Global Scientific JOURNALS
GSJ: Volume 10, Issue 10, October 2022, Online: ISSN 2320-9186
www.globalscientificjournal.com

PHYTOCHEMICAL SCREENING, INVESTIGATION OF ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF LEAF 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 typical traditional medicinal plant in Amhara and Benishangul regions, Ethiopia used for treatment of most of fungal and protozoan diseases. The purpose of this study was to conduct phytochemical screening and evaluate Total antioxidant capacity (TAC), Total flavonoid content (TFC), Total phenolic content (TPC), and microbial activities of leaf extracts of Dorstenia foeteda. The phytochemical screening result showed the presence of most secondary metabolites in the extracts. The TAC, TFC, and antioxidant analysis result also showed a strong positive correlation between 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 leaf extracts lied from, 49.57 ± 1.35 (in hexane) to 76.63 ± 1.40 µg AAE /mL (in acetone). While Ferric reducing power leaf extracts varied from 331 ± 0.02 (hexane) to 554.3 ± 0.02 mg AAE /50g (in acetone). Phosphomolybdate total reducing the power of extracts varied from 32 ± 0.64 g(in hexane) to 38.4 ± 0.50 g AAE /kg (in acetone) dried powder of extract. Similarly, the TFC of extracts of Dorstenia varied from 47 ± 0.017 (hexane) to 64.3 ± 0.044 mg QE/g (in methanol) for the extracts dry powder of extracts. Surprisingly methanol crude extract showed the highest zone of inhibition (mm) which was better than the standard disc gentamycin and tetracycline in both grams negative and positive bacteria. 30.60 ± 0.03, 27.20 ± 0.5, 26.60 ± 0.69, 27.00 ± 0.53 Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus and Streptococcus Pyogenes respectively. The cumulative investigation of the study leads us to the conclusion that the plat has metabolites that contribute medicinal value. Therefore, it is recommended strongly that to isolate metabolites and antioxidant components.

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

1. INTRODUCTION

1.1 Background of the study

Ethiopia is one of the oldest nations of the world and has a rich history of traditional medicine (TM) and indigenous practices. Various kinds of literature indicated the significant role of medicinal plants in primary health care delivery in Ethiopia where 80% of humans and 90% of livestock population depend on traditional medicine. In the Ethiopian context, there seems to be no village, town, or city where traditional medicine is not involved in the provision of health care, since it is an integral part of the local culture and accessible to the majority of the population, even when there is demonstrably efficient and less costly alternative care. TM has been practiced in Ethiopia for a long time ago. The knowledge, largely oral, has been transferred from one generation to the next through professional healers, knowledgeable elders, or ordinary people. The Ethiopian population is still dependent on traditional medicine, which essentially involves the use of plants[1].

TM is regarded as a combination of knowledge, skill and practice originated from theories, experiences, and attitudes native to various cultures that are utilized to treat and diagnose enormous types of physical and mental complications and maintain health [2]. Many people in the world rely on traditional medicine for their primary health care needs. It is well understood that several medical techniques and procedures have been discovered from the use and knowledge of traditional medicines. This is because the major part of traditional therapy involves the use of plant extracts or their active principles. As a result, natural products have recently become of immense interest owing to their diverse application [3]. In indigenous African communities, traditional doctors are well known for treating the patient holistically. They (the traditional doctors) usually attempt to reconnect the social and emotional equilibrium of patients based on community rules and relationships [4].

Unlike medical doctors who only treat diseases in patients. In many of these communities, traditional healers often act, in part, as an intermediary between the visible and invisible worlds; between the living and the dead or ancestors, sometimes to determine which spirits are at work and how to bring the sick person back into harmony with the ancestors. However, the arrival of the Europeans marked a significant turning point in the history of this age-long tradition and culture. The trends and challenges of African traditional medicine are examined with emphasis on the efforts towards the integration of TM into the mainstream of health care systems [4].

Discourses about the impact of colonialism in Africa are clouded by a mixture of 'fortune' and 'agony'. Some scholars believe that the process of modernization in Africa is intrinsically connected with foreign intervention particularly in areas of health and democracy. Most scholars argue that the period between

1840 and 1860 marked a significant and rapid innovation in tropical medicine, particularly, the invention of quinine to stem the scourge of malaria in the most endemic region of the world.

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 [5]. All but one species of *Dorstenia* are restricted to the Neotropics or Africa, and it is the only group in the family with an almost equal distribution of species on either side of the Atlantic Ocean [5].

The Moraceae have been particularly well studied in part due to the unique pollination syndromes present in the family and it has been hypothesized that *Dorstenia* may represent an intermediate form in the evolution from open inflorescence and wind pollination in MorusL.(mulberries) to the specialized syconium and obligate pollination mutualism of Ficus [6]. Despite *Dorstenia'*s unique distribution, and evolutionary position within the Moraceae family, little is known about its evolutionary history, biogeography, or reproductive ecology.

The *Dorstenia Moraceae* are comprised of 37 genera and 1100 species. The family exhibits a cosmopolitan distribution, with the majority of extant species found in the Old World tropics [6]. 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 [7].

The family is thought to have radiated during the mid-Cretaceous with an estimated date of 89.1 million years ago (mya), suggesting that it probably diversified after the break-up of Africa and South America (105 mya) [8]. *Dorstenia* species are distinct from the majority of the Moraceae family in morphological characteristics as well as geographic distribution. Ninety percent of *Dorstenia* species are herbaceous, as compared to the entire Moraceae family in which 90% of species exhibit a woody habit and only 10% are herbaceous. Life forms within the genus are variable, with geophytes (defined by the presence of underground storage organs), hemicryptophytes (plants with perennating buds located at the soil surface), and phanerophytes (in which the perennating buds are positioned well above the soil surface). Stems range from calescent (well developed and above ground) to acaulescent (having only a very short below-ground stem or lacking one altogether[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 foeteda is a typical succulent plant in the genus dorstenia which is native to eastern Africa and Arabia [10]. It is mainly found in Ethiopia, Somalia, Kenya, Tanzania, Saudi Arabia, Yemen & Oman [10]. In Ethiopia, it is found in Benishangul Gumuz, afar region around Asayta, Arbaminch, west Gojjam zone north mecha woreda, Awi zone in Jawi, and zigem woreda. Most of west Gojjam, Awi, and Shinasha peoples used it for different purposes the Shinasha, Gumuz, Agew-Awi, and Amhara peoples in northwest Ethiopia and most of the traditional drug users typically sorcerers (debtera's) used it for several diseases treatments such as cancer, malaria, fungal, snake bites, typhoid, amoeba, giardia, rat poison, stomach disorder, headache, stroke, gonorrhea, syphilis, evil eye, asthma, common cold, and others.

2. METHODOLOGY

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),10% ferric chloride (99% BDH), Iodine in potassium iodide (Lobal chemia India) , (54%, 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 Description of the study area

Agew Awi zone, which consists of nine woredas' and 7 city administrations, is one of the 10 Zones in the Amhara Region of Ethiopia. Awi zone covers the area of 9148.43 km^2. It is bordered on the west by

Benishangul-Gumuz Region, on the north by North Gondar Zone, and on the east by west Gojjam. Topographically speaking, Agew Awi is relatively flat and fertile, whose elevations vary from 1,800 to 3,100 m above sea level with an average altitude of about 2,300 m. Based on the 2007 Census conducted by the Central Statistical Agency of Ethiopia (CSA), this Zone has a total population of 982,942, an increase of 37.07% over the 1994 census, of whom 491,865 are men and 491,077 women. With an area of 9,148.43 square km, Agew Awi has a population density of 107.44; 123,014 or 12.51% are urban inhabitants. Most people use traditional medicine for the treatment of different diseases with the help of Agew Kunfel and sorcerers (debtera's). Worq bemeda is the typical traditional drug in this zone. The samples were collected from the two woreda's in the Agew Awi zone, Zigem, and Jawi woredas'.

