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Antibacterial Properties of Traditional Medicinal Flora From The Benishangul-Gumuz Region, Ethiopia

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ABSTRACT

In Ethiopia, the utilization of medicinal plants for the treatment of different illnesses has been common, although their effectiveness and doses are not unknown. Aralia elata, Coffee arabica, Croton macrostachyus, *and* Ficus sycomorus *are some of the common cultural medicinal plants in the Benishangul-Gumuz region of Ethiopia. Leaves of these plants were extracted using solvents such as methanol, chloroform, and petroleum ether. The inhibition effect of the crud extracts was tested against pathogenic bacterial strains such as* Staphylococcus aureus *ATCC 25926,* Escherichia coli *ATCC 25922,* Shigella boydii *ATCC 12022,* Salmonella typhi *ATCC 10535 and* Pseudomonas aeruginosa *ATCC 248 using disc diffusion method. The Minimum Inhibitory Concentration (MIC) of the crud extracts was determined by the microplate dilution method. The highest biological yield was 2.32% from the chloroform extract of Ficus sycomorus. The maximum inhibition zone for petroleum ether extracts was 15 mm against E. coli from Ficus sycomorus at a concentration of 100 mg/mL. 18 mm in diameter of Staphylococcus aurous growth was inhibited at 100 mg/ml of chloroform extract of Croton macrostachyus. Methanol extract from the plants inhibited the growth of the five pathogens with an inhibition range of 12 mm to 21 mm in diameter. The lowest inhibitory concentration was 12.5 mg/mL by the methanol and chloroform extract of the plant's leaves against the pathogens. In general, the methanolic-based crude extract of the plants was the most effective and efficient in ranges of inhibition.*

Keywords: Crude extract, Antibacterial, Medicinal plants, Cultural medicine, Disc diffusion.

1. INTRODUCTION

The ever-evolving drug-resistant microbes urged researchers to look for potential antibiotics that are capable of killing or inhibiting the pathogens. Therefore, searches for effective medicinal plants that are known to have the potential to inhibit or kill pathogens are becoming the major work of therapeutic industries.

Ethiopia is located in the horn of Africa within a wide range of latitude (3 and 15°) and longitude (33 and 48°). The country is also characterized by a wide range of ecological, edaphic, and climatic condition that contributes to diverse floral composition. Besides, Ethiopia is known for its ancient civilization and is home to diverse ethnomedicinal utilization.

The extensive utilization of *Aralia elata* in the treatment of various ailments, including hepatitis, rheumatoid arthritis, bruising, lumps, and carbuncles, has been documented in nations such as China, Japan, and South Korea (Xu et al., 2021). Research indicates that extracts from different parts of Coffea arabica exhibit antibacterial properties (Duangjai et al., 2016). Notably, hexane and ethyl acetate extracts derived from coffee leaves have demonstrated efficacy against bacterial pathogens, including Edwardsiella tarda, Streptococcus agalactiae (Mewaba et al., 2019), and Vibrio cholerae (Rawangkan et al., 2022). Furthermore, methanol, ethyl acetate, and butanol extracts from the stem bark of Croton macrostachyus have shown significant antibacterial effects against human pathogens such as *Escherichia coli*, *Salmonella typhi*, Klebsiella pneumoniae, Enterobacter aerogenes, and Listeria monocytogenes (Obey et al., 2016). In a similar vein, crude leaf extracts of Ficus sycomorus L., using ethanol, methanol, and acetone, have proven effective against resistant strains of *Staphylococcus aureus*, Acinetobacter baumannii, *Salmonella typhi*, and *Escherichia coli* (Saleh et al., 2015).

