPC)

Phenolic compounds are secondary metabolites that are produced in the shikimic acid of plants and pentose phosphate through phenylpropanoid metabolization [24]. The total phenolic content of the crude extract was determined by using the Folin-Ciocalteau method [24].

Folin Ciocalteau reagent (FCR) was prepared from sodium tungstate and sodium molybdate in the presence of 85% phosphoric acid following the standard procedure[24].

The total phenolic content of the extract samples measured in triplicate was reported as mg GAE /g of dried powder of extract.

250 mL of 120 µg/mL stock solution of gallic acid was prepared by dissolving 30 mg of gallic acid powder in distilled water. Finally, 20-100 µg/mL working solutions were prepared by serial dilution of the stock solution [25]. To each 1 mL standard gallic acid working solution, 5 mL of Folin-Ciocalteau reagent was added and allowed to stand for 6 minutes. Then, 4 mL of 10% sodium carbonate was added to the reaction mixture (to change the phenol group to phenol oxide unless Folin cannot react with phenols). The absorbance was recorded at 765 nm after being incubated for 30 minutes. The absorbance of the plant extracts was measured following the same procedure as for the gallic acid using which the total phenolic content of the methanol, acetone, chloroform, and hexane extract of the root and leaf parts of the plant material was calculated using the equation below as Gallic acid equivalents (µg/mL).



. $$T = C\frac{V}{M}$$

Where T is the total phenolic content in mg/g of the extracts as GAE, C is the concentration of Gallic acid established from the calibration curve in mg/mL, V is the volume of the extract solution in mL and M is the weight of the ex-tract in g. All the experiments were performed in triplicate results.

### 2.7.3 Antibacterial test

An important task of a clinical microbiological laboratory is the performance of antimicrobial susceptibility testing of significant bacterial isolates. The goal is to detect drug resistance in common pathogens and to assure susceptibility to drugs of choice for a particular infection. not only this but also it is very important in the textile and leather industry.

Agar diffusion method

5 mm diameter sterile discs obtained from Whatman No 3 paper were placed on the surface of the inoculated agar in Petri dishes on to which 20 µL of each test solution ware applied. After the

addition of test solutions on the discs, the test solution was allowed to diffuse for 5 minutes and the plates were kept in an incubator at 37 °C for 24 hrs. The antibacterial activity was evaluated by measuring the zone of growth inhibition surrounding the discs in millimeters with a ruler and results were expressed as mean ± SD of replicate tests [26,25]. The disk diffusion assay was used as a preliminary test to select the most efficient extracts.

Data Management and statistical analysis

Most experiments results are expressed as mean ± SD values for triplicate measurements. Origin Pro 8software to draw the calibration curve for the standard solutions and SPSS software version 22 for one-way ANOVA analysis were used and evaluate the statistical significance, respectively.

# 3 RESULTS AND DISCUSSION

## 3.1 Yield of the extracts of Dorstenia foeteda



The mean mass of crude extract, and the percent yield of the extracts of Dorstenia foeteda root extracts (50 g dry powder) are summarized in Table 1.

Table 1**:** Summary of the mass and percent yield of crude extracts of *Dorstenia foeteda* using different solvents.

| Type of solvent | Plant part | Mass of crude extract (g) | % yield |
|---|---|---|---|
| Acetone | Root | 4.7±0.1 | 9.4 |
| Hexane | Root | 4.2±0.02 | 8.4 |
| Chloroform | Root | 3.36±0.01 | 6.7 |
| Methanol | Root | 10.2±0.18 | 20.4 |

The highest yield was obtained from methanol extract of Dorstenia foeteda. This might be due to the high extracting ability of the solvent. Therefore methanol is good extracting solvent. Whereas the lowest result was obtained from hexane extracts of Dorstenia foeteda. This might be the presence of polar metabolites in the extracts which are not extracted easily by nonpolar solvents. Generally, four extracting solvents have better-extracting ability.

### 3.1.1 preliminary phytochemical analysis

Preliminary phytochemical tests are helpful in finding information about chemical constituents present in the plant material [15] which facilitates quantitative estimation and bioassay-guided separation of pharmacologically active compounds from the plant. Phytochemical screening of the crude extracts of the Dorstenia foeteda using the four solvents revealed the presence of all the investigated secondary metabolites in almost all the extracts although with different signal intensity signifying their concentrations (Table 2). As can be seen from the table, while tannins, phenols, Coumarins, and glycosides were detected in all the extracts, the remaining components (alkaloids, flavonoids, terpenoids, and saponins) were only detected in some of the extracts.