2.4 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 green leaf of *Dorstenia Foeteda* were collected from the selected Zigem (wingi) and Jawi sites.

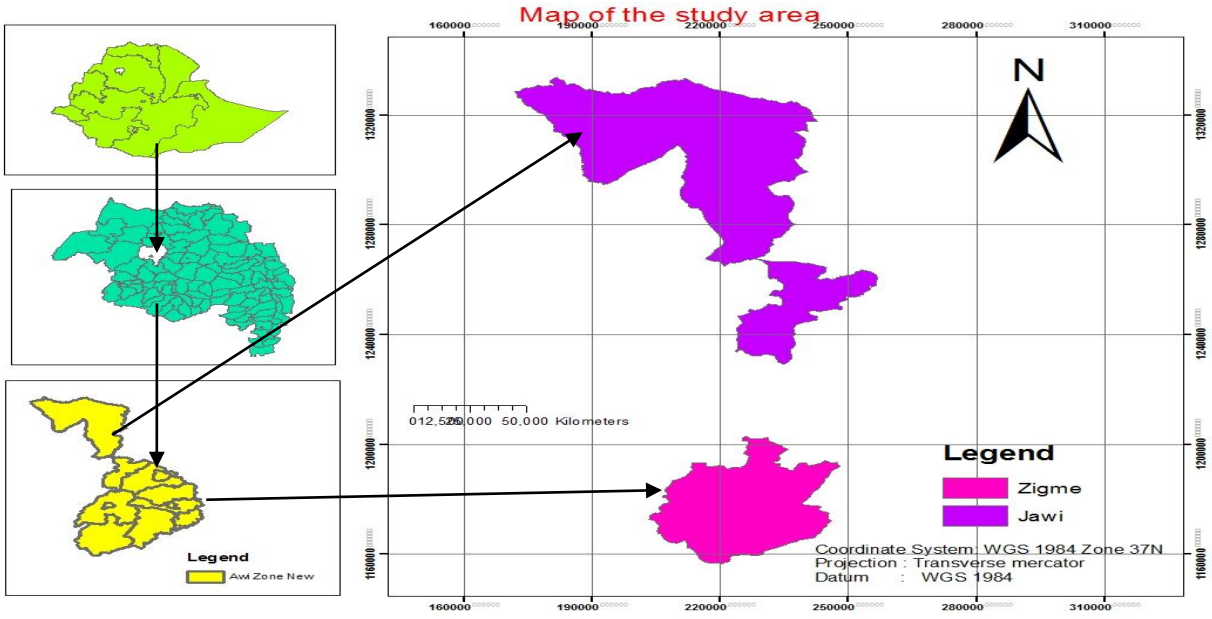


Figure 1: Sampling site area map

The samples were collected and kept in an 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 72 hours (leaf). Finally, the dried leaf 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 [11].

50 g of separate leaf 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 a three-hour interval shaking. While the filtrate for each was collected in a separate flask, the residue was further extracted for two rounds following the same procedure. The solvent was then removed by using a low-pressure rotary evaporator with a speed of 180 rpm at a temperature of 45 ^{0}C. The dried crude extract was carefully transferred from the rotary into a pre-weighed sample holder and weighed. While 9.40, 6.23, 5.52, and 17.79 g of leaf extract of *Dorstenia foeteda* was also obtained using the same solvents (acetone, chloroform, hexane, and methanol, respectively).

1000 ppm stock solution for each extract was prepared by dissolving the appropriate mass of the extract in a 250 mL flask from which crude extracts of 20, 40, and 60 ppm samples (working solutions) were prepared through serial dilution.

2.6 Phytochemical screening

Phytochemical screening refers to the extraction, screening, and identification of the medicinally active substances found in plants [12]. Some of the bioactive substances that can be derived from plants are flavonoids, alkaloids, carotenoids, tannin, Coumarins, terpenoids saponins glycosides, antioxidants, and phenolic compounds. Although the knowledge of how these substances provide medicinal value to humans reflects a relatively recent scientific understanding, the use of plants and plant extracts to heal, relieve pain and promote good health dates back to before the beginning of medical science. It is believed that there may be about 4,000 phytochemicals contained in plants that can be used to prevent, minimize or remedy medical conditions such as strokes, cancer, or metabolic syndrome. The evidence obtained through current scientific research does not appear to demonstrate that the use of phytochemical supplements supports long-term health as well as consuming the actual fruits, grains, and vegetables from which they were taken. The long-term use of phytochemical supplements as a substitute for their natural food sources should only be considered after consulting a doctor, as noted by the American Cancer Society. Phytochemical analysis of the plants is very important commercially and has a great interest in pharmaceutical companies for the production of new drugs for curing various diseases. This study was focused on screening 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). The formation of brown/reddish precipitate indicates the presence of alkaloids [13].

ii) Detection of phenols (Ferric Chloride Test)

Extracts were treated with 3-4 drops of ferric chloride solution. The formation of bluish-black color indicates the presence of phenols [14].

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. The appearance of yellow color formed which indicates the presence of flavonoid [13].

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 were added. The formation of a reddish-brown interface confirms the presence of terpenoids [14].

v) Detection of steroids (Salkowski's test)

2 mL of chloroform extract, 1 mL of concentrated H_2SO_4 acid were added carefully along the sides of the test tubes. A **red color** was produced in the chloroform layer and confirms the presence of steroids [15].

vi) Detection of saponins

To a little amount of each of the samples in a test tube, 2 mL of distilled water was added and vigorously shaken for 15 minutes. The formation of 1 cm foam confirms a positive result [13].

vii Detection of glycosides

A small amount of extract was dissolved in 1 mL of water and the aqueous NaOH solution was added. The formation of **yellow** color indicates the presence of glycosides [14].

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 [14].

xi 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 [16].

2.7 Analytical methods

2.7.1 Determination of antioxidant activity

2.7.1.1 DPPH radical scavenging assay

The free radical scavenging activities of the extracts were determined by using the 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 [17]. 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.

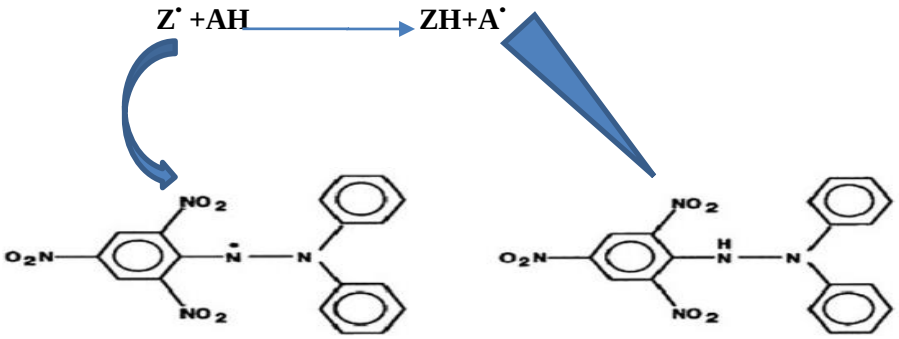


Figure 2 a: Diphenyl-1-picrylhydrazyl (free radical) Figure 2b: Di phenylhydrazine (nonradical)

An antioxidant compound donates the electron to DPPH thus causing its reduction and in reduced from its color changes from deep violet to yellow [18]. 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). 1.5 mL of the ascorbic acid and extracts (20, 40, and 60 ppm) was mixed with a 3 mL solution of DPPH and allowed to stand in darkness for 15 minutes. Control was prepared by taking 3mL of DPPH and 1.5 mL methanol and its absorbance were recorded at 517nm. The assay was performed in triplicates. Percentage inhibition of free radical DPPH was calculated based on control. The absorbance was again recorded at 517 nm. The percentage inhibition of DPPH by extracts was calculated by using the following formula.

$$\% \textbf{ inhibition} = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \times 100$$

Where A control is the absorbance of pure DPPH in oxidized form and A sample is the absorbance of the sample taken after 15 minutes of reaction with DPPH [18].