In Ethiopia, particularly in the Benishangul-Gumuz region, different parts of plants have been used for the treatment of different diseases. However, the effectiveness of the plant's extract in inhibiting some of the pathogens is scientifically not proven. Therefore, this experiment is aimed at investigating the antibacterial effect of cultural medicinal plants extracted from leaves of *Aralia elata, Coffee arabica, Croton macrostachyus,* and *Ficus sycomorus.*

2. MATERIALS AND METHODS

2.1. Description of the Sampling Area

Leave samples of the medicinal plants were collected from Mao-Komo district, Benishangul-Gumuz Region of Ethiopia. Mao-Komo district is found within the coordinates of 9^{0}00N- 9'4^{0}00N latitude and 34^{0} 00"-4'4'00'E longitude (Fig. 1). The altitude and temperature of the area are in the range of 15-23 m a.s.l. and 17-27 °C, respectively. The district has an unimodal type of rainfall that ranges from 900 to 1400 mm; the highest rain was during the summer (June, July, and August). Mao-Komo district is bordered by two countries: South Sudan in the west and Sudan Republic in the north. Bamboo is an abundant plant in the district, along with some other indigenous plant species.

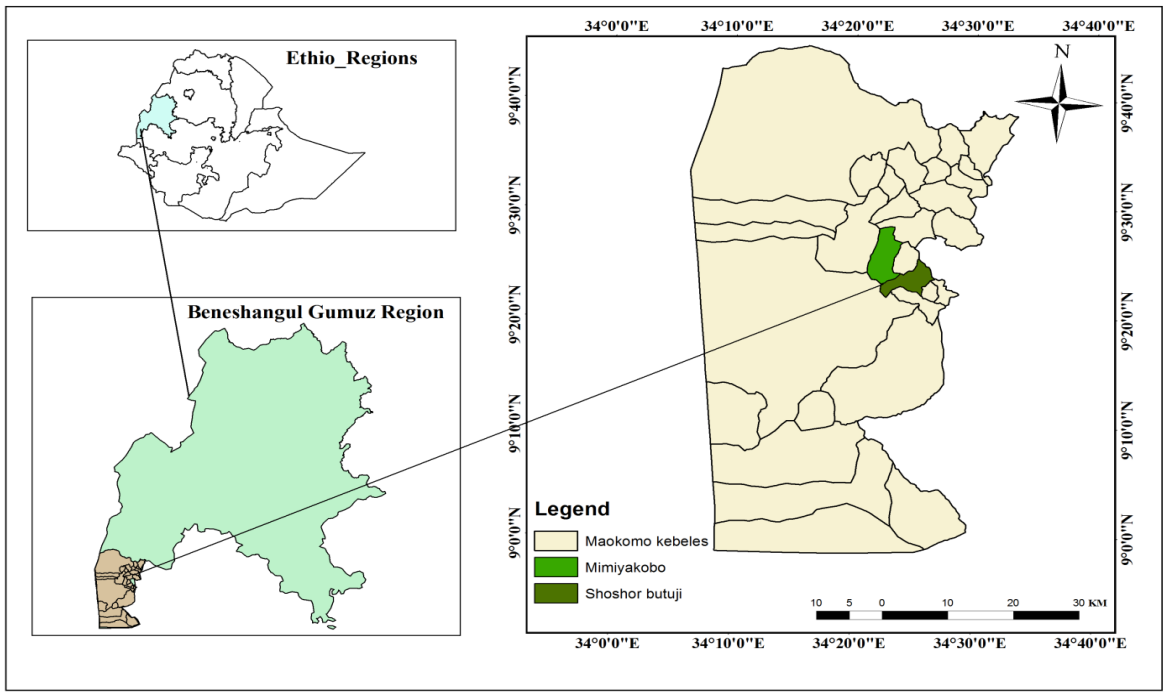

Fig. 1. Map of the sampling area in Ethiopia, Mao-Komo, Benishagul Gumuz region (Source Ethio- GIS)

The major ethnic groups living in the district are Mao and Komo, although Oromo, Barta, and Fedhashi are also living in the area. The livelihood of the indigenous community is mixed small-scale farming, and they are mostly vegetarian.

2.2. Plant Specimen Collection and Identification

Ethnobotanical data were collected from elders and herbalists of the district using semi-structured interviews. Thus, information regarding the local names of medicinal plants, specific locations, parts of plants commonly used as medicine, preparation, and method of utilization was organized based

on the consent of confidentiality. Five major plant species known for their medicinal value were identified, and leaf samples of the plants were taken to Wollega University Herbarium for identification as shown in Fig. 2. The plants were identified as *Coffee arabica (fig. 2a), Aralia elata (fig. 2b), Croton macrostachyus (fig. 2c),* and *Ficus sycomorus (fig. 2d).* According to herbalists, leaf extracts of these plants are commonly used for wound healing and relief of stomachaches among the indigenous people.