Table 2: Summary results of phytochemical screening analysis

| Phytochemicals | reagent | Solvents | | | |
|---|---|---|---|---|---|
| | | Methanol | Acetone | Chloroform | Hexane |
| Alkaloids | Wagner | + | + | + | + |
| Flavonoids | $H_2SO_4$ | + | + | - | + |
| Tannins | ferric chloride | + | + | + | + |
| Terpenoids | Salkowski's test | + | + | + | + |
| Phenol | ferric chloride test | + | + | + | + |
| Coumarins | NaOH test | + | + | + | + |
| Glycosides | NaOH test | + | + | + | + |
| Saponins | Foam test | - | + | - | - |



### 3.2.1 Antioxidant analysis

#### 3.2.1.1 DPPH radical scavenging assay

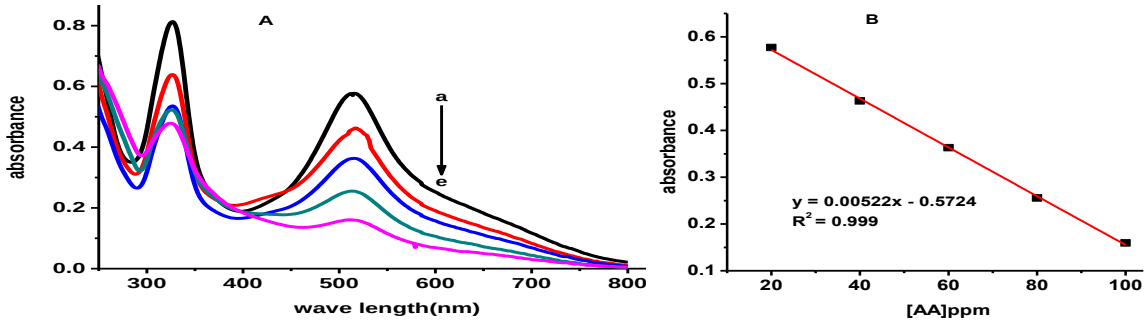


**Figure 3.1:** UV-Vis spectra of (A) ascorbic acid of various concentrations (a-e: 20, 40, 60, 80, and 100 µg/mL, respectively) all spiked with 0.002% DPPH in methanol, and (B) plot of absorbance vs concentration of ascorbic acid.

During the experiment, color change of the DPPH from purple to yellow was observed with the addition of AA, indicating scavenging (protonation) of the DPPH by the AA added (Fig. 3.1A) [21]. Moreover, Fig 3.1B revealed linear dependence of the absorbance of the DPPH on the concentration of AA in the range 20-100 ppm with a determination coefficient (R2) of 0.999.

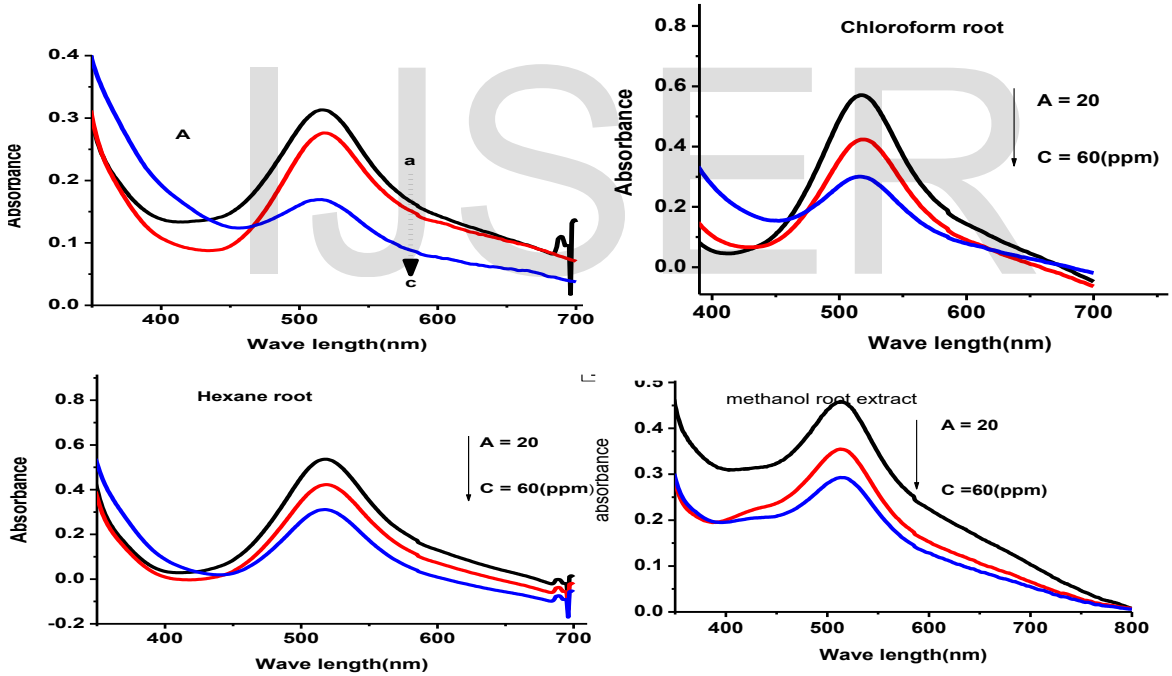


**Figure 3.2:** UV-Vis spectra of crude root extracts in various solvents (A-D: acetone, chloroform, hexane, and methanol, respectively) of various concentrations (a-c: 20, 40, and 60 ppm, respectively).

The UV-Vis spectra of absorbance of various concentrations of crude extracts using different solvents mixed with a constant amount of DPPH were recorded (Fig3.2) and the resulting absorbances were compared against the absorbance of the control (DPPH) (Table 3). As can be seen from the table, the absorbance for different concentrations of extracts using the same

solvent decreased with increasing concentration of the crude extracts irrespective of the type of plant part and solvent used for extraction. While acetone extract of the sample showed the least antioxidant property, Methanol extract showed the highest antioxidant property among all the studied samples and solvents. With this regard, the concentration of antioxidant activity of the crude plant extract as calculated using the regression equation ranged between 334.8 µg / mL in chloroform extract to 563 µg /mL acetone extract.

