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An Analysis of the Anti-Mycobacterial Activity of some Medicinal Plants

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Abstract - The objective of this study is to provide a rational scientific justification for the anti-mycobacterial activity of medicinal plant extracts. Mycobacterium-suppressing characteristics may be found in a variety of plants, including well-known species like Aloe vera and Ocimum sanctum as well as notorious and swiftly spreading species like Lantana camara and Acacia senegal.

Keywords - Plants, Meditional plant, Anti microbial activity

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INTRODUCTION

Plants that have been used to treat sickness for millennia have a good chance of keeping up with demand. Both the raw and prepared forms have found considerable success. Due to its abundance of medicinal plants and long history of using herbal medicine, India stands out as a site for those seeking treatment for a wide range of disorders. As a consequence, it makes sense to integrate recent scientific findings into conventional knowledge. Amazingly, many common plants have beneficial effects when used medicinally. About 70,000 plant species have been employed historically for their medicinal benefits. It is possible to synthesise or isolate conventional drugs from plants. More over five thousand traditional Chinese medicines (TCMs) are derived from plants, and over two thousand Ayurvedic physicians believe that particular plant species have therapeutic properties. Even though entire plants are seldom used, over 500 herbs are still included into traditional treatment.

REVIEW OF LITERATURE

Donfack, et al., (2019) This research sought to assess the antimycobacterial efficacy of plants extracted using organic solvents from plants chosen for their ethnobotanical value. The Resazurin Microtiter Assay was used to assess the in vitro viability of *Mycobacterium smegmatis, Mycobacterium avium, Mycobacterium bovisBacille Calmette Guerin (BCG)*, *Mycobacterium TB*, and *Mycobacterium ulcerans*. Human lung fibroblast cells (MRC5) and bone marrow-derived macrophages were tested for MTS tetrazolium's cytotoxicity (BMDM). BMDM infected with *M. smegmatis* was used to assess the most promising Annickiachlorantha stem bark extract (ACsbI) for intracellular antimycobacterial activity against *M. smegmatis*. 19 Cameroonian medicinal herbs were used to yield sixty crude extracts, 19 fractions, and two refined compounds. The interface fractions from the stem bark and stem of *A. chlorantha*, respectively, exhibited Minimal Inhibitory Concentrations (MIC) of 1.95 and 7.81 g/ml and showed the highest activity against *M. ulcerans* as a result. The results also showed that crude extracts mostly inhibited BCG. At dosages of 3.9 g/ml and 62.5 g/ml, respectively, two compounds (SJfr 3.6 and SJfr 4.5) produced from Sorindeiajuglandifolia fruits have shown efficacy against BCG and *M. ulcerans*. Finally, ACsbI inhibited intracellular *M. smegmatis* growth while being safe for MRC5 cells and BMDM. The findings of this study support the traditional uses of these herbs and the necessity for more investigation into them to find viable substitutes for the present antimycobacterial medications.

Nguta, et al., (2016) There were five distinct medicinal plants studied for their crude extracts' cytotoxicity and antimycobacterial properties. Microplate alamar blue assay (MABA) was used to examine antimycobacterial activity, while the CellTiter 96s AQueous Assay was used to examine cytotoxicity. For cytotoxicity tests, a novel tetrazolium chemical known as CellTiter 96s is used. The correlation coefficients were used to compare the efficiency of crude extracts against pathogenic and nonpathogenic strains of Mycobacterium TB subsp. tuberculosis. The minimal inhibitory concentration results demonstrated that crude extracts were effective against all three mycobacterial strains. To combat M. tuberculosis strain H37Ra (ATCCs

25,177TM), the leaves of Solanum torvumSw were found to have the lowest inhibitory doses, at only 156.3 mg/mL. (Solanaceae). There was a wide range of cytotoxicity across the extracts, although the leaves of S. torvum were the most selective. The Mycobacterium tuberculosis H37Ra strain was the most accurate predictor of success against the virulent Mycobacterium tuberculosis subsp. tuberculosis. Conclusions from this study support the use of certain medicinal plants in the treatment of tuberculosis. To battle drug-sensitive and drug-resistant forms of Mycobacterium TB, further research into Solanum torvum leaves is required.