(a) *Coffee Arabica* (b) *Aralia elata*

(c) *Croton macrostachyus* (d) *Ficus sycomorus*

Fig. 2(a-d). Pictures of medicinal plants used for the study

2.2.1. Leaf Sample Collection And Preparation

Sufficient plant leaves from their actively growing part were collected in a paper bag and washed with sterile water to get rid of soil particles. The samples were dried in open air under shade for two weeks (Ebadi et al., 2015). The dried leaves were meshed with a hand and ground to 400 μm in an electric miller. It was sieved to screen out the appropriate size (Azwanida, 2015).

2.2.2. Plant Crude Extraction

The maceration technique was used for the extraction of plants' leaves using methanol, chloroform, and petroleum ether as solvents at room temperature. 2 g of each sample was dissolved separately in 100 ml of the solvents in flasks, and the solution was shaken on a rotary shaker for 48 hr.

2.2.3. Filtration and Concentration

The extract was filtered using filter paper (Whatman 1), and the filtrate was concentrated using a rotary evaporator. Afterward, the product was air-dried and the yields were recorded. The yield was obtained by dividing the weight of the dry extract by the weight of the sample used for extraction (Alshammaa, 2016). The extracts have been kept in a cooling room (4 °C).

2.2.4. Source of Test Organisms

The test organisms utilized in this study included *Staphylococcus aureus* ATCC 25926, *Escherichia coli* ATCC 25922, *Shigella boydii* ATCC 12022, *Salmonella typhi* ATCC 10535, and *Pseudomonas aeruginosa* ATCC 248. These bacterial strains were originally sourced from the Ethiopian Public Health Institute (EPHI) and were preserved through freeze preservation in the microbiology laboratory at Wollega University. To activate the cultures, they were incubated in Mueller-Hinton broth media at 37 °C for 48 hours in a rotary incubator shaker. The purity and viability of the cultures were confirmed by subculturing on selective and differential agar media.

2.2.5. Antimicrobial Test of the Crude Extracts

The experiments were carried out on Mueller-Hinton agar media utilizing the disc diffusion technique. 1 ml of actively growing bacterial strains, estimated to have around 10^7 CFU ml^{-1} (0.5

McFarland Standard), was evenly spread across the agar plates using cotton swabs. The inoculated plates were allowed to sit at room temperature for 6 minutes to ensure the surface moisture dried before the extract was applied. Concurrently, sterile filter paper disks (Whatman No. 1, 6 mm in diameter) were immersed in a 100 mg/mL solution of the plant crude extract prepared with 10% DMSO. Once the extracts were absorbed, the filter paper disks were placed onto the culture plates. Control disks of Amoxicillin (30 µg), Erythromycin (15 µg), and Ciprofloxacin (20 µg), along with a disk soaked in 10% DMSO, were also included. After an incubation period of 18 hours at 37°C, the diameters of the growth-free zones, also known as zones of inhibition, were measured in mm using a Vernier caliper (De Zoysa et al., 2019).

2.3. Minimum Inhibitory Concentration (MIC) Determination

The antimicrobial efficacy of the crude plant extracts that exhibited inhibitory effects on pathogen growth was evaluated for the minimum inhibitory concentration (MIC) utilizing the microplate dilution method with slight modifications. A series of two-fold dilutions of the plant extracts were prepared in 10% DMSO. Subsequently, 300 µl of each plant extract was combined with 100 µl of bacterial suspensions, which were prepared from Mueller-Hinton broth, in Eppendorf tubes. Control tubes were included, containing bacterial suspensions with various concentrations of antibiotics (positive control) and tubes containing the plant extract without bacterial suspension (negative control). The cultures were incubated at 35 ± 2°C for 24 hours. The assessment of antimicrobial activity was conducted by measuring absorbance at 630 nm using a UV-VIS spectrophotometer (Drawell, DU-8800R). The negative control served as the blank for these measurements (Mishra et al., 2017).