Table 3: Summary of absorbance of various concentrations of root extracts in various solvents spiked with constant DPPH

| Root extract solution (ppm) | Absorbance | | | | | |
|---|---|---|---|---|---|---|
| | AA/ methanol | type of solvent for extraction | | | | Control (DPPH) |
| | | methanol | acetone | chloroform | Hexane | |
| 20 | 0.4634 | 0.4556 | 0.3130 | 0.5670 | 0.5384 | |
| 40 | 0.4632 | 0.3547 | 0.2761 | 0.4192 | 0.4170 | 0.57725 |
| 60 | 0.2560 | 0.2499 | 0.1695 | 0.3249 | 0.3133 | |



Table 4: % Inhibition of *Dorstenia foeteda* root extracts

| Type of solvent used for extraction | crude extract (ppm) | % of inhibition |
|---|---|---|
| Acetone | 20 | 45.5 |
| | 40 | 52.2 |
| | 60 | 70.6 |
| chloroform | 20 | 1.77 |
| | 40 | 27.4 |
| | 60 | 43.7 |
| Hexane | 20 | 6.73 |
| | 40 | 27.75 |
| | 60 | 45.7 |
| Methanol | 20 | 21.1 |
| | 40 | 38.6 |
| | 60 | 56.6 |

Table 4: Presents the DPPH % inhibition calculated for each extract using the four solvents utilized. Among all the solvents considered in this study, acetone extract, in particular, showed the highest DPPH

% inhibition indicating its extracting ability of the compounds responsible for scavenging the DPPH and hence antioxidant property.

Acetone extract > methanol extract > hexane chloroform extract is decreasing the order of DPPH scavenging power of extracts. Except hexane chloroform extract the other solvents showed great DPPH scavenging power than the standard (at 20-60 µg/mL). This indicates the presence of potent antioxidants in the extract of Dorstenia foeteda. As the concentration of the sample increased, its DPPH scavenging power also increased. Acetone extract showed the highest scavenging power than the others. Whereas Chloroform and hexane, extracts showed the lowest scavenging power than the others. Methanol extract has competitive scavenging power of DPPH with the standard (ascorbic acid

Table 5: Summary of the absorbance and corresponding DPPH % inhibition of 60 µg/mL plants extracts using different solvents

| Plant part | Solvents | Crude extract concentration(µg/mL) | Absorbance | % DPPH inhibition* |
|---|---|---|---|---|
| Root | Acetone | 60:1 | 0.168 | 70.87 ± 1.320 |
| | | 60:2 | 0.160 | |
| | | 60:3 | 0.175 | |
| | Chloroform | 60:1 | 0.554 | 23.67 ± 0.709 |
| | | 60:2 | 0.549 | |
| | | 60:3 | 0.547 | |
| | Hexane | 60:1 | 0.413 | 29.01 ± 1.028 |
| | | 60:2 | 0.440 | |
| | | 60:3 | 0.403 | |
| | Methanol | 60:1 | 0.251 | 57.41 ± 0.913 |
| | | 60:2 | 0.240 | |
| | | 60:3 | 0.247 | |
| | *Mean ±SD for 60µg/mL, n =3 | | | |



As can be seen from Table 5, the DPPH % inhibition ability of the crude plant extracts was compared by taking 60 µg/mL crude extracts of the four solvents.

Comparing the % inhibition of the extracts, while chloroform extract showed the least DPPH % inhibition (23.67%), acetone extract showed the highest % inhibition 70.87 ± 1.320 which might be due to the presence of potent antioxidants with medium polarity in plant extract [21].

### 3.2.1.2 **Ferric reducing antioxidant po**wer

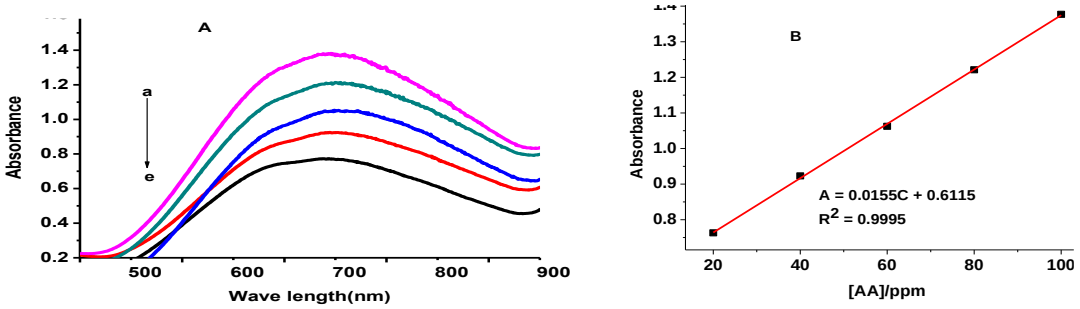


**Figure 3.3:** (A) UV-vis spectra of various concentrations of AA (a-e: 100, 80, 60, 40, and 20 µg/mL, respectively) at a constant amount of FRAP reagent, (B) plot of absorbance of AA vs concentration of ascorbic acid.