Abuzeid, et al., (2014) In this research, 50 ethanolic extracts from various parts of 46 chosen medicinal plants—which have historically been used in Sudan to treat infectious diseases—were evaluated for their antimycobacterial capabilities. After the plants were harvested, ethanolic extracts were created. Hydrophilic and hydrophobic solvent fractionation were used for different extracts. The growth of mycobacteria in primary human macrophages and broth cultures was assessed using a luminometry-based test in the presence or absence of plant extracts and extract fractions, respectively. Additionally, it was established whether or not the active plant extract fractions were cytotoxic. An avirulent strain of *Mycobacterium TB* was significantly inhibited by three of the tested extracts at the initial screening concentrations (125 and 6.25 g/ml) (H37Ra). Leaf extract from *Rosmarinus officinalis L*. and leaf and bark extract from *Khaya senegalensis* were also used. Additional fractions of these plant extracts were able to continue to function in the presence of the solvents n-hexane, chloroform, ethanol acetate, n-butanol, and water. Additional research was conducted since it was shown that the chloroform fraction of the bark from *Khaya senegalensis* was not hazardous to human monocyte-derived macrophages and other cell types at the levels used. Further research on the *Khaya senegalensis* bark's chloroform percentage is required in light of these findings.

MATERIAL AND METHODS

Selection of medicinal plants

The study's plants were selected because they are plentiful around the Integral University campus and are often used in Unani therapy. Particular plants were selected for this study as follows *Acacia arabica, Emblica officinalis*, *Ficus bengalensis*,*Lantana camara, Lawsonia inermis, Myristica fragrans, Nyctanthus arbortristis*, *Ocimum sanctum, Ricinus communis* and *Ziziphus jujuba*

Extract preparation

All of the plant leaves were rinsed in distilled water to remove any lingering dust. These cleaned leaves were air dried at room temperature, out of direct sunshine. After the leaves had dried completely, they were powdered, measured, and stored at room temperature.

Mycobacterial strains

The Standard for Comparison *Mycobacterium tuberculosis* H37Ra (MTCC 300) and Mycobacterium avium (MTCC 1723) culture strains were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), a national institute funded jointly by the Department of Biotechnology (DBT) and the Council of Scientific and Industrial Research (CSIR), Government of India.

Revival of strains

In accordance with the standard operating procedures (SOPs) provided by MTCC, IMTECH, and ATCC Guidelines, lyophilized strains were revived in L-J Medium and Middlebrook 7H9 broth medium.

RESULT AND DISCUSSION

Results for *m tuberculosis*

Comparison of Mycobacterium tuberculosis means growth and % inhibition in extract-containing and extract-free controls after being kept at 37 degrees Celsius for 42 days, the slants L-J were measured. Both the water and ethanol extracts of the medicinal plants were shown to inhibit the growth of the *M. tuberculosis* standard strain. *M. tuberculosis* was inhibited by both water and alcoholic extracts of the plant's *Lantana camara, Ocimum sanctum, Aloe vera,* and *Acacia senegal*. The anti-mycobacterial properties of Ocimum sanctum were tested on Mycobacterium tuberculosis. With 2%, 4%, and 6% concentrations of extract in LJ medium, the percentage of inhibition was 19, 40, and 62 for water extract and 28, 57, and 69 for alcoholic extracts, respectively. *Lantana camara* water extract showed 13, 31, and 48 percent inhibition in 2%, 4%, and 6% LJ medium, whereas *Lantana camara* ethanol extract showed 19, 44, and 56 percent inhibition in the same concentrations. There was anti-mycobacterial action in *Acacia Senegal* against Mycobacterium tuberculosis. Water extract showed 19%, 31%, and 40% inhibition at 2%, 4%, and 6% in the medium, whereas alcohol extract showed 28%, 40%, and 48% inhibition at the same concentrations. Both the water and alcohol extracts of Ocimum sanctum showed much higher levels of anti-mycobacterial activity than those of any other plant tested. All plants benefited more from an alcoholic extract than a water one.