2.4. Data Analysis

All experiments were conducted in triplicate. The inhibition zone data were analyzed using SPSS version 20.0 software, and the mean performances were compared employing Tukey's b test at a significance level of $\alpha = 0.05$.

3. RESULTS AND DISCUSSION

The yield of the crude extract exhibited variation depending on the type of solvents utilized for extraction. The highest yield recorded was 2.32% from the methanol extract of Ficus sycomorus, followed by the petroleum ether extract of Coffee arabica at 1.93%. The lowest yield was observed

at 1.33% for *Aralia elata* and *Croton macrostachyus*, using methanol and petroleum ether solvents, respectively (Fig. 3). All experiments were conducted in triplicate. Inhibition zone data were analyzed using SPSS version 20.0 software, and their mean performance was compared employing Tukey's b at $\alpha = 0.05$.

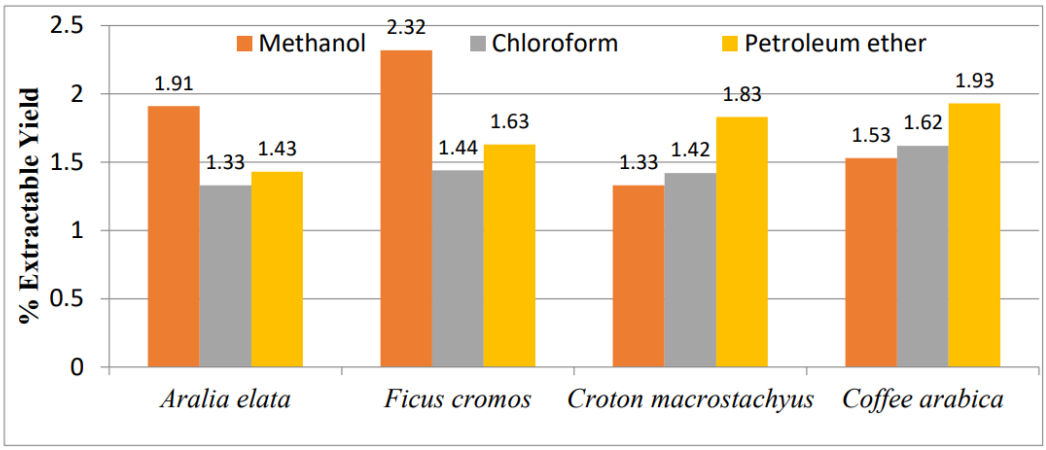

Fig. 3. Leaf Extractable Yield (%) of *Aralia elata, Ficus sycomorus, Croton macrostachyus*, and *Coffee Arabica* by three different solvents

Several factors can influence the yield of crude plant extracts. According to Nawaz et al. (2020), an increase in solvent polarity correlates with a higher extractable yield in protein-rich seeds. In the present study, the extractable yield of *Aralia elata* and *Ficus sycomorus* improved with increasing solvent polarity, while the opposite trend was observed for *Croton macrostachyus* and *Coffee arabica* (Fig. 3). Previous research has demonstrated a 72% variation in extractable yields for different plants using a single solvent (El Mannoubi, 2023). Furthermore, the extractable yield is also contingent on the specific parts of the plant employed for extraction, such as leaves, seeds, or fruits. For instance, Cha et al. (2009) reported a yield difference of up to 53% in crude extraction between the leaves and shoots of *Aralia elata* when using the same solvent and extraction method, with the leaves yielding the highest amount. The crude extraction yield reported in this study is comparatively lower than previously documented findings; this may be attributed to factors such as the sample-to-solvent ratio, extraction method, extraction temperature, and duration of extraction or maceration (Che Sulaiman et al., 2017).

3.1. Antibacterial Activities of Petroleum Ether-Based Crude Extracts

100 mg/ml of the plant extract using petroleum ether as solvent didn't inhibit the strains of *Shigella boydii* and *Staphylococcus aureus*. The strains *E. coli* and *Pseudomonas aeruginosa* were inhibited only by

Ficus sycomorus and *Aralia elata* leaf extracts (Fig. 4), with an average inhibition zone of 15 mm and 7 mm, respectively.