As indicated in figure 3. 5(A), the absorbance of ascorbic acid in the presence of the constant amount of the FRAP reagent described under the methodology part increased with increasing ascorbic acid concentration [22]. Dependence of the absorbance on concentration showed a linear correlation with a determination coefficient ($R^2$) of 0.9995 (Figure 3.3B).

Sample extracts of various concentrations were also prepared using different solvents which were further treated with the FRAP reagent as described under the experimental part.



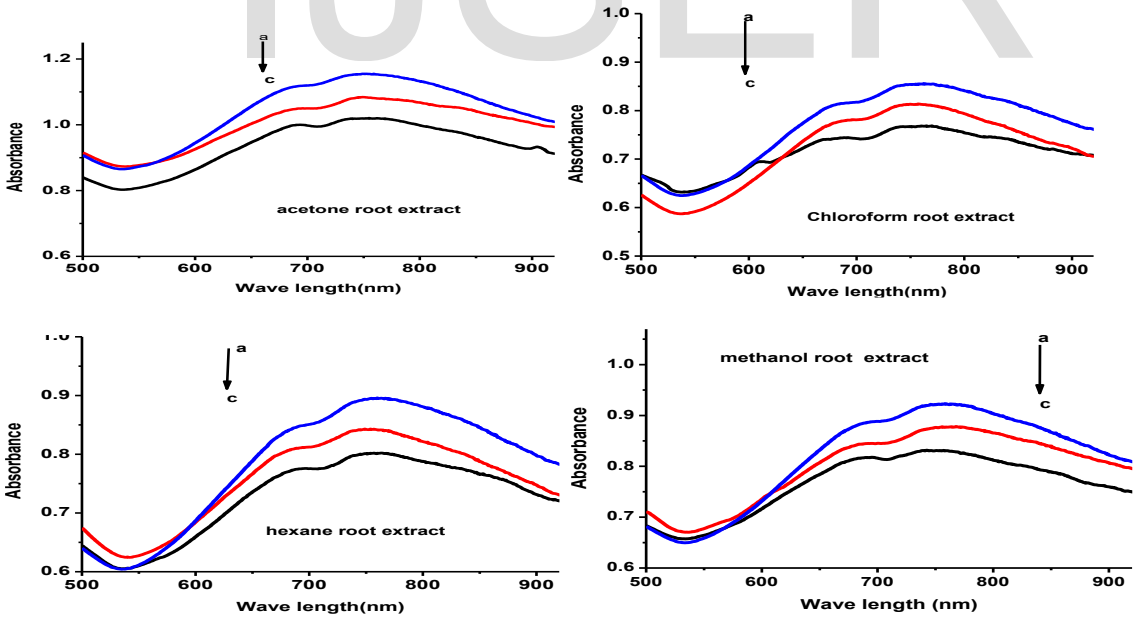


**Figure 3.4:** UV-Vis spectra of crude extracts (a-c: 60, 40, and 20 µg/mL, respectively) and plant extracts using various solvents for FRAP antioxidant determination.

Figure 3.4 presents the UV-Vis spectra of the plant extracts with different solvents. The absorbance at the characteristic wavelength of 700 nm for each analyzed sample was recorded from which reducing power was calculated using the regression equation.

**The** ability of the crude extract to reduce $Fe^{+3}$ to $Fe^{+2}$ (reducing effect) which served as an indicator of its antioxidant activity was determined following the method described [22]. Ascorbic acid, which is a dietary antioxidant was used for comparison. The absorbance of ascorbic acid mixed with the FRAP reagent showed a linear dependence on the concentration of ascorbic acid in the range 20-100 ppm with a determination coefficient ($R^2$) of 0.9995. With regard to this, the concentration of the antioxidant activity of the crude plant extract as calculated using the regression equation. As summarized in Table 6, while the reducing power of the extracts ranged between 55 in hexane extract to 197.4 µg AAE /mL acetone extract.

Table 6: Summary of absorbance of the plant extracts of various concentrations (20-60 µg/mL) treated with FRAP reagent and corresponding %reducing power

| Plant part | Type of solvent | Conc. (ppm) | absorbance | % Ferric reducing antioxidant power* |
|---|---|---|---|---|
| Root Sample | Acetone | 20 | 0.999 | 64.91±0.44 |
| | | 40 | 1.052 | 67.17±0.90 |
| | | 60 | 1.121 | 67.823±09 |
| | Chloroform | 20 | 0.809 | 53.60±0.93 |
| | | 40 | 0.841 | 58.43±0.45 |
| | | 60 | 0.865 | 57.77±0.97 |
| | Hexane | 20 | 0.754 | 54.07±1.10 |
| | | 40 | 0.793 | 56.47±0.50 |
| | | 60 | 0.827 | 58.26±0.91 |
| | Methanol | 20 | 0.872 | 61.57±1.40 |
| | | 40 | 0.953 | 63.37±0.35 |
| | | 60 | 1.070 | 67.60±1.22 |
| *Mean ±SD: n=3, | | | | |

The percent reducing power of each extract was calculated using equation below.

$$\frac{A-B}{A} \times 100\%$$

Where A = Absorbance of sample, B = Absorbance of blank

Absorbance of blank = 0.3552

% Reducing antioxidant power of acetone, chloroform, hexane and methanol extracts of Dorstenia foeteda were determined and the results were shown in the table 6. Among the four solvents used for extraction Acetone extract (20, 40 and 60 µg/mL) showed the highest reducing power whereas hexane and chloroform extracts showed comparably the least % reducing power (Table 6). The higher absorbance the higher would be reducing power. Although to a different extents, increment of reducing power was observed with concentration of crude extracts for all the studied solvents which is in agreement to the literature [22].