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 ^{0}C [17].

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.

2.7.1.2 Ferric reducing antioxidant power (FRAP) assay

The reducing power of the crude extracts was determined according to the standard method [11] with minor modification.

Percentage (%) reduction power = $\frac{(A-B)}{B} \times 100$

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

Preparation of standard solutions and samples

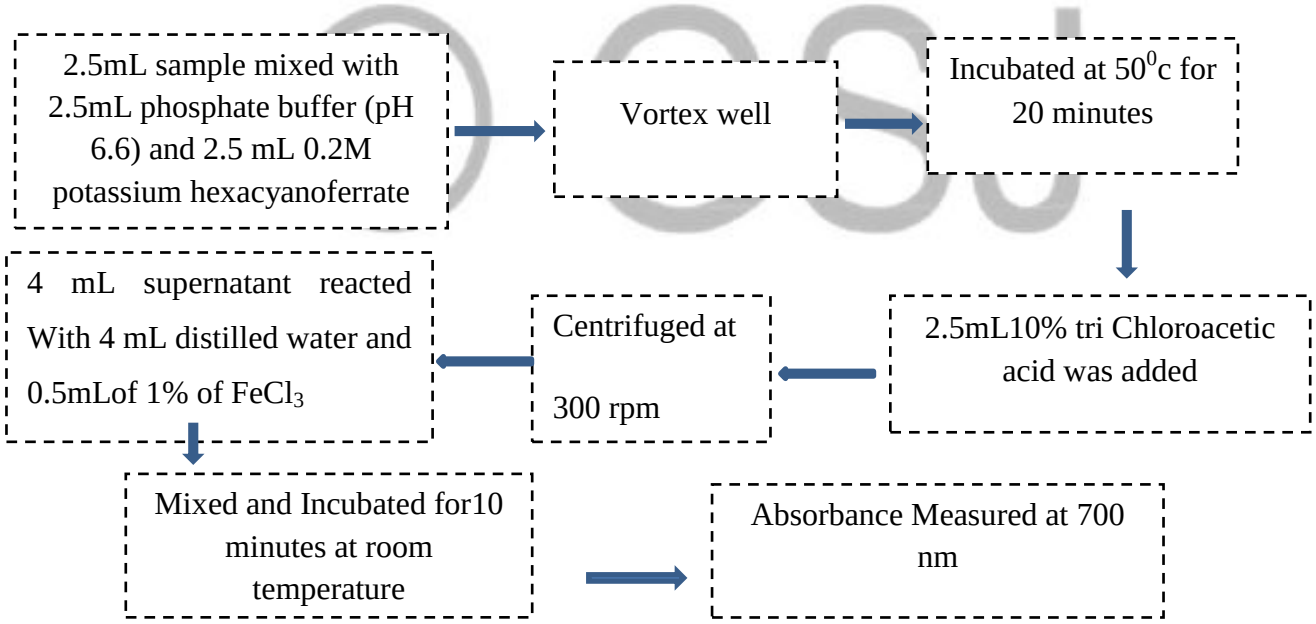


Figure: 3 Preparation *of standard solutions* and samples

2.7.1.3 Determination of Total antioxidant activity by Phosphomolybdate assay

To carry out a Phosphomolybdate assay, the procedure reported elsewhere was followed [11]. 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. Various concentrations of ascorbic acid (20, 40, 60, 80 and100 µg/mL) were prepared and the same protocol was carried out to plot a standard curve. The results were expressed as g of ascorbic acid equivalent (AAE) per kg of the dried weight of the sample as determined from the equation of the standard calibration curve.

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 [18].

2.7.2.1 Quercetin stock solution preparation

It was prepared by dissolving 30 mg of quercitin in 250 mL of distilled water. Standard solutions of 20, 40, 60, 80, and 100 µg/mL working solutions were prepared by serial dilution of the stock solution. From each working solution of the quercetin, 1 mL quercitin was added to 0.3 mL of 5% of potassium acetate, incubated for 5 minutes to which 0.3 mL of 10% aluminum chloride was added. The mixture was incubated for 5 minutes to which 2 mL 1 M sodium hydroxide and 2.4 mL distilled water were added sequentially. The absorbance was then measured at 420 nm [19].

250 mL of 400 µg/mL solution of each crude extract was prepared using the four solvents (acetone, chloroform, hexane, and methanol) the absorbance was measured for each solvent extract. Total flavonoid content was calculated as Quercetin equivalents (mg QE/g) [19].

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 [19]. The total phenolic content of the crude extract was determined by using the Folin-Ciocalteau method [20].

The fact that phosphotungstic acid and phosphomolybdic acid in the reagent have the property to react with nonphenolic compounds like ascorbic acid, some sugars, and amino acids, Folin Ciocalteu assay method is known to overestimation of the total phenolics. Due to its simplicity, and low cost compared to the chromatographic methods, the method remains useful and is largely used to evaluate the relative contents of total polyphenolic compounds in varieties of plant extracts [21].

Folinciocalteau reagent (FCR) was prepared from sodium tungstate and sodium molybdate in the presence of 85% phosphoric acid following the standard procedure [21]. The total phenolic content of the extract samples measured in triplicate was reported as mg GAE /g of dried powder of extract.

2.7.3.1Preparation of Folinciocalteau reagent

A mixture of 25 g of sodium tungstate and 6.25 g of sodium molybdate was dissolved in 200 mL of distilled water placed in a 1500 mL flask. To the solution, 12.5 mL of phosphoric acid and 25 mL HCl were added and refluxed for10 hours. After cooling, 37.5 g of lithium sulfate, 12.5 mL of distilled water, and 2drops of bromine water were added and allowed to stand for 2 hours. The solution was boiled for 15 minutes and cooled before filtration. The pale yellowish solution was then filtered [22]. Finally, FC reagent was prepared from the above solution in a 1:10 ratio of the solution to distilled water.

Preparation of gallic acid standard for total phenolic content detection

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 [20].

To each 1 mL standard gallic acid working solution, 5 mL of Folinciocalteau 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.

3.7.4 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.

2.7.4.1 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. Standard discs of the antibiotic disc (Gentamycin 10 µg and tetracycline, 30µg/disc) were serving as the negative and positive antibacterial control. For negative control, the same volume (20 µL) sample extract was poured on a paper disk. Antibacterial activity was recorded if the zone of inhibition was greater than 6 mm [22,25]. The disk diffusion assay was used as a preliminary test to select the most efficient extracts.

2.8 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

3.7 The yield of the extracts of *Dorstenia foeteda*

The mean mass of crude extract and the percent yield of the extracts of *Dorstenia foeteda* leaf extracts (50 g dry powder) are summarized in table 3.1.

Table 3.1: 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	Leaf	9.4±0.09	18.8
Chloroform	Leaf	6.23±0.04	12.46
Hexane	Leaf	5.52±0.03	11.4
Methanol	Leaf	17.9±0.2	35.8

The highest yield was obtained from methanol extract of *Dorstenia foeteda* leaf. 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 and chloroform extracts of *Dorstenia foeteda*. This might be the presence of polar metabolites in the extracts which are not extracted easily by nonpolar solvents.