Results for m *avium*

Comparison of *M. avium* mean growth and % inhibition in extract-containing and control samples After 21 days of incubation at 37°C, the slants L-J were measured. All plant extracts tested, whether dissolved in water or ethanol, inhibited *M. avium* isolates. Inhibitory action against *M. avium* was seen in all four plants. Water Extract of *Lantana camara* showed 40%, 52%, and 63% inhibition in 2%, 4%, and 6% extract containing medium, whereas Alcoholic Extract showed 47%, 59%, and 67% inhibition in the same conditions. Using 2%, 4%, and 6% concentrations of extract-containing LJ medium, we found that Ocimum sanctum exhibited anti-mycobacterial activity. The percentage of inhibition was 45, 61, and 71 for the water extract and 59, 68, and 75 for the alcoholic extracts, respectively. Additionally, anti-mycobacterial efficacy against *M. avium* was observed in *Acacia senegal*. The percentages of inhibition in water extract-containing mediums at 2%, 4%, and 6% were 20, 33, and 40, whereas those in alcoholic extract-containing media were 26, 40, and 47. The proportion of *M. avium* growth inhibited by 2%, 4%, and 6% LJ medium containing pure Aloe vera gel was 20, 26, and 33%, respectively. Both the water and alcohol extracts of *Ocimum sanctum* showed much higher levels of anti-mycobacterial activity than those of any other plant tested. All plants benefited more from the alcoholic extract than the water extract. Plant extracts tested for anti-mycobacterial activity in Lowenstein-Jensen (L-J) medium.

Table 1: Mean CFU of *Mycobacterium tuberculosis* on Extract containing L J Medium

Plant Botanical Name	Part Used	ExtractType	Control Drug Free Media	Isoniazide Drug Media 0.2 µg/ml	L-J proportionmethod		
					Mean cfu onmedia		
					Plant Extract		
					2%	4%	6%
Lantanacamara	Leaf	Water	55	0	48	38	29
		Methanolic	55	0	45	31	24
Ocimum sanctum	Leaf	Water	55	0	45	33	21
		Methanolic	55	0	40	24	17
Acacia Senegal	Leaf	Water	55	0	45	38	33
		Methanolic	55	0	40	33	29
Aloe vera	Leaf	Pure Gel	55	0	41	36	24

DISC DIFFUSION METHOD FOR DETERMINING ANTI-MICROBIAL ACTIVITY IN AQUEOUS AND ORGANIC EXTRACTS

***Acacia arabica* Extract**

Acacia arabica's aqueous and organic *extracts significantly reduced the growth of Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Proteus vulgaris, Saccharomyces cerevisiae, Staphylococcus epidermidis, and Saccharomyces cerevisiae was the target of the most activity, whereas Escherichia coli was the target of the least activity. The anti-bacterial activity in* different extracts was found decreasing in the following order aqueous > chloroform > acetone > ethanol > methanol > cyclohexane > ethyl acetate extract. Against any of the examined bacterial strains, the benzene extract of *Acacia arabica* exhibited no anti-bacterial action. Table contains the zone of inhibition data for Acacia arabica. All of the strains were significantly inhibited by the aqueous extract of Acacia arabica.