Table 7**:** Summary of ferric reducing power of 60 µg/mL crude extracts of *Dorstenia foeteda* using the four solvents (60 µg/mL)

| Plant part | Types of solvent | Mean absorbance | Reducing power (in mg /g Dried powder)* |
|---|---|---|---|
| Root | Acetone | 1.131 | 555± 0.05 |
| | Chloroform | 0.868 | 271.5 ± 0.61 |
| | Hexane | 0.827 | 227 ± 0.02 |
| | Methanol | 1.073 | 492 ± 0.25 |
| *Mean ±SD for 60 µg/mL, n = 3; | | | |

The reducing power of the crude extracts of Dorstenia foeteda using the four solvents is presented in table 7. As can be seen from the table, acetone extract showed the highest reducing capacity (555 ± 0.05) than the others. In contrast to the others, hexane extract showed relatively the least reducing activity (227± 0.025 mg/g of dry powder of extract).

3.2.1.3 **Phosphomolybdate assay**

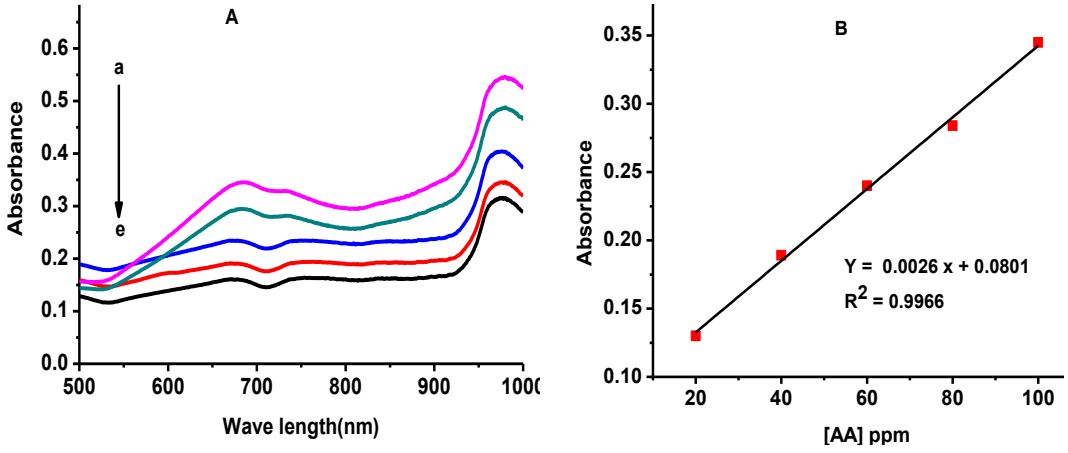

Figure 3.5: (A) UV-vis spectra of various concentrations of AA (a-e: 100, 80, 60, 40, and 20 µg/mL, respectively) at a constant amount of Phosphomolybdate reagent, (B) plot of absorbance of AA vs concentration of ascorbic acid.

The total antioxidant capacity of the different extracts of Dorstenia foeteda was also evaluated using the Phosphomolybdate method [22]. This assay is based on the reduction of Mo (VI) to Mo (V) in presence of the antioxidant compounds leading to the formation of a green phosphate/Mo (V) complex in acidic pH whose absorbance is measured at 695 nm. Figure 3.5(A) presents the UV-Vis spectra of various concentrations of ascorbic acid (20-100 µg/mL) in the presence of the constant amount of the Phosphomolybdate reagent. As can be seen from Figure 3.5(B), the absorbance of the resulting complex showed linear dependence with a determination coefficient of 0.9966. The measured absorbance's were then converted using the regression equation to the total antioxidant capacity of Dorstenia foeteda extracts expressed as equivalents of ascorbic acid (mg/mL). The total antioxidant capacity of the extracts was expressed as µg/mL AAE. The calculated AAE total antioxidant capacity of extracts ranged from 12.6 in hexane to 306 µg/mL in acetone extract confirming that while hexane extract is the least, acetone extract is the highest in terms of AAE total antioxidant capacity



In this study, acetone extract showed the highest total antioxidant capacity while hexane showed the least total antioxidant capacity. Generally, the total antioxidant activity of various samples decreased in the following order:

acetone extract > methanol extract > chloroform extract > hexane extract. Which was analogous to FRAP, TFC, and TPC assay.

Among all the solvents considered in this study, acetone extract, in particular, showed the highest total antioxidant capacity indicating its ability to extract components that are responsible reduce Mo (VI) to Mo (V) that the other solvents do.

### 3.3 Total phenolic content estimation

Phenolics or polyphenols are an important class of compounds found in the plant extract. The total concentration of phenols in a plant extract is highly dependent on the plant type and source [23,27].

The total phenolic content of the samples of DF in this study was estimated as Gallic acid equivalent.