3.1.1 Preliminary phytochemical analysis

Preliminary phytochemical tests are helpful in finding information about chemical constituents present in the plant material [24] 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 3.2). 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 3.2: Summary results of phytochemical screening analysis

Phyto Chemicals	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	+	+	-	+

Phenol Detection Saponin Detection Tannin Detection Terpenoids Detection

Figure 3.1: Qualitative phytochemical screening results

3.8 Antioxidant analysis

3.2.1 DPPH radical scavenging assay

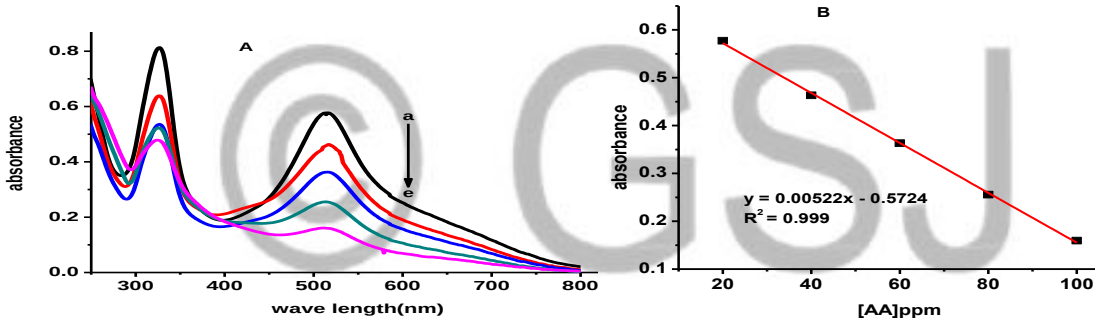


Figure 3.2: Uv-Vis spectra of A) ascorbic acid of various concentrations (a-e: 20, 40, 60, 80, and 100 µg/mL, respectively) spiked with 0.002% DPPH in methanol absorbance vs wave length, and (B) Plot of absorbance of vs concentration of ascorbic acid.

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. 3.2A) [17]. Moreover, Fig. 3.2B revealed linear dependence of the absorbance of the DPPH on the concentration of AA in the range 20-100 ppm with a determination coefficient (R^2) of 0.999.

The UV-Vis spectra of absorbance of various concentrations of crude leaf and root extracts using different solvents mixed with the constant amount of DPPH were recorded (Fig.3.3) and the resulting absorbance were compared against the absorbance of the control (DPPH) (Table 3.4). As can be seen from the table, the absorbance for different concentrations of both plant parts using the same solvent decreased with increasing concentration of the crude extract irrespective of the type of plant part and solvent used for

extraction. While acetone extract of leaf 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 from 394.5µ g /mL hexane extract to 582.9 µg / mL.

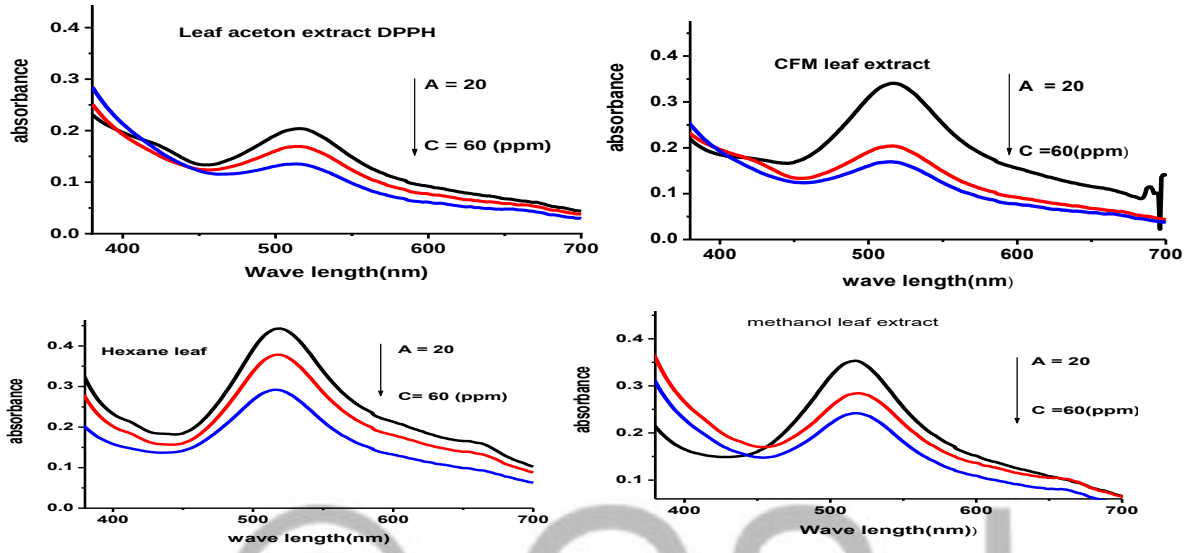


Figure 3.3: UV-Vis spectra of crude extracts (a-c: 20, 40, and 60 ppm, respectively) and plant part using various solvents

Table 3.3: DPPH –Leaf extracts absorbance

plant sample	Conc. (ppm)	Absorbance					
		AA/ methanol	type of solvent for extraction				Control (DPPH)
			Methanol	Acetone	chloroform	Hexane	
Leaf	20-	0.46324	0.3528	0.2040	0.3408	0.4632	
	40	0.3631	0.2844	0.1693	0.2040	0.3785	0.57725
	60	0.2560	0.2419	0.1354	0.1682	0.2922	

Table 3.4: % Inhibition of *Dorstenia foeteda* leaf extracts

Type of Solvent used for extraction	crude extract concentration (ppm)	% inhibition
Acetone	20	64.7
	40	70.7
	60	76.7
Chloroform	20	41
	40	64.7
	60	70.8
Hexane	20	18.9
	40	34.4
	60	49.4
Methanol	20	38.9
	40	50.7
	60	58.1

Table 3.4: Presents the DPPH % inhibition calculated for each extract using the four solvents utilized. Among all the solvents considered in this study, acetone leaf extract showed the highest DPPH % inhibition indicating its extracting ability of the compounds responsible for scavenging the DPPH and hence antioxidant property.

Acetone extract > chloroform extract > methanol extract > hexane is decreasing the order of DPPH scavenging power of leaf extracts. Except for hexane 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 leaf extract of *Dorstenia foeteda*. As the concentration of the sample increased, its DPPH scavenging power also increased. Methanol extract has competitive scavenging power of DPPH with the standard (ascorbic acid).

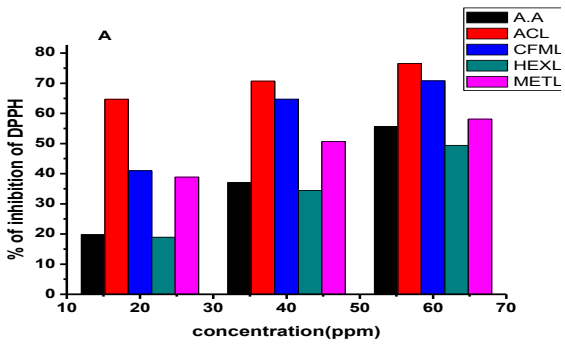


Figure 3.4: (A) comparison of **%** inhibition leaf extract with the standard in 3 mL DPPH

As indicated in figure 3.4 (A) except hexane extract the others showed better scavenging activity than the standard AA.

Table 3.5: Summary of the absorbance and corresponding DPPH % inhibition of 60 µg/mL plant extracts using different solvents

Plant part	Solvents	Crude extract concentration(µg/mL)	Absorbance	% DPPH inhibition*
Leaf	Acetone	60:1	0.135	76.63 ± 1.401
		60:2	0.127	
		60:3	0.143	
	chloroform	60:1	0.169	70.56 ± 1.563
		60:2	0.171	
		60:3	0.179	
	Hexane	60:1	0.292	49.57 ± 1.348
		60:2	0.298	
		60:3	0.283	
	methanol	60:1	0.242	58.47 ± 1.387
		60:2	0.231	
		60:3	0.246	
	*Mean ±SD for 60µg/mL, n =3			

As can be seen from Table 3.5, the DPPH % inhibition ability of the crude plant extracts was compared taking 60 µg/mL crude extracts of the four solvents.

