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In Vitro Antibiotic Potential and Antioxidant Activity of Ethanol and Acetone Excerpts of *Sesbania grandiflora* (L.) Pres. (Agastya) Bark

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ABSTRACT

Sesbania grandiflora (L.) Pres. is a commonly used ethnomedicinal plant belonging to the family Fabaceae. In this study, the antibiotic potential of bark extracts from this plant was evaluated against one fungus *(Candida albicans)*, four gram-positive bacteria (*Lactobacillus acidophilus, Bacillus subtilis, Enterococcus faecalis, & Streptococcus mutans*), and one gram-negative bacteria (*Pseudomonas aeruginosa*). The bark extracts exhibited the highest zone of inhibition against *Candida albicans* (230.81 mm^2) in ethanol solvent, and the lowest against *Enterococcus faecalis* (30.70 mm^2). In acetone solvent, the highest inhibition was observed against *Bacillus subtilis* (136.79 mm^2), and the lowest against Pseudomonas aeruginosa (13.96 mm^2). Antibiotic potential against *Lactobacillus acidophilus, Bacillus subtilis*, & *Streptococcus mutans* is not reported yet. The values of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) displayed variability, reaching the highest point against *Bacillus subtilis* (acetone extracts) and the lowest against *Lactobacillus acidophilus* (ethanol extracts). Moreover, the antioxidative potential of the ethanol and acetone bark extracts was evaluated through DPPH and CUPRAC antioxidant assays. The ethanol extracts demonstrated maximal inhibition against *Candida albicans* (41.08 %) and minimal inhibition against *Enterococcus faecalis* (11.15 %), whereas the acetone extracts exhibited maximal inhibition against *Enterococcus faecalis* (50.02 %) and minimal inhibition against *Lactobacillus acidophilus* (4.58 %). Additionally, the most significant scavenging activity against DPPH was observed at a concentration of 30 µg/ml (19.50 % for ethanol extracts and 15.60 % for acetone extracts), while the least activity was observed at 5 µg/ml (1.96 %) for both solvents. Similarly, in the CUPRAC assay, the highest scavenging activity was noted at a concentration of 100 µg/ml, with the lowest at 2 µg/ml for both ethanol and acetone extracts. The primary objective of this investigation is to explore the phototherapeutic antibiotics and antioxidants inherent in the extracts derived from the bark of Sesbania grandiflora, which offer significant benefits with minimal or negligible adverse effects.

Keywords: *Sesbania grandiflora* "(L.)"; Antibacterial; MIC; MBC; Antioxidant

1. INTRODUCTION

In contemporary healthcare practices of the 21st century, the significance of medicinal plants is on the rise as an increasing number of individuals seek safer remedies and healthcare approaches. The global demand for various herbal products, including herbal medicines, health products, pharmaceuticals, nutraceuticals, supplements, and cosmetics, is witnessing a notable surge. This trend is fuelled by the growing recognition of these products as predominantly non-toxic, with fewer side effects, better compatibility with physiological flora, and affordability. Throughout history, the world population has relied significantly on medicinal plants, with approximately 80 %[1-3] of the population utilizing them since ancient times. Among these, *Sesbania grandiflora* (L.) Pres. stands out as a well-known ethnomedicinal plant of the Fabaceae family,[4] renowned for its pharmacological activities [5-12] and nitrogen-fixing ability[13,14]. It is found scattered across tropical and subtropical regions, including Thailand, India, the Philippines, Sri Lanka, Indonesia, and Malaysia[15]. In India, it is predominantly found in northeast states, Bihar, West Bengal, Jharkhand, Karnataka, and Assam.16 Known by various names such as August, Hummingbird tree, Agastya, Turi, and Agathi, this plant holds rich medicinal properties documented in Ayurvedic, Unani, and Chinese literature. The leaves, flowers and bark of this plant are visualised in photograph, which is shown in Fig. 1.