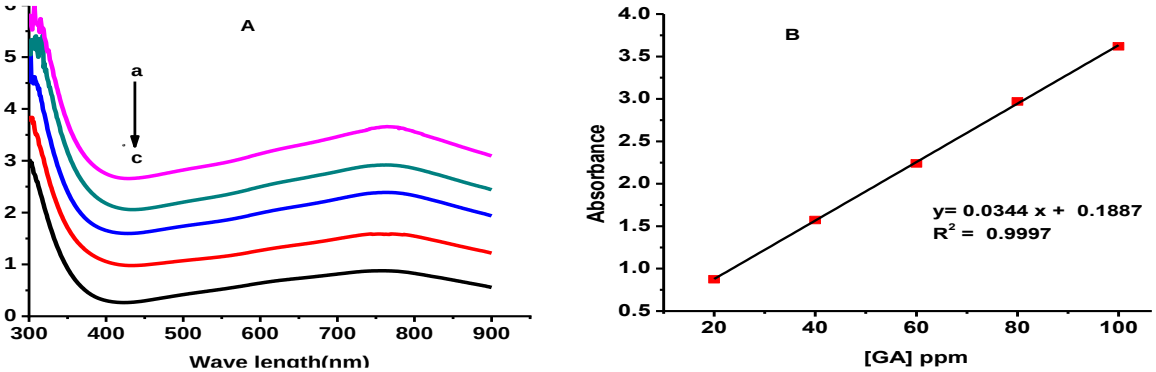


**Figure 3.6:** (A) UV-vis spectra of various concentrations of gallic acid (GA) (a-e: 100, 80, 60, 40, and 20 µg/mL respectively) in the presence of 5 mL of Folin Ciocalteau reagent, (B) plot of absorbance of GA vs concentration of GA.

Fig. 3.6(A) presents the UV-Vis spectra of five working Gallic acid standard solutions (20-100 µg/mL) while figure 3.6(B) is presenting the corresponding calibration curve. The absorbance of Gallic acid in the presence of 5 mL of Folin Ciocalteau reagent measured at its characteristic wavelength (765 nm) showed a linear dependence on its concentration in the studied range with a determination coefficient of 0.9997.

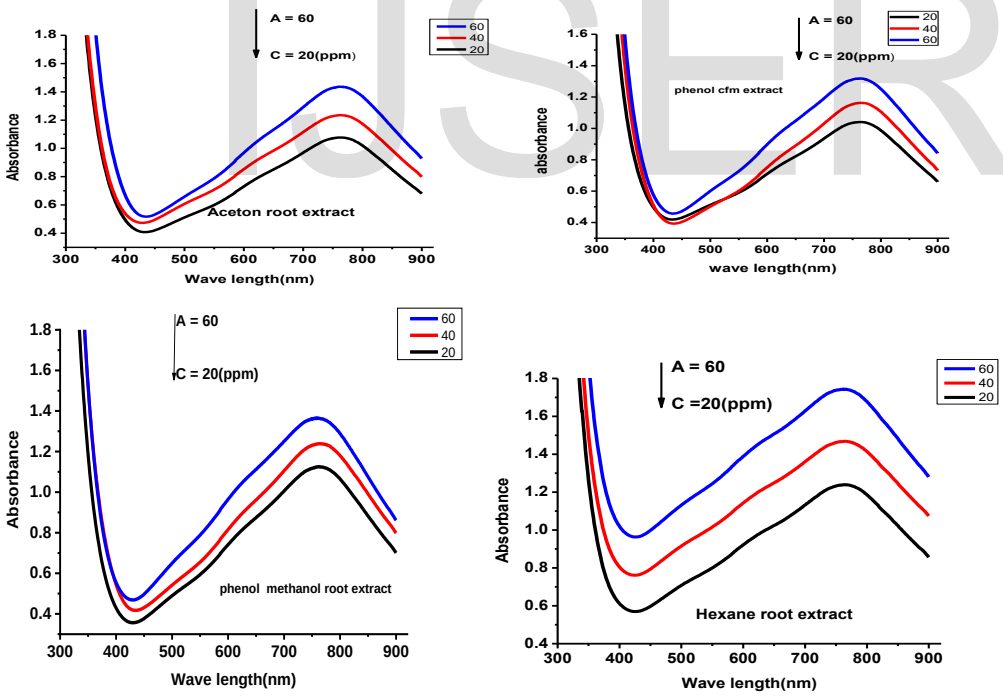


Figure 3.7: UV-Vis spectra of crude extracts of DF (a-c: 60, 40, and 20 µg/mL respectively) and plant parts using various solvents for phenol determination.

Figure 3.7 presents the UV-Vis spectra of various concentrations (20, 40, and 60 µg/mL) of the crude extracts of Dorstenia foteda. The concentration of total phenols in the Dorstenia foeteda calculated using the regression equation was ranged between 31.04 in chloroform extract to 450 µg GAE / mL in hexane extract .

Table 8: Total phenolic content estimation

| Plant part | Solvent | Mean absorbance | Total Phenolic content in (mg /50 g)* |
|---|---|---|---|
| Root | Acetone | 1.406 | 589.8± 0.026 |
| | Chloroform | 1.268 | 523 ± 0.042 |
| | Hexane | 1.701 | 732.7 ± 0.037 |
| | Methanol | 1.329 | 552.5 ± 0.035 |
| *Mean ±SD for 60 µg/mL, n = 3; | | | |

. The total phenolic content of the plant extracts in terms of the total phenols per mass of extracted crude dry powder is summarized in Table 8.