Comparing the % inhibition of the extracts, acetone leaf extract showed the highest % inhibition (76.63%) which might be due to the presence of potent antioxidants with medium polarity in plant extract [18].

3.3.2 Ferric reducing antioxidant power

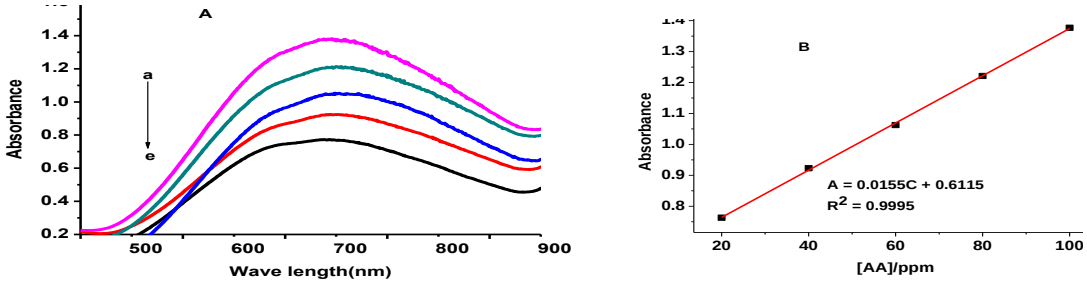


Figure 3.5: (A) UV-vis spectra of various concentrations of AA (a-e: 100, 80, 60, 40, and 20 µg/mL, respectively) at a constant amount of FRAP reagent, (B) plot of absorbance of AA vs concentration of ascorbic acid.

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 [11]. Dependence of the absorbance on concentration showed a linear correlation with a determination coefficient (R^2) of 0.9995 (Figure 3.5B).

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 3.6 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 [11]. 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 was calculated using the regression equation. As summarized in Table 4.6, while the reducing power of the extract ranged between 51 in hexane extract to 200 µg AAE / mL in acetone extract.

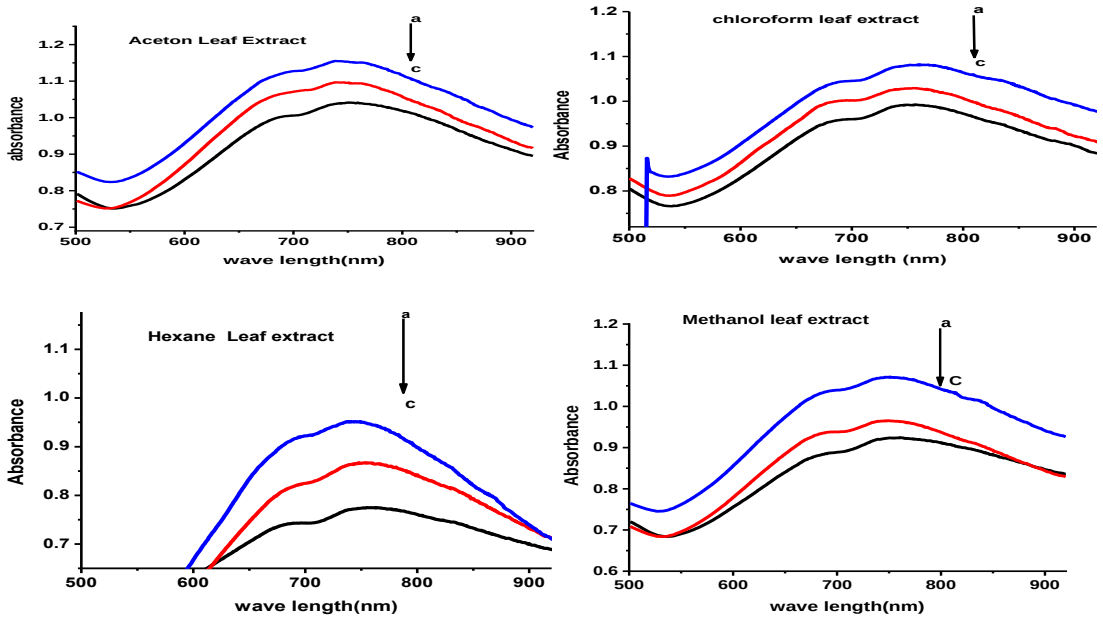


Figure 3.6: UV-Vis spectra of crude extracts (a-c: 60, 40, and 20 µg/mL, respectively) and plant part using various solvents for FRAP antioxidant determination

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

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

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

Absorbance of blank = 0.3552

Table 3.6: 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*
Leaf Sample	Acetone	20	1.011	65.34±0.47
		40	1.074	66.64±0.56
		60	1.128	68.54±0.55
	Chloroform	20	0.962	63.50±0.50
		40	1.001	64.77±0.25
		60	1.099	68.17±0.72
	Hexane	20	0.744	52.09±1.01
		40	0.862	57.93±0.90
		60	0.917	61.51±0.86
	Methanol	20	0.892	61.17±0.90
		40	0.935	62.73±0.70
		60	1.092	67.17±1.04
*Mean ±SD: n=3,				

% Reducing antioxidant power of acetone, chloroform, hexane, and methanol extracts of leaf *Dorstenia foeteda* were determined and the results were shown in table 3.6. Among the four solvents used for extraction of *Dorstenia foeteda*, acetone extract (20, 40, and 60 µg/mL) showed the highest reducing power whereas hexane and extract showed comparably the least % reducing power (Table 3.6). The higher absorbance the higher would be reducing power. Although to a different extent, increment of reducing power was observed with a concentration of crude extracts for all the studied solvents which are in agreement with the literature [11].

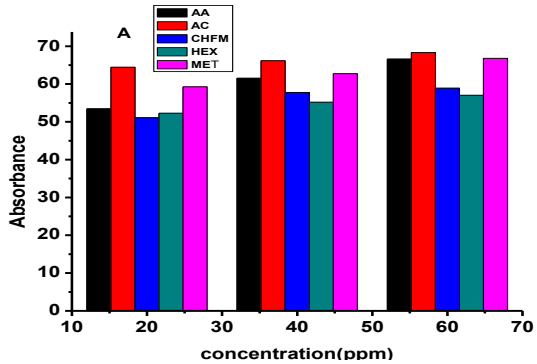


Figure 3. 7: (A) comparison of 20-60 µg/mL leaf extracts (acetone, chloroform, hexane, and methanol respectively) with standard AA

Table 3.7: Summary of ferric reducing power of 60 µg/mL root and leaf 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)*
Leaf	Acetone	1.131	554 ±0.092
	Chloroform	1.099	525 ± 0.02
	Hexane	0.92	331 ± 0.01
	Methanol	1.094	514.5± 0.02
*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 3.7. As can be seen from the table, acetone extract of leaf showed the highest reducing capacity (554 ± 0.092) than the others. In contrast to the others, hexane extract showed relatively the least reducing activity (331±0.0 mg/g of dry powder of extract).