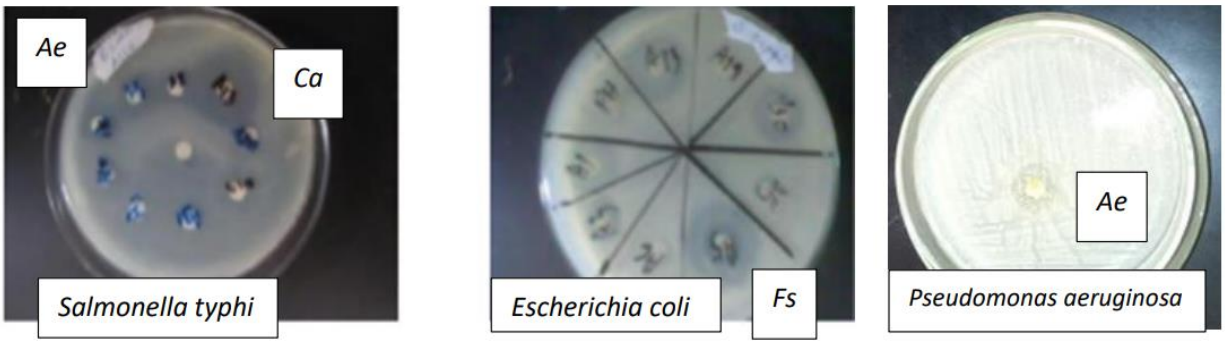

Fig. 4. Inhibition potential of the plant extract using Petroleum ether as a solvent against bacterial pathogens

A strain of *Salmonella tyhpi* was inhibited by 100 mg/ml of leaf extracts of *Coffee arabica* and *Aralia elata* with an inhibition zone of 10 mm and 8 mm, respectively (Table 1). The highest inhibition was by extracts of *Ficus sycomorus* against the strain of *E. coli,* which was comparable with inhibition by Ciprofloxacin (20µg/ml). Similarly, the growth inhibition of *Coffee arabica* extract against the stain of *Salmonella tyhpi* was comparable with Erythromycin (15µg/ml), and the inhibition potential of *Aralia elata* extract against the strain of *Salmonella tyhpi* was not significantly smaller than Erythromycin (15µg/ml) (Table 1).

Table 1: *Antibacterial effect of plants' leaf extract using petroleum ether as a solvent on disk diffusion method (zone of inhibition)*

No	Plant species	Concentration	Inhibition of the test strains of the pathogens (mm)				
			E.coli	*Salmonella tyhpi*	*Shigella boydii*	*Staphylococcus aurous*	*Pseudomonas aeruginosa*
1	*Coffee Arabica*	100mg/ml	-	10 ± 0.1^{c}	-	-	-
2	*Aralia elata*	100mg/ml	-	8 ± 0.4^{cd}	-	-	7 ± 1^{c}
3	*Ficus sycomorus*	100mg/ml	15 ± 0.8^{cd}	-	-	-	-
4	*Croton macrostachyus*	100mg/ml	-	-	-	-	-
5	Amoxicillin	30 µg/ml	27 ± 0.6^{a}	18 ± 0.7^{a}	14 ± 0.8^{d}	25 ± 0.1^{b}	25 ± 0.51^{b}
6	Erythromycin	15 µg/ml	23 ± 0.1^{b}	12 ± 0.2^{bc}	31 ± 0.4^{a}	27 ± 0.3^{a}	10 ± 0.2^{d}
7	Ciprofloxacin	20 µg/ml	17 ± 0.2^{c}	14 ± 0.1^{b}	12 ± 1^{d}	24 ± 0.1^{c}	30 ± 0.6^{a}

Foot Note: Inhibition zones (mm) designated by the different letters across the column are significantly different from each other at a=0.05

There is no previous report on the antibacterial characteristics of the plants' extract using petroleum ether against the four pathogens. However, an experiment showed that 10 mg/ml of leaf extracts of *Mimosa pudica* using petroleum ether have inhibited *Staphylococcus aurous* up to 8 mm in diameter (Akter et al., 2010). Therefore, the current result showed the possibility of obtaining bioactive compounds from the plants using proportional optimization.