Table 2: *Acacia arabica* extract's zone of inhibition (mm) in different solvents

Plant Extract	Concentration of Extract	Zone of Inhibition (mm)						
		Bacillus subtilis	*Escherichia coli*	*Staphylococcus epidermidis*	*Saccharomyces cerevisiae*	*Proteus vulgaris*	*Bacillus cereus*	*Staphylococcus aureus*
Aqueous	40 mg/ml	17 ±0.5	8 ±0.1	23 ±0.05	16 ±0.05	15 ±0.05	14 ±0.32	15 ±0.1
	30 mg/ml	16 ±0.1	4 ±0.5	20 ±0	15 ±3.45	13 ±4.76	13 ±0.42	13 ±0
	20 mg/ml	14 ±0.2	2 ± 0.5	19 ±0	14 ±0	12 ±0	11 ±0.22	12 ±0.1
	10 mg/ml	10 ±0.2	Zone not found	17 ±3.3	12 ±0	9 ±9.0	8 ±0.05	11 ±0.45
	5 mg/ml	Zone not found	Zone not found	10 ±4.5	Zone not found	Zone not found	Zone not found	9 ±5.78
Acetone	40 mg/ml	17 ±0.50	19 ±2.2	17 ±3.2	16 ±2.56	17 ±0	16 ±0.2	20 ±0.1
	30 mg/ml	15 ±0.05	17 ±2.3	15 ±2.6	14 ±3.27	16 ±0.1	13 ±0.56	17 ±0.2
	20 mg/ml	14 ±0.05	15 ±6.7	12 ±2.2	13 ±0.05	12 ±0.5	12 ±4.32	16 ±0.1
	10 mg/ml	12 ±0.05	12 ±9.3	11 ±3.4	12 ±0.05	10 ±0.22	8 ±3.23	15 ±4.56
	5 mg/ml	10 ±0.05	10 ±3.4	7 ±0.05	10 ±0.5	Zone not found	Zone not found	7 ±0.05
Benzene	40 mg/ml	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found
	30 mg/ml	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found
	20 mg/ml	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found
	10 mg/ml	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found
	5 mg/ml	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found
Chloroform	40 mg/ml	17 ±4.23	10 ±1.3	18 ±3.34	15 ±2.33	14 ±2.33	19 ±0.5	17 ±3.2
	30 mg/ml	14 ±0.5	7 ±5.4	16 ±0	12 ±0	12 ±0.1	15 ±0.5	13 ±6.7
	20 mg/ml	12 ±6.8	4 ±1.6	13 ±2.3	8 ±2.30	9 ±3.44	10 ±0.5	9 ±0.5
	10 mg/ml	9 ±0.5	NZ	10 ±4.2	2 ±1.80	6 ±2.13	8 ±0.05	4 ±0.05
	5 mg/ml	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	2 ±0.05
Cyclohexane	40 mg/ml	9 ±3.42	14 ±4.5	Zone not found	Zone not found	Zone not found	12 ±0	Zone not found
	30 mg/ml	7 ±3.56	13 ±0	Zone not found	Zone not found	Zone not found	11 ±0	Zone not found
	20 mg/ml	4 ±4.78	12 ±0	Zone not found	Zone not found	Zone not found	10 ±0.1	Zone not found
	10 mg/ml	2 ±0	10 ±3.2	Zone not found	Zone not found	Zone not found	8 ±0.5	Zone not found
	5 mg/ml	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found

Ethanol	40 mg/ml	10 ±2.3	10 ±4.3	17 ±0.5	12 ±0	10 ±2.3	15 ±4.32	9 ±4.33
	30 mg/ml	7 ±4.5	9 ±0.2	15 ±4.45	11 ±0.1	8 ±0	12 ±2.11	6 ±2.56
	20 mg/ml	2 ±6.7	4 ±0.5	12 ±3.23	10 ±0	4 ±0.1	10 ±0.99	4 ±0.5
	10 mg/ml	Zone not found	2 ±0.5	10 ±5.6	7 ±2.89	3 ±0.2	7 ±5.42	2 ±0.05
	5 mg/ml	Zone not found	Zone not found	5 ±0.05	Zone not found	Zone not found	5 ±0	Zone not found
Ethyl acetate	40 mg/ml	Zone not found	9 ±1.2	12 ±0.05	Zone not found	10 ±0.2	Zone not found	13 ±6.32
	30 mg/ml	Zone not found	8 ±0.1	10 ±0.05	Zone not found	8 ±0.4	Zone not found	12 ±4.32
	20 mg/ml	Zone not found	Zone not found	4 ±0.05	Zone not found	Zone not found	Zone not found	10 ±0
	10 mg/ml	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	8 ±0
	5 mg/ml	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found
Methanol	40 mg/ml	Zone not found	6 ±0.5	10 ±0.05	15 ±3.44	12 ±0.39	19 ±0.65	10 ±0.1
	30 mg/ml	Zone not found	10 ±5.6	9 ±0.05	12 ±5.60	9 ±0.32	15 ±0.1	8 ±0.11
	20 mg/ml	Zone not found	8 ±0.5	8 ±4.50	10 ±2.90	8 ±0.43	9 ±0.41	7 ±0.11
	10 mg/ml	Zone not found	3 ±0.5	7 ±4.32	9 ±0.05	Zone not found	6 ±0.22	5 ±0.1
	5 mg/ml	Zone not found	Zone not found	4 ±5.69	Zone not found	Zone not found	5 ±0.05	Zone not found