The result of total phenolic content was expressed as Gallic acid equivalent in mg /g of dried powder of the extract. As can be observed from the table, hexane extract showed the highest total phenolic content this might be the presence of poly methylated long-chain phenolic compounds since the length of the chain from the functional group is one determinant factor for the solubility of compounds. The total phenolic content of the extracts in decreasing order of magnitude for extracts was:

Hexane extract > Acetone extract > methanol extract > chloroform extract.

### 3.4 Estimation of total flavonoid content

Aluminum chloride complex forming assay was used to determine the total flavonoid content of the extracts [24]. In this method, quercetin was used as standard and hence flavonoid content of the extracts was estimated as quercetin equivalent.

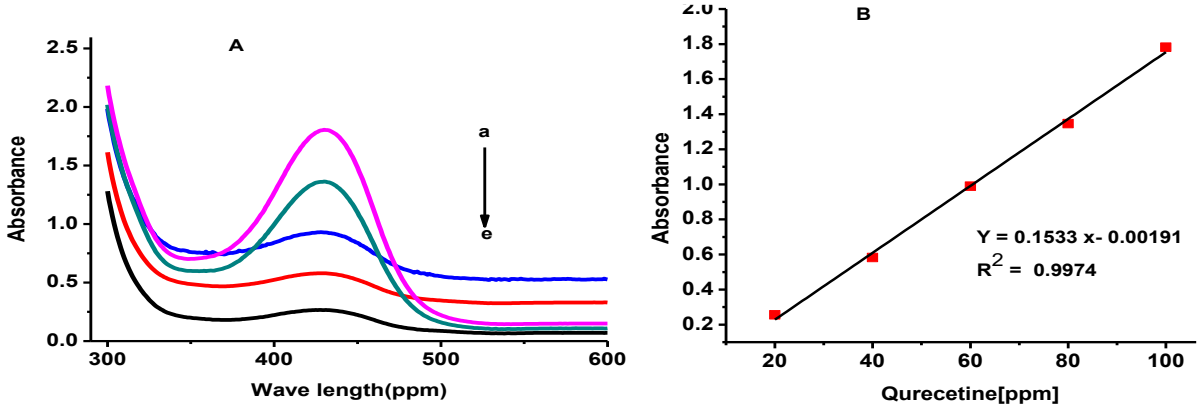


**Figure 3.8:** (A) UV-vis spectra of various concentrations of qurecitene (a-e: 100, 80, 60, 40, and 20 µg/mL, respectively) at constant amount of $AlCl_3$ reagent, (B) plot of absorbance of qurecitene vs concentration of qurecitene.

Figure 3.8(A) presents the UV-Vis spectra of various concentrations of quercetin mixed with 0.3mLof $AlCl_3$ while Figure 3.8(B) presenting the corresponding calibration curve.

The absorbance of the formed complex showed linear dependence on the concentration of qurecitene with determination coefficient of 0.9974 confirming the reliability of determining the total flavonoid content in the sample using the method.

The calculated total flavonoids corresponding to the absorbance at 420 nm of each extract of Dorstenia foeteda is summarized in Table 9. While the total flavonoid content of ranged from 2.88 in chloroform extract to 4.1QE µg/mL in hexane extract.

Table.9: Total flavonoid contents

| Plant part | Solvent | Mean absorbance | Total Flavonoid content (mg /50 g dried extract) * |
|---|---|---|---|
| Root | Acetone | 0.552 | 52.6 ± 0.015 |
| | Chloroform | 0.442 | 48 ± 0.007 |
| | Hexane | 0.618 | 33 ± 0.017 |
| | Methanol | 0.52 | 56 ± 0.044 |

*Mean ±SD for 60 µg/mL, n = 3.

The total flavonoid content was expressed as mg QE/ g. Methanol extract in particular showed the highest total phenolic content, which ranged from56 ± 0.044 methanol extract to 33 ± 0.017g

QAE/50 g hexane extract. In this study hexane extract showed least TFC content. However, its flavonoid content is highest as compared to other plant extracts within 50g yield. The decreasing order of TFC of extract of Dorstenia foeteda showed as follows:

Methanol extract > Acetone extract > Chloroform extract > Hexane extract

The trend was completely competitive with TPC in all extracts. The triplicate measurement of the four solvent extracts of TPC was presented in table 10.

### 3.5 Antimicrobial Activity

The extracts of Dorstenia foeteda using different solvents demonstrated antibacterial activities against gram-positive and gram-negative bacteria strains. The methanol extract exhibited maximum zone of inhibition against gram positive and gram negative bacteria followed by acetone, chloroform and hexane extracts as indicated table10 and in appendix. While streptococcus pyogenes gram negative bacteria was found not susceptible to hexane and chloroform extracts, staphylococcus aureus was also not susceptible for hexane and chloroform extracts(table). Among the gram negative bacteria, Escherichia coli was the most susceptible followed by Klebsiella pneumonia. The crude extracts of methanol and acetone extract have shown zone of inhibition completely better than that of standard drug tetracycline, and competitive with gentamycin .Even in some cases better than gentamycin. The antibacterial activity of most dilutions of each extract was statistically significant ($P \leq 0.05$) as compared to the negative control gentamycin and positive control tetracycline. Most of them displayed potency greater than that of tetracycline, which was used as a standard positive control drug in this study. The antimicrobial activity of methanol extracts of Dorstenia foeteda was stronger than acetone, chloroform and hexane extracts towards the tested pathogens. This substantiates that more polar bioactive compounds are extracted more by methanol than the other solvents. In other words, alcoholic (methanol) extracts of tested plants provided better antibacterial effect on both gram positive and gram negative bacteria than other solvents. Thus, the present study showed that the different extracts of Dorstenia foeteda possessed significant antibacterial activity and provides possible rationalization to the traditional anti-infection, anticancer and antityphoid use of the plant. Dose dependent zone of inhibition of both gram negative and gram positive bacteria was presented in the table 10.