3.2. Antibacterial Properties of Chloroform-Based Crude Extracts

100 mg/ml of the four plants' crude extract using chloroform did not show any inhibition against the strain of *E. coli* but on *Shigella boydii, Staphylococcus aureus, and Pseudomonas aeruginosa (Fig. 5).* However, some crude extracts showed selective inhibition against the other bacterial strains. As shown in Table 2, 100 mg/ml of chloroform-based leaf crude extract of *Aralia elata* has inhibited the Salmonella *tyhpi* strain comparable to the inhibition made by Ciprofloxacin (20µg/ml) and significantly higher than the inhibition made by Erythromycin (15µg/ml).

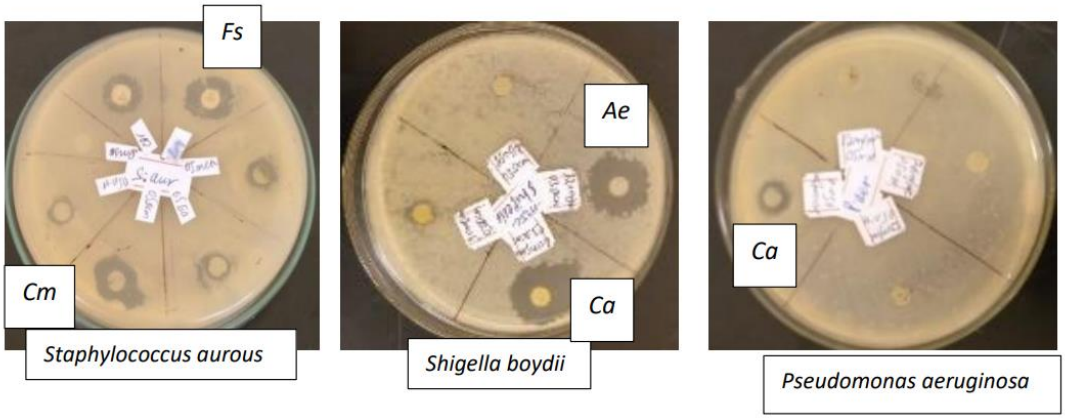

Fig. 5. Inhibition potential of the plant extract using Chloroform as solvent against bacterial pathogens

Leaf extracts from *Ficus sycomorus* and *Croton macrostachyus* by Chloroform showed comparable growth inhibition against the strain of *Staphylococcus aureus* with an inhibition zone of 17 mm and 18 mm, respectively (Table 2). Previous reports also indicated the bioactive properties of *Ficus sycomorus* leaf extract using the solvent against *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa* at 15–

60 µg/disc, with the highest inhibition of 19 mm on *E. coli* (Mudi et al., 2015). Besides, the leaf extract of *Coffee arabica* using chloroform showed growth inhibition against *Pseudomonas aeruginosa.*

Table 2: *Antibacterial Effect of plants' leaf extract using chloroform as a solvent on disk diffusion method (zone of inhibition)*

No	Plant species	Concentration	Inhibition of the test strains of the pathogens(mm)				
			E.coli	*Salmonella tyhpi*	*Shigella boydii*	*Staphylococcus aurous*	*Pseudomonas aeruginosa*
1	*Coffee Arabica*	100mg/ml	-	-	17±1^{c}	-	10±0.6^{d}
2	*Aralia elata*	100mg/ml	-	14±0.9^{b}	12±0.3^{d}	-	-
3	*Ficus sycomorus*	100mg/ml	-	-	-	17±0.3^{c}	-
4	*Croton macrostachyus*	100mg/ml	-	-	-	18±0.1^{c}	-
5	Amoxicillin	30 µg/ml	27±0.6^{a}	18±0.3^{a}	14±1^{d}	25±0.1^{b}	25±0.4^{b}
6	Erythromycin	15 µg/ml	23±0.2^{b}	12±0.6^{c}	31±0.1^{a}	27±0.3^{a}	10±0.8^{d}
7	Ciprofloxacin	20 µg/ml	17±0.3^{c}	14±0.5^{b}	12±0.6^{d}	24±0.2^{b}	30±0.7^{a}

Foot Note: Inhibition zones (mm) designated by the different letters across the column are significantly different from each other at a=0.05

Although there is no report on the antimicrobial activities of the chloroform extract of *Coffee arabica* leaf against *Pseudomonas aeruginosa;* the current result is comparable with the Chloroform extract of *Cornus macrophylla* leaf against the pathogen *(*Akbar *et al.*, 2020).