Bacillus subtilis, Escherichia coli, Staphylococcus epidermidis, Saccharomyces cerevisiae, Proteus vulgaris, Bacillus cereus, and *Staphylococcus aureus* were the bacteria employed, and their concentrations were 40, 30, 20, 10, and 5 mg/ml, respectively. Values are expressed as mean standard deviation (n = 3).

AGAR WELL DIFFUSION METHOD FOR ASSESSING ANTIMICROBIAL ACTIVITY IN AQUEOUS AND ORGANIC EXTRACTS

Acacia arabica Extract's Anti-Microbial Activity

Bacteria including *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Proteus vulgaris, Saccharomyces cerevisiae,* and *Staphylococcus epidermidis* were all strongly suppressed by a number of aqueous and organic Acacia arabica *extracts. Staphylococcus epidermidis* was found to be the most successful target, whereas *Escherichia coli* was almost useless, according to the data. The cyclohexane and benzene extracts did not work as well as the methanol and ethanol extracts did. It was shown that certain strains of bacteria were resistant to the effects of unrefined plant extracts. The Acacia arabica aqueous extract inhibited the bacterial strains *Staphylococcus epidermidis, Bacillus cereus, Staphylococcus aureus, Bacillus subtilis, Saccharomyces cerevisiae, Proteus vulgaris,* and *Escherichia coli* at doses of 40 mg/ml, with zones of inhibition of 23, 20, 19, 17, 16, and 10 mm, respectively.

Table 3: *Acacia arabica* extract's Zone of inhibition (mm) in different solvents