3.3.3 Phosphomolybdate assay

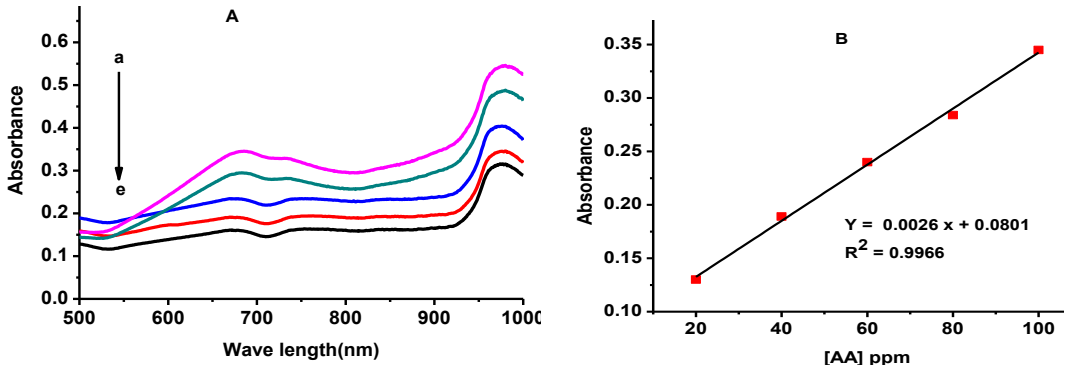


Figure 3.8: (A) UV-vis spectra of various concentrations of AA (a-e: 100, 80, 60, 40, and 20 µg/mL, respectively) at a constant amount of Phosphomolybdate reagent, (B) plot of absorbance of AA *vs* concentration of ascorbic acid.

The total antioxidant capacity of the different extracts of *Dorstenia foeteda* was also evaluated using the Phosphomolybdate method [11]. 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 3.8 (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 3.8(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).

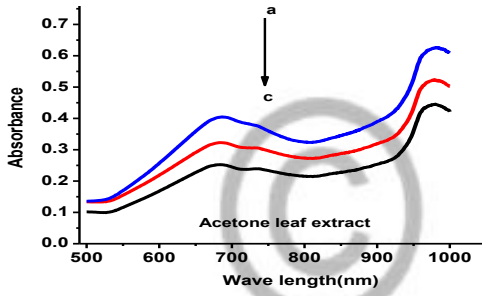


Figure 3.9: UV-Vis spectra of crude acetone leaf extract of DF (a-c: 60, 40, and 20 µg/mL, respectively) mixed with Phosphomolybdate reagent.

The total antioxidant capacity of the extracts was expressed as µg/mL AAE. The calculated AAE total antioxidant capacity of leaf extracts ranged from 18 in hexane to 115.7 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.

Table3.8: Summary of Phosphomolybdate assay total antioxidant reducing power (60 ppm)

Plant part	Types of solvents	Mean absorbance	Mean ± SD (g/1kg) total antioxidant power
Leaf	Methanol	0.380	38.4 ± 2.08
	Chloroform	0.343	33.7 ± 0.76
	Hexane	0.330	32 ± 0.64
	Acetone	0.337	33 ± 0.50
	*Mean ±SD for 60 µg/mL, n = 3;		

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

Methanol extract > chloroform extract > acetone extract >hexane extract. Which was analogous to FRAP,TFC, and TPC assay. Among all the solvents considered in this study, methanol leaf 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. Figure 3.10 presents the mean total antioxidant capacity of the crude plant extracts of *Dorstenia foeteda* in the solvents.

There is a strong correlation between the DPPH, FRAP, and Phosphomolybdate assay values of measured total antioxidant activity antioxidant activities.

3.9 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 [19,25].

The total phenolic content of the samples of DF in this study was estimated as gallic acid equivalent. Fig. 3.11(A) presents the UV-Vis spectra of five working gallic acid standard solutions (20-100 µg/mL) while figure 3.11(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.

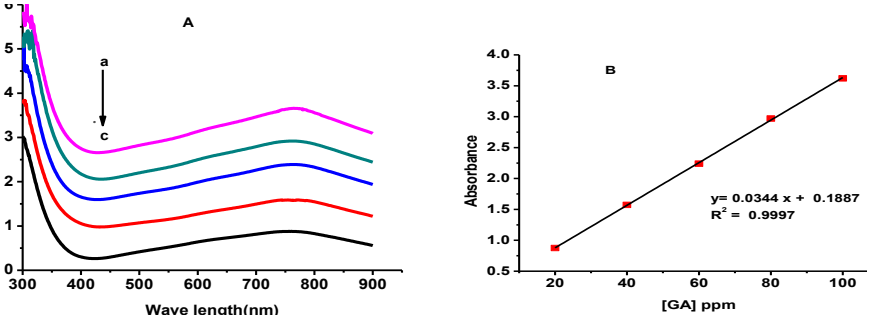


Figure 3.11: (A) UV-vis spectra of various concentrations of gallic acid (GA) (a-e: 100, 80, 60, 40, and 20 µg/mL respectively) in the presence of 5 mL of Folin Ciocalteau reagent, (B) plot of absorbance of gallic acid *vs* concentration of gallic acid.

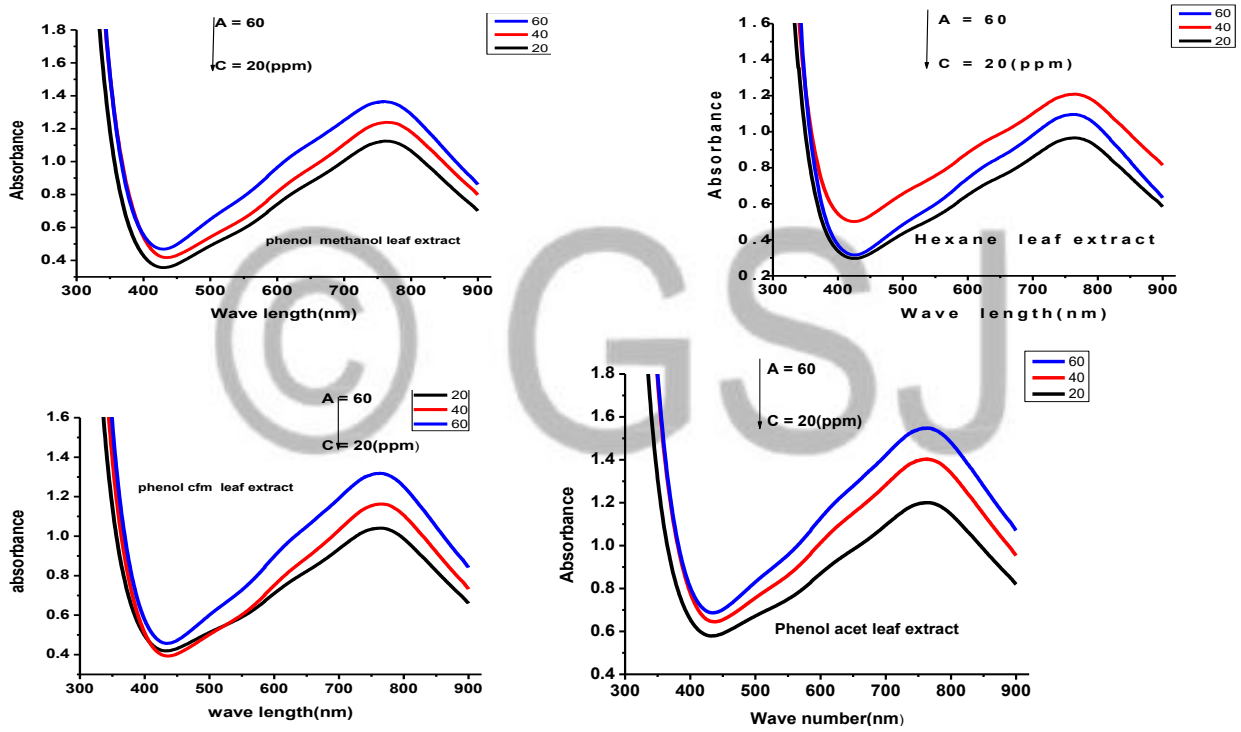


Figure 3.12: UV-Vis spectra of crude extracts of DF (a-c: 60, 40, and 20 µg/mL respectively) and plant part using various solvents for phenol determination

Figure 3.12 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 extract of *Dorstenia foeteda* calculated using the regression equation was ranged between 310 in hexane extract to 40 µg GAE / mL in acetone extract. 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 3.9.