3.3. Antibacterial Properties of Methanol-Based Crude Extracts

Methanol extract of *Aralia elata* leaf (100mg/ml) inhibited the growth of the five strains of bacterial species. *Salmonella tyhpi, Shigella boydii,* and *Pseudomonas aeruginosa* were inhibited by the methanol extract of the four plant species (Fig. 6).

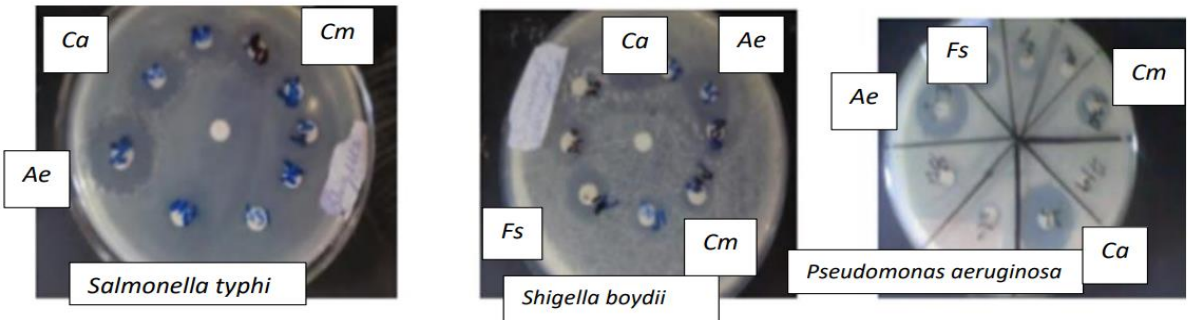

Fig. 6. Inhibition potential of the plant extract using methanol as a solvent against bacterial pathogens

Foot Note: *Coffee Arabica (Ca), Aralia elata (Ae), Croton macrostachyus (Cm), and Ficus sycomorus (Fs).*

The maximum growth inhibition was 21mm from *Coffee arabica* and *Aralia elata* extract against the strains of *Pseudomonas aeruginosa* and *Salmonella tyhpi,* respectively; with the latter significantly higher than the inhibition by the antibiotics (Table 3).

Table 3: *Antibacterial Inhibition Effect of methanol extracts of the plant leaves using disk diffusion method (zone of inhibition)*

No	Plant species	Concentration	Inhibition of the test strains of the pathogens(mm)				
			E.coli	*Salmonella tyhpi*	*Shigella boydii*	*Staphylococcus aurous*	*Pseudomonas aeruginosa*
1	*Coffee arabica*	100mg/ml	-	14 ± 0.2^{c}	16 ± 1^{cd}	-	21 ± 0.2^{c}
2	*Aralia elata*	100mg/ml	14 ± 0.1^{d}	21 ± 0.3^{a}	17 ± 1^{c}	12 ± 0.6^{d}	19 ± 0.4^{cd}
3	*Ficus sycomorus*	100mg/ml	-	8 ± 0.9^{d}	14 ± 0.8^{d}	17 ± 0.1^{c}	10 ± 0^{e}
4	*Croton macrostachyus*	100mg/ml	-	10 ± 0.4^{d}	14 ± 0.7^{cd}	18 ± 0.1^{c}	8 ± 1^{e}
5	Amoxicillin	30 µg/ml	27 ± 0.6^{a}	18 ± 0.6^{b}	14 ± 0.6^{cd}	25 ± 0.3^{a}	25 ± 0.1^{b}
6	Erythromycin	15 µg/ml	23 ± 0.3^{b}	13 ± 0.2^{c}	31 ± 0.4^{a}	24 ± 0.4^{a}	10 ± 0.2^{e}
7	Ciprofloxacin	20 µg/ml	17 ± 0.2^{c}	14 ± 0.3^{c}	12 ± 0.3^{e}	24 ± 0.5^{a}	30 ± 0.7^{a}

Foot Note*: Inhibition zones (mm) designated by the different letters across the column are significantly different from each other at a=0.05*