Plant Extract	Concentration of Extract	Zone of Inhibition (mm)						
		Bacillus subtilis	*Escherichia coli*	*Staphylococcus epidermidis*	*Saccharomyces cerevisiae*	*Proteus vulgaris*	*Bacillus cereus*	*Staphylococcus aureus*
Aqueous	40 mg/ml	17±0.05	10 ±1	23±0.05	16 ±0.11	16±0.05	20 ±0	19±2
	30 mg/ml	16±0.5	9 ±2	20±0.05	15 ±4	15 ±0.05	19 ±0	18±0
	20 mg/ml	14±0.05	4 ±0.1	19 ±0.11	14 ±0.05	12 ±0.1	15 ±0.56	15 ±0.14
	10 mg/ml	10 ±0	1±0.05	17 ±0.11	12 ±0.2	9 ±0.3	11 ±0	12 ±5
	5 mg/ml	Zone not found	Zone not found	10 ±0.3	Zone not found	Zone not found	7 ±3	11 ±2
Cyclo hexane	40 mg/ml	10 ±0.05	14 ±0.1	Zone not found	Zone not found	Zone not found	12 ±2	Zone not found
	30 mg/ml	9 ±0.1	13 ±1	Zone not found	Zone not found	Zone not found	11 ±3	Zone not found
	20 mg/ml	9 ±0.2	12 ±0.1	Zone not found	Zone not found	Zone not found	10 ±4	Zone not found
	5 mg/ml	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found
Benzene	40 mg/ml	Zone not found	10 ±0.5	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found
	30 mg/ml	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found
	20 mg/ml	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found
	10 mg/ml	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found
	5 mg/ml	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found
Chloro form	40 mg/ml	18 ±0.11	10 ±1	20 ±0	16 ±4	16 ±2	21±7	19 ±0.1
	30 mg/ml	16 ±0.11	7 ±2	18 ±4	15 ±0.5	15 ±0.2	15 ±4	14 ±0.2
	20 mg/ml	14 ±0.3	4 ±1	15 ±0	10 ±9	10 ±5	10 ±3	10 ±0.1
	10 mg/ml	10 ±0.1	Zone not found	11 ±0	2 ±1	6 ±7	8 ±2	8 ±1
	5 mg/ml	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	4 ±0.1
Acetone	40 mg/ml	15 ±1	20 ±2	25 ±4	20 ±0.5	20 ±0.2	17 ±2.3	20 ±3.1
	30 mg/ml	12 ±3.1	17 ±0.5	22 ±0.1	16±0.6	18 ±2	16 ±4	18 ±6.1
	20 mg/ml	9 ±4	15 ±0.2	18 ±0	14 ±0	15 ±6	15 ±5	15 ±2
	10 mg/ml	4 ±1	12 ±4	16 ±1	10 ±4	9 ±0	10 ±3	10 ±0.1
	5 mg/ml	Zone not found	10 ±4	12 ±7	Zone not found	Zone not found	9±3	Zone not found

Ethyl acetate	40 mg/ml	13 ±0	10 ±0.1	20 ±0.2	Zone not found	11 ±1	11 ±2	13 ±6
	30 mg/ml	9 ±2	8 ±4	16 ±7	Zone not found	8 ±1	4 ±2	12 ±0.1
	20 mg/ml	4 ±0.2	Zone not found	14 ±0.2	Zone not found	Zone not found	Zone not found	10 ±0
	10 mg/ml	Zone not found	Zone not found	12 ±0	Zone not found	Zone not found	Zone not found	8 ±1
	5 mg/ml	Zone not found	Zone not found	6±5	Zone not found	Zone not found	Zone not found	Zone not found
Ethanol	40 mg/ml	12 ±2	11 ±0.1	19 ±0.11	15 ±0.2	12 ±1	18 ±6	12 ±2.9
	30 mg/ml	7 ±2.4	9 ±0.05	15±0.2	12 ±0.1	8 ±0.05	15 ±1	6 ±2
	20 mg/ml	2 ±2.1	4 ±0.1	12 ±0.2	10 ±0.1	4 ±5	10 ±2	4 ±1
	10 mg/ml	Zone not found	2 ±0.2	10 ±0.1	7 ±1	3 ±2	7 ±1	2 ±0.1
	5 mg/ml	Zone not found	Zone not found	5 mm	Zone not found	Zone not found	5 mm	Zone not found
Methanol	40 mg/ml	13 ±8.0	12 ±0.1	22 ±0	15 ±0.1	12 ±0	19 ±0.2	11±0.1
	30 mg/ml	9 ±1	10 ±2	20 ±0	12 ±0.2	9 ±3	17 ±6	8 ±1
	20 mg/ml	4 ±0	8 ±2	17 ±0.1	10 ±0.1	6 ±0	15 ±6	7 ±3
	10 mg/ml	Zone not found	3 ±0.1	14 ±3	9 ±1	3 ±6	9 ±0.1	5 ±2
	5 mg/ml	Zone not found	Zone not found	8 ±1	Zone not found	Zone not found	5 ±0.2	2 ±0.1

Bacillus subtilis, Escherichia coli, Streptococcus epidermidis, Streptococcus cerevisiae, Proteus vulgaris, Staphylococcus aureus, and *Bacillus cereus* were utilised at concentrations of 40, 30, 20, 10, and 5 mg/ml, respectively. These numbers represent the mean SD (n=3).