The rampant misuse and overuse of antibiotics have led to the emergence of antimicrobial resistance, rendering many existing medications ineffective. Consequently, the discovery of new antibiotics has become an urgent objective. Plant-based products are pivotal in this pursuit, considering the potential negative consequences associated with prolonged usage of allopathic medications. The quest for plants exhibiting potent antimicrobial properties has become a central focus of research endeavours aimed at addressing the risks linked with infectious diseases, while simultaneously reducing or eliminating side effects.

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Free radicals such as singlet oxygen species, hydroxyl ions, superoxide ions, and hydrogen peroxide are the result of the unpairing of electrons from any molecules and atoms. These free radicals are the main cause of the imbalance of our immune system which leads to many deadly diseases such as inflammation, ageing, diabetes, heart problems, and many more. This toxic and highly reactive free radical are overcome by antioxidant-rich food and medicine. The word antioxidant comes from the Greek word 'anti' and the English word 'oxidant' which means the molecules/substances which are oxidizing in nature.[17] These antioxidants either balance the unpaired electrons of free radicals or scavenge them. Antioxidants are of two types synthetic (retrieved from chemical processes) and natural (retrieved from nature). While both types of antioxidants are dependable, plant-based antioxidants are preferable due to their minimal occurrence of side effects.

Antioxidants gained prominence in the 1990s when scientists recognized their ability to mitigate the damage inflicted by free radicals, which play a pivotal role in the initial stages of atherosclerosis—a condition characterized by the narrowing of arteries. This pathological process has been linked to various chronic diseases, such as cancer, vision impairment, and others. Studies have demonstrated that individuals with a low consumption of antioxidant-rich fruits and vegetables are at a higher risk of developing these chronic conditions compared to those who include such foods abundantly in their diets. As a result, research into the protective effects of antioxidants continues to be a global priority.[18]

According to estimates by the World Health Organization (WHO), approximately 140 million people worldwide suffer from alcohol dependence, with the disease accounting for 3.8% of global mortality and 4.6 % of Disability-Adjusted Life Years (DALYs). Alcohol-related Liver Disease (ALD) is a consequence of liver damage resulting from the accumulation of acetaldehyde and oxidative stress induced by alcohol consumption.[19]

2. MATERIAL AND METHODS

2.1 Collection and Identification

The bark of *Sesbania grandiflora* was sourced from Vidya Nagar, Harmu, Ranchi, Jharkhand. The plant was identified and authenticated by the National Institute of Science Communication and Policy Research, New Delhi, in 2022, with the authentication number NIScPR/RHMD/Consult/2022/4258-59-2.

2.2 Extract Preparation

The fresh bark was collected, washed, and air-dried for approximately 1-1.5 months. Once dried, the bark was ground into powder. Subsequently, 20 grams of the bark powder was mixed with 200 ml of 80 % methanol solution prepared in distilled water. The mixture was then placed in a shaker incubator at 34 °C and 100 rpm for 3-4 days. After incubation, the solvent-powder mixture was filtered using What man filter paper 1 and then placed in an incubator at 38°C until the methanol was completely evaporated. The resulting dry bark extract was then collected, scratched, and stored in centrifuge tubes inside a refrigerator (stock). This extraction method, commonly referred to as the cold extraction method, was utilized for both the antibiotic and antioxidant assays of *Sesbania grandiflora* bark.[21]

2.3 Anti-microbial Assay

2.3.1 *Preparation of Extract Solution*

The antimicrobial efficacy of *Sesbania grandiflora* (L.) Pres. bark was evaluated according to the methodology delineated by Bauer, A. W. et al. Initially, 8 mg of *Sesbania grandiflora* bark extract was mixed with 1000 µl of both ethanol and acetone solutions. This mixture was then subjected to agitation using a thermo-mixer set at 30 °C and 1000 rpm for a duration of 30 minutes. Following agitation, the resultant mixture underwent centrifugation at 28 °C and 1800 rpm for 20 minutes,

Figure 1. a-plant, b-leaves, c-flowers and d- bark.

after which the supernatant was meticulously collected for subsequent analysis.