Methanol extract of *Coffee Arabic* (100 mg/mL) inhibited *Salmonella tyhpi* proportional to Erythromycin (15 µg/ml) and Ciprofloxacin (20 µg/ml) (Table 3). Similarly, growth inhibition of *Pseudomonas aeruginosa* by methanoic leaf extract of the plants was significantly higher or proportionate to inhibition of the bacterial species by Erythromycin (15 µg/ml) (Table 3). The anti-*Salmonella tyhpi* of methanoic extract of *Croton macrostachyu*s in the present experiment was higher than the antibacterial potential in the bark of the plant species (Obey *et al*., 2016). Although there is variation in the antimicrobial potential of crude extracts from different plant parts, variation could also depend on strains of the pathogen and the type of solvents used for extraction (Adeonipekun *et al*., 2014).

3.4. Minimum Inhibitory Concentration (MIC) of the Crude Leaf Extracts

The minimum inhibitory concentration of the crud extract showed variation. *E. coli* was sensitive to 100 mg/ml of methanoic-based *Aralia elata* crude extract and petroleum ether extracts of *Ficus sycomorus*. Whereas *Staphylococcus aureus* was resistant to 100 mg/ml crude extract of *Coffee arabica.* The lowest crude concentration that inhibited the growth of the pathogens was 12.5 mg/mL (Table 4).

Relatively, methanol-based crude extracts of the plants were effective against most pathogens at lower concentrations.

Table 4: *Minimum Inhibitory Concentration (mg/ml) of leaf Crude Extracts from four Plants using three Solvent types*

Bacteria	*Coffee arabica*			*Aralia elata*			*Ficus sycomorus*			*Croton macrostachyus*		
	MeOH	Chlo	Pet.E	MeOH	Chlo	Pet.E	MeOH	Chlo	Pet.E	MeOH	Chlo	Pet.E
E.coli	-	-	-	25	-	-	-	-	25	-	-	-
Salmonella typhi	25	-	25	12.5	25	50	50	-	-	25	-	-
Shigella boydii	12.5	12.5	-	12.5	25	-	25	-	-	25	-	-
Staphylococcus aureus	-	-	-	25	-	-	12.5	12.5	-	12.5	12.5	-
Pseudomonas aeruginosa	12.5	25	-	12.5	-	50	25	-	-	50	-	-

***Foot Note**: MeOH, Methanol; Chlo, Chlorophorm; Pet.E, Petrolium Ether*

In this experiment, 12.5 mg/ml of methanolic crude extract of *Aralia elata* was able to inhibit the growth of *Salmonella typhi.* Similarly, the growth of *Shigella boydii* was inhibited by 12.5 mg/mL methanol and chloroform crude extracts of *Coffee arabica* and *Aralia elata.* The lowest concentration for the methanolic extract of *Aralia elata, Ficus sycomorus,* and *Croton macrostachyus* that inhibited *Staphylococcus aureus* was 12.5 mg/ml (Table 4). The MIC that inhibited *Pseudomonas aeruginosa* was 12.5 mg/mL by methanolic crude extract of *Coffee arabica* and *Aralia elata.* Previous research on *Croton macrostachyus* stem bark showed that 62.5 mg/mL and 125 mg/mL of methanol extract were effective in inhibiting *Escherichia coli* and *Staphylococcus aureus*, respectively, whereas *Staphylococcus aurous* was inhibited at 250 mg/mL of the chloroform extract of the plant (Minyamer and Belay, 2018). This showed that the leaf crude extract contains comparatively higher bioactive compounds as compared to the crude extract from stem bark.

4. CONCLUSION

Leaf extracts of *Aralia elata, Coffee Arabica, Croton macrostachyus,* and *Ficus sycomorus* using solvents such as petroleum ether, chloroform, and methanol have shown antibacterial activities. However, antibacterial activities depend on the concentration of the crude extract, the type of solvents used for extraction, and the nature of the bacterial pathogens. 100 mg/mL of methanolic crude extracts

from the leaves of the plants were effective in inhibiting the growth of *Salmonella tyhpi, Shigella boydii,* and *Pseudomonas aeruginosa*. The MIC of most of the crude extracts was 12.6 mg/mL.

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