ZONE OF INHIBITION IN STANDARD ANTIBIOTICS AGAINST BACTERIAL STRAINS

The effectiveness of the plant extracts against various bacterial strains was evaluated in comparison to dimethyl sulphoxide (DMSO) and conventional antibiotics as positive and negative controls, respectively. When plant extracts were tested for their ability to kill bacteria and compared to antibiotics, it was shown that *Emblica officinalis, Acacia arabica,* and *Lawsonia inermis* extracts had strong anti-bacterial activity and had zones of inhibition that were comparable to those of conventional antibiotics. Table provides the zone of inhibition of common antibiotics and dimethyl sulfoxide as a harmful control.

Table 4: Zone of inhibition (mm) of the standards

Standards	*Bacillus subtilis*	*Escherichia coli*	*Staphylococcus epidermidis*	*Saccharomyces cerevisiae*	*Proteus vulgaris*	*Bacillus cereus*	*Staphylococcus aureus*
Amoxicillin	Zone not found	Zone not found	12 ±3.21	Zone not found	Zone not found	Zone not found	Zone not found
Penicillin G	Zone not found	Zone not found	Zone not found	Zone not found	20 ±0.91	Zone not found	Zone not found
Tetracycline	27 ± 0.05	24 ± 0.1	28 ±0	23 ±0.2	25 ±0.43	16 ±0.5	25 ±0.1
Streptomycin	20 ±0.5	16 ±0	Zone not found	20 ±0.05	20 ±0.1	21 ±0.63	15 ±0.11
Dimethyl Sulfoxide	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found	Zone not found

Amoxicillin, Penicillin G, Tetracycline, and *Streptomycin* are the antibacterial medications that are utilised as a positive control. The negative control substance utilised was dimethyl sulphoxide. Values are expressed as mean standard deviation (n = 3).

PICTURES OF PLATES OF ANTI-MICROBIAL ACTIVITY BY AGAR WELL DIFFUSION METHOD

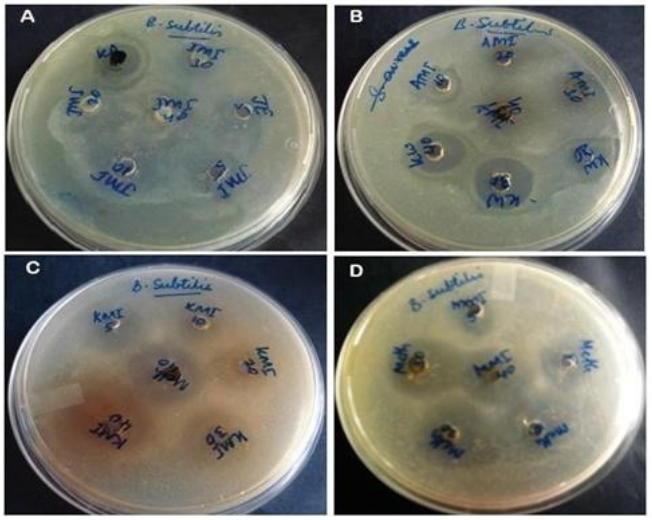

Figure 1: Zone of inhibition (mm) of different plant extracts against *Bacillus subtilis* at different concentrations by well diffusion method.

(A) *Myristica fragrans* methanolic extract (JMI) 40, 30, 20, 10 and 5 mg/ml; *Acacia arabica* acetone extract (KA) 5 mg/ml. (B) *Ricinus communis*methanolic extract (AMI) 40, 30, 20 and 10 mg/ml; *Acacia arabica* aqueous extract (KW) 40, 30 and 20 mg/ml (C) *Acacia arabica* methanolic extract (KMI) 40, 30, 20, 10 and5 mg/ml; *Lawsonia inermis* ethyl acetate extract (METH) 40 mg/ml (D) *Lawsonia inermis* ethyl acetate extract (METH) 30 mg/ml, 20, 10, 5 mg/ml; *Lawsonia inermis* methanolic extract (MMI) 40 and 5 mg/ml.

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