2.3.2 *Microbial Strains and Inoculation*

The antimicrobial activity of *Sesbania grandiflora* bark extract was evaluated against one fungus, *Candida albicans* (MCC 1152), and four gram-positive bacteria: *Lactobacillus acidophilus* (MCC LA), *Bacillus subtilis* (MCC 2511), *Enterococcus faecalis* (MCC 3040), and *Streptococcus mutans* (MCC SM), as well as one gram-negative bacterium, *Pseudomonas aeruginosa* (MCC 3973). These microorganisms were chosen due to their Biosafety level 1 classification, ensuring safety during experimentation.

2.3.3 *Antibiotic Assay Procedure*

In this assay, 50 µl of different microbial inoculums were spread onto Petri plates containing nutrient agar (for bacteria) and PDA media (for fungus) using a glass rod spreader. Following this, 6 mm paper discs made from Whatman filter paper 1 were positioned onto the media. Various volumes (5, 10, and 15 µl) of *Sesbania grandiflora* bark extracts, for a concentration of 8 mg/ml, were applied onto sterilized Petri plates alongside positive controls (90 % ethanol for ethanol extracts and 90 % acetone for acetone extracts) and negative controls (tetracycline for bacteria and thioquest for fungus). The loaded Petri plates were then incubated for 18 hours at 36°C to allow the diffusion of the plant extracts into the media, and inhibition of microbial growth was observed using the Disc diffusion method[22]. Subsequently, the diameters of inhibition zones and the percentage of inhibition were measured. The concentrations of the drugs present in 5, 10, and 15µl were 40, 80, and 120µg, respectively.

2.3.4 *Calculations*

Triplicates of each Petri plate was meticulously prepared following the standard protocol. The zone of inhibition was quantified in square millimetres (mm^2) employing the following formula:

$$\text{Zone of inhibition } (mm^2) = \text{Area of inhibited zone } (\pi r_1^2) - \text{Area of disc } (\pi r_2^2)$$

Standard Deviation (SD), Standard Error (SE), and p-values were calculated using one-way ANOVA. The percentage inhibition was calculated using the following formula:

$$\% \text{ inhibition } = \frac{Zone\ of\ sample}{Zone\ of\ standard} * 100$$

(Where Control Diameter represents the diameter of growth in the negative control)

2.4 MIC and MBC

A separate experiment was conducted to ascertain the Minimum Inhibitory Concentration (MIC) and Maximum Bacterial Concentration (MBC) values of ethanol and acetone bark extracts of *Sesbania grandiflora* (L.) Pres. For this method, the following materials were necessary: 100 µl of nutrient broth, 20 µl of bacterial inoculum, and 0.5 % of 5 µl of TTC (2,3,5-triphenyl tetrazolium chloride) freshly prepared in distilled water.[22]

2.4.1 *Procedure*

1. Different volumes, ranging from 2 4, 6, 8, 10, 12, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 & 40 µl of the concentration 5 mg/ml of the bark extracts were placed into 96-well microtiter plates which is equivalent to 10, 20, 30, 40, 50, 60, 80, 90, 100, 110,120,130,140, 150, 160,170,180,190 & 200µg.
2. Each well was then supplemented with 100 µl of nutrient broth and 20 µl of bacterial inoculum.
3. Subsequently, 5 µl of 0.5 % TTC solution was added to each well.
4. The microtiter plates were then incubated for eighteen hours at 36°C inside the incubator.
5. After incubation, the plates were examined for the presence or absence of pink colouration. The appearance of pink colour indicated microbial growth, while the absence of colour indicated microbial inhibition.

2.4.2 *Interpretation*

The MIC value was noted as the lowest concentration of the bark extract at which no pink colour was observed, indicating complete inhibition of microbial growth.

The culture wells where no colour was observed were subculture onto nutrient agar media and incubated until no microbial growth was observed. The concentration of the bark extract corresponding to this well represented the MBC value, indicating the concentration at which microbial growth was completely inhibited.

2.5 Anti-oxidant Assay

2.5.1 *DPPH Antioxidant Assay*[23,24]

The DPPH (2,2-Diphenyl-1-picrylhydrazyl) antioxidant assay is widely recognized as a reliable method for evaluating the antioxidant activity of sample extracts. In executing this assay, 5 to 30 µg of *Sesbania grandiflora* bark stock was meticulously mixed with 1000 µl of ethanol and acetone solvents, utilizing the same extraction procedures as those applied in the antimicrobial assay.