Table 3.9: Total phenolic content estimation

Plant part	Solvent	Mean absorbance	Total Phenolic content in (mg /50 g)*
Leaf	Acetone	1.557	662.9± 0.0351
	Chloroform	1.321	533 ± 0.136
	Hexane	1.254	500± 0.121
	Methanol	1.354	564.6 ± 0.030
*Mean ±SD for 60 µg/mL, n = 3;			

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 least total phenolic content this might be the presence of poly methylated long-chain phenolic compounds since the length of the chain from a 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:

Acetone extract >methanol extract > chloroform extract > Hexane extract

3.10 Estimation of total flavonoid content

An aluminum chloride complex-forming assay was used to determine the total flavonoid content of the extracts [18]. In this method, quercetin was used as standard and hence flavonoid content of the extracts was estimated as quercetin equivalent. Figure 3.13(A) presents the UV-Vis spectra of various concentrations of quercetin mixed with 0.3mLof $AlCl_3$ while Figure 3.13(B) presents the corresponding calibration curve.

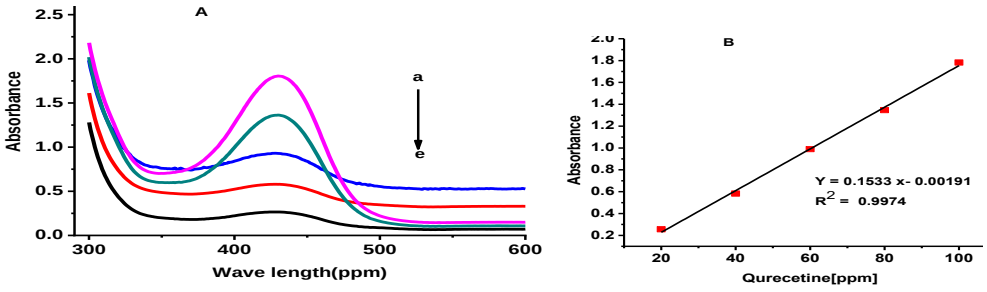


Figure 3.13: (A) UV-vis spectra of various concentrations of quercetin (a-e: 100, 80, 60, 40, and 20 µg/mL, respectively) at a constant amount of $AlCl_3$ reagent, (B) plot of absorbance of quercitin vs concentration of quercetin.

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

Figure 3.14 presents UV-Vis spectra for leaf acetone extracts of various concentrations (20, 40, and 60 µg/mL) as a representative case. The calculated total flavonoids corresponding to the absorbance at 420 nm of each extract of *Dorstenia foeteda* are summarized in Table 3.10. While the total flavonoid content ranged from 2.9 in hexane leaf extract to 3.9 µg QE/mL in acetone extract

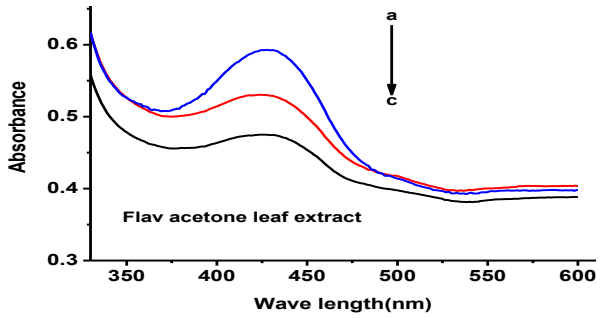


Figure 3.14: UV-Vis spectra of crude acetone leaf extract of *Dorstenia foeteda* absorbance *vs* wavelength (a-c: 60, 40, and 20 µg/mL, respectively) using acetone solvent for TFC determination.

Table3.10: Total flavonoid contents.

Plant part	Solvent	Mean absorbance	Total Flavonoid Content (mg /50 g dried extract) *
Leaf	Acetone	0.568	61.5 ±0.011
	Chloroform	0.462	50 ± 0.041
	Hexane	0.438	47.4 ± 0.028
	Methanol	0.593	64 ± 0.013

*Mean ±SD for 60 µg/mL, n = 3;

The total flavonoid content was expressed as mg QE/ g. Methanol leaf extract showed the highest total phenolic content, which ranged from 47.4 ± 0.017 hexane root extract to 64 ± 0.013g QAE/50 g methanol leaf extract. In this study, hexane extract showed the least TFC content. However, its flavonoid content is highest as compared to other plant extracts within a 50g yield. The decreasing order of TFC of leaf 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 3.1

Table 3.11: Summary of regiration equations and correlation coefficients

Type of analysis	**Regiration equation**	**R^2**
DPPH	y=0.00522x-0.5723	0.999
FRAP	y=0.153x+06115	0.9995
Phosphomolybdate	y=0.0026x+0.0801	0.9966
Total phenol	y=0.0344x+0.1887	0.9996
Total flavonoid	y=0.1533x-0.0191	0.997

3.6. Antimicrobial Activity

Leaf extracts of *Dorstenia foeteda* using different solvents demonstrated antibacterial activities against gram-positive and gram-negative bacteria strains. The methanol extract exhibited a maximum zone of inhibition against gram-positive and gram-negative bacteria followed by acetone, chloroform, and hexane extracts. While Streptococcus pyogenes gram-negative bacteria was found not susceptible to hexane and chloroform extracts, staphylococcus aureus was also not susceptible to hexane and chloroform extracts. 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 a zone of inhibition completely better than that of standard drug tetracycline, and are 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 extract 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 a 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 table 3.12.**Table3.12: Dose-dependent crude extracts and standards bacterial zone of inhibition.**

Bacteria	solvent	Zone of Inhibition in mm		
			Standards	
		Leaf extract	Gentamycin	Tetracycline
		10µg	10µg	30µg
Escherichia coli	Acetone	22.00±0.02	26.4±0.56	16.04± 0.52
	Chloroform	18.00±0.01		
	Hexane	14.00±0.01		
	Methanol	30.00±0.03		
Klebsiella Pneumonia	Acetone	20.00±0.03	23.00±0.37	
	Chloroform	18.00±0.45		
	Hexane	12.40±0.23		
	Methanol	27.20±0.57		15.07± 0.70
Staphylococcus Aureus	Acetone	20.30±0.07	24.00±00	
	Chloroform	14.10±0.22		
	Hexane	11.30±0.57		
	Methanol	26.60±0.69		< 5 mm
Streptococcus Pyogenes	Acetone	23.6±0.78	26.00±00	
	Chloroform	16.60±0.34		< 5 mm
	Hexane	10.23±0.32		
	Methanol	27.00±0.53		

4.**CONCLUSION AND RECOMMADATION**

4.1 Conclusion

The results of phytochemical analysis revealed the presence of alkaloids, phenols, flavonoids, tannins, saponin, and glycosides in different concentrations for each extract of the plant. The outcome of the investigation indicated that *Dorstenia foeteda* extracts were to find a high amount of phenolic content which plays a 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 leaf and root showed the highest activity than the others. Phytochemicals and antioxidants found in the extracts of *Dorstenia foeteda* extracts might be highly polar. The combination of the above results proves the effectiveness of the plant for its antibacterial, antioxidant activities, and use in traditional medicine.

4.2 Recommendation

The presence of a 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.*

Acknowledgments

The author is grateful to acknowledge Debre Markos and Bahir Dar University for providing facilities required to carry out this work.

Conflicts of Interest

The authors declared that there have no conflict of interest for this research.

Author Contributions

The authors have made substantive intellectual contribution to this study in data collection, identification of plants, preparation of the manuscript and authors have read and approved the final manuscript.

Funding

No findings has been received to conduct this research.