Table10: Dose dependent crude and bacterial extracts inhibition standards zone of

| Bacteria | Solvent | Plant Extract | Standards | |
|---|---|---|---|---|
| | | Zone of inhibition in mM | | |
| | | Root | Gentamycin | Tetracycline |
| | | 10µg | 10µg | 30µg |
| Escherichia coli | Acetone | 21.40±0.40 | 26.4±0.56 | 16.04± 0.52 |
| | chloroform | 15.00±0.02 | | |
| | Hexane | 10.20±0.40 | | |
| | methanol | 28.20±0.10 | | |
| Klebsiella Pneumonia | Acetone | 20.00±0.30 | 23.00±0.37 | 15.07± 0.70 |
| | chloroform | 15.50±0.78 | | |
| | Hexane | 11.1±0.59 | | |
| | methanol | 26.50±0.21 | | |
| Staphylococcus Aureus | Acetone | 20.00±0.26 | 24.00±00 | |
| | Chloroform | 14.00±0.10 | | |
| | Hexane | 11.60±0.20 | | |

| | | | | |
|---|---|---|---|---|
| | | Methanol | 26.00±0.50 | < 5 mm |
| Streptococcus Pyogenes | | Acetone | 22.60±0.48 | 26.00±00 |
| | | Chloroform | 16.20±0.58 | < 5 mm |
| | | Hexane | 10.70±0.41 | |
| | | Methanol | 27.70±0.64 | |

# 4 CONCLUSION AND RECOMMADATION

## 4.1 Conclusion

The results of phytochemical work revealed the presence of alkaloids, phenols, flavonoids, tannins, saponin, and glycosides in different concentration for each extract of the plant. The outcome of the investigation indicated that Dorstenia foeteda extracts were to found high amount of phenolic content which play great role in the control of oxidation. Not only this but also the extracts revealed that significant antioxidant activities against various tests (Phosphomolybdate, FRAP and DPPH). Likewise, the extracts revealed significant antibacterial activities. In phenolic, flavonoid content, ferric reducing power, Phosphomolybdate assay methanol extract showed highest activity than the others. Phytochemicals and antioxidants found in the extracts of Dorstenia foeteda might be high polar. The combination of the above results proves the effectiveness of the plant for its antibacterial, antioxidant activities and its use in traditional medicine.



## 4.2 Recommendation

The presence of high amount of phenolic, antioxidant and antibacterial activities led to the conclusion that the plant has medicinal values. Therefore, it is recommended strongly to isolate metabolites and antioxidant components of extracts of Dorstenia foeteda.

## Data Availability

The dataset generated/analyzed during the current study are available from the auther on reasonable request.

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### Conflicts of Interest

The author declares that there is no conflict of interest for this research.

### Author Contributions

Abrham Biresaw carried out all the manuscript preparation, analyzed the data, and wrote the paper.

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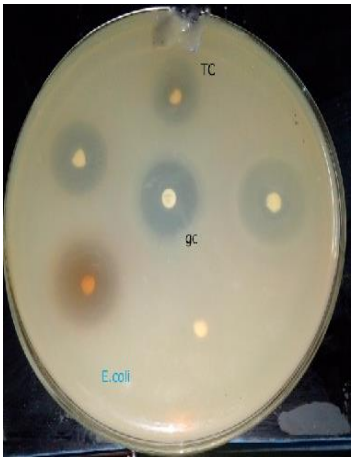
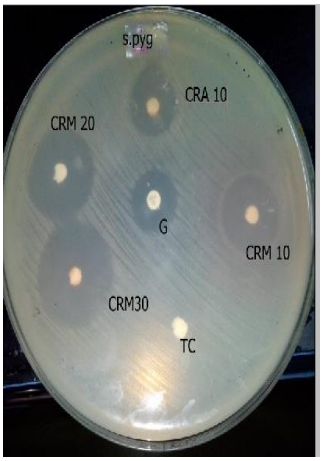
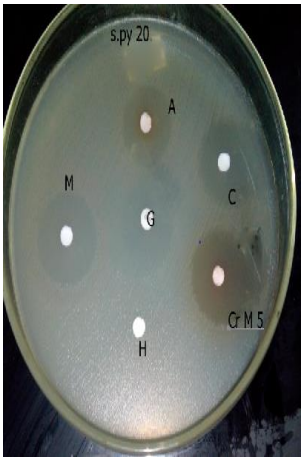
## APPENDIX




*Escherichia coli root extracts* *S. Pyogenes Pyogenes* *(crud root extracts10µg)*

*(crude root methanol extract).*

**(A= acetone, C = chloroform,** **C= chloroform, M= methanol, H= hexane**

**M= methanol, H= hexane, TC = tetracycline** **TC = tetracycline, G= gentamycin)**

Appendix 7. 4 antibacterial test for different concentration and standards