5. REFFERENCES

[1]. Abebe, D., Hagos, E., 1991. Plants as a primary source of drugs in the traditional health Practices of Ethiopia. In: Engles, J.M.M., Hawkes, J.G., MelakuWorede (Eds.), Plant Genetic Resources of Ethiopia. Cambridge University Press, Cambridge, pp. 101–113

[2]. Hammer, K.A., Carson, C.F. and Riley, T.V., 1999. Antimicrobial activity of essential oils and other plant extracts. *Journal of applied microbiology*, *86*(6), pp.985-990.

[3]. Giday, M., Asfaw, Z., Elmqvist, T. and Woldu, Z., 2003. An ethnobotanical study of medicinal plants used by the Zay people in Ethiopia. *Journal of ethnopharmacology*, *85*(1), pp.43-52.

[4]. Hillenbrand, E., 2006. Improving traditional-conventional medicine collaboration: Perspectives from Cameroonian traditional practitioners. Nordic Journal of African Studies, 15(1).

[5]. Misiewicz, T.M. and Zerega, N.C., 2012. Phylogeny, biogeography and character evolution of Dorstenia (Moraceae). Edinburgh Journal of Botany, 69(3), pp.413-440

[6]. Datwyler, S.L. and Weiblen, G.D., 2004. On the origin of the fig: phylogenetic relationships of Moraceae from ndhF sequences. *American Journal of Botany*, *91*(5), pp.767-777.

[7]. Datwyler & Weiblen, 2004; Zerega et al., 2005; Clement & Weiblen, 2009Clement, W.L. and Weiblen, G.D., 2009. Morphological evolution in the mulberry family (Moraceae). Systematic Botany, 34(3), pp.530-552.).

[8]. Clement, W.L. and Weiblen, G.D., 2009. Morphological evolution in the mulberry family (Moraceae). Systematic Botany, 34(3), pp.530-552.

[9]. Horwood, F.K., 1974. Some notes on the genus Dorstenia. *Cac Suc J*.

[10]. Rzepecky, A., 2016. Dorstenia horwoodii Rzepecky sp.nov.from Nudum to Novum, a Fortyish Year Hiatus. Cactus and Succulent Journal, 88(2), pp.66-75.

[11]. Umamaheswari, M. and Chatterjee, T.K., 2008. In vitro antioxidant activities of the fractions of Coccinia grandis L. leaf extract. *African Journal of Traditional, Complementary and Alternative Medicines*, *5*(1), pp.61-73.

[12]. Huang, T., Gao, P. and Hageman, M.J., 2004. Rapid screening of antioxidants in pharmaceutical formulation development using cyclic voltammetry-potential and limitations. *Current Drug Discovery Technologies*, *1*(2), pp.173-179.

[13]. Sochor, J., Ryvolova, M., Krystofova, O., Salas, P., Hubalek, J., Adam, V., Trnkova, L., Havel, L., Beklova, M., Zehnalek, J. and Provaznik, I., 2010. Fully automated spectrometric protocols for determination of antioxidant activity: Advantages and disadvantages. *Molecules*, *15*(12), pp.8618-8640.

[14]. Shrestha, P., Adhikari, S., Lamichhane, B.Gov inda, B.S. 2015. Phytochemical Screening of the Medicinal Plants of Nepal. IOSR-JESTFT, 1:11-17.

[15]. Banu, K.S. and Cathrine, L., 2015. General techniques involved in phytochemical analysis. *International Journal of Advanced Research in Chemical Science*, *2*(4), pp.25-32.

[16]. Bansode T. S. and Salalkar B .K., 2015. Phyto chemical analysis of some Selected Indian medcienal plants.International Jornal of Pharma and Bioscience, 6 (1)550-556

[17]. Shirazi, O.U., Khattak, M.M.A.K., Shukri, N.A.M. and Nasyriq, M.N., 2014. Determination of total phenolic, flavonoid content and free radical scavenging activities of common herbs and spices. *Journal of Pharmacognosy and Phytochemistry*, *3*(3), pp.104-108.

[18]. Chantiratikul, P., Meechai, P. and Nakbanpotec, W., 2009. Antioxidant activities and phenolic contents of extracts from Salvinia molesta and Eichornia crassipes. *Research Journal of Biological Sciences*, *4*(10), pp.1113-1117.

[19]. Randhir, R., Lin, Y.T. and Shetty, K., 2004. Stimulation of phenolics, antioxidant and antimicrobial activities in dark germinated mung bean sprouts in response to peptide and phytochemical elicitors. *Process Biochemistry*, *39*(5), pp.637-646.

[20]. Odabasoglu, F., Aslan, A., Cakir, A., Suleyman, H., Karagoz, Y., Halici, M. and Bayir, Y., 2004. Comparison of antioxidant activity and phenolic content of three lichen species. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *18*(11), pp.938-941.

[21]. Chua, L.S., Rahaman, N.L.A., Adnan, N.A. and Eddie Tan, T.T., 2013. Antioxidant activity of three honey samples in relation with their biochemical components. *Journal of analytical methods in chemistry*, *2013*.

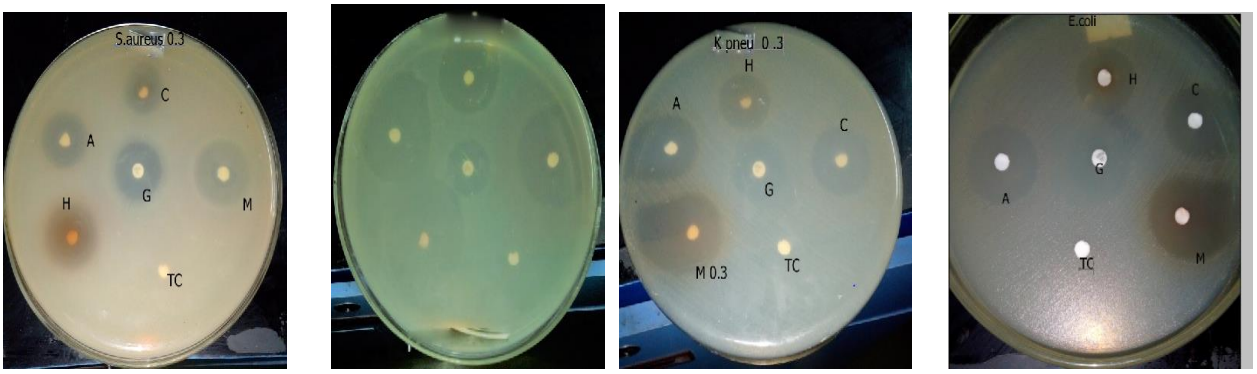
[22]. Wikler, M.A., 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard. *CLSI (NCCLS)*, *26*, pp.M7-A7.

[23]. Teymuri, A. and Amin, S., ANTIMICROBIAL EFFECT OF ROSMARINUS OFFICINAL EXTRACTS ON BIOFILM OF SOME IMPORTANT HUMAN BACTERIAL PATHOGENS.

[24]. Akinyemi, K.O., Oladapo, O., Okwara, C.E., Ibe, C.C. and Fasure, K.A., 2005. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant Staphylococcus aureus activity. *BMC complementary and alternative medicine*, *5*(1), pp.1-7.

[25]. Hilawea, K.T. and Desta, Z.Y., 2020. Determination of Biological Activities of the Root Bark of Senna singueana. *Asian Journal of Chemical Sciences*, pp.25-34.

APPENDIX



Staphylococcus aureus DF leaf | **k. pneumonia crude leaf extracts** | **K. pneumonia 0.3mg/mL leaf extract** | **E. coli leaf extracts**

Extracts (A=acetone, C= chloroform, M= methanol, H= hexane, TC = tetracycline ,G= gentamycin)

Appendix 7. 4 antibacterial test for different concentration and standards