A fresh stock solution of DPPH (0.5 mM) was meticulously prepared in a 90 % methanol solvent and utilized in the experiment. To test the sample extracts, 100 µl of this fresh DPPH stock solution was combined with 10 µl of extracts of varied concentrations, prepared in the two distinct solvents. Following an incubation period of 30-35 minutes in darkness to prevent light interference, absorbance was gauged at 517 nm. It's crucial to note that DPPH exhibits high sensitivity to light; thus, all experimental procedures were conducted under dark conditions. DPPH served as the control, while ascorbic acid was employed as the standard.

DPPH antioxidant assay: DPPH (0.5mM) + Test sample= absorbance (517nm)

Mena, SD, SE, and IC^{50} values were computed, and

the p-value was determined through one-way ANOVA. The percentage inhibition in this assay was calculated using the following formula:

$$\text{Inhibition } \% = \frac{\text{Control Absorbance} - \text{Plant Extract Absorbance}}{\text{Control Absorbance}} X\ 100$$

2.5.2 CUPRAC Antioxidant Assay[25]

The CUPRAC (cupric reducing antioxidant capacity) antioxidant assay was performed to assess the antioxidant activity of *Sesbania grandiflora* bark extracts. Extracts ranging from 2 to 100 µg were combined with 1000 µl of two polar solvents (ethanol and acetone), following the same preparation steps outlined in the antimicrobial activity assay.

For this assay, three reagents were required: ammonium acetate (1 mM), neo-cuproine (7.5 mM), and copper II chloride dihydrate (10 mM). Reagents 1 and 3 were dissolved in distilled water, while reagent 2 was dissolved in ethanol. 150 µL of plant extracts of varying concentrations were mixed with 150 µL each of 7.5 mM neo-cuproine, 1 mM ammonium acetate, and copper (II) chloride dihydrate in a centrifuge tube, and then incubated for 30 minutes at room temperature. After incubation, the absorbance of the reaction mixture was measured at 450 nm using a spectrophotometer.

A calibration curve was constructed using standard concentrations of ascorbic acid ranging from 25 µg/ml to 100 µg/ml, plotted against the absorbance recorded at a wavelength of 450 nm. The total CUPRAC reducing capacity was determined using the standard calibration curve based on linear regression analysis. The concentration of the analyte in the extracts was calculated from the equation derived from the calibration curve and expressed in terms of ascorbic acid equivalents. The detailed protocol of this method (slide modification) is provided below:

CUPRAC antioxidant assay: Test sample + Reagent 1+ Reagent 2 + Reagent 3 = Absorbance (450nm)

3. RESULTS

3.1 Antimicrobial Activity

All selected bacteria and fungi exhibited positive responses against both ethanol and acetone bark extracts (refer to Fig. 2 & 3). In ethanol solvent, the bark extracts demonstrated the maximum zone of inhibition against MCC 1152, with a diameter of 230.81 mm^2, and the minimum against MCC 3040, with a diameter of 30.70 mm^2 (see Table 1). Conversely, in acetone solvent, the maximum inhibition was observed against MCC 2511, with a diameter of 136.79 mm^2, while the minimum was against MCC 3973, with a diameter of 13.96 mm^2 (see Table 2).

Ethanol solvent extracts exhibited higher antibiotic potential against MCC 1152, MCC LA, MCC SM, and MCC 3973 compared to acetone extracts (see Table 3). Additionally, the maximum percentage inhibition was reported against *Candida albicans*, with a value of 41.08 %, and the minimum against *Enterococcus faecalis*,with a value of 11.15 %, in ethanol extracts (see Table 4). In contrast, acetone extracts demonstrated the maximum inhibition against *Enterococcus faecalis*, with a value of 50.02 %, and the minimum against *Lactobacillus acidophilus*, with a value of 4.58 % (see Table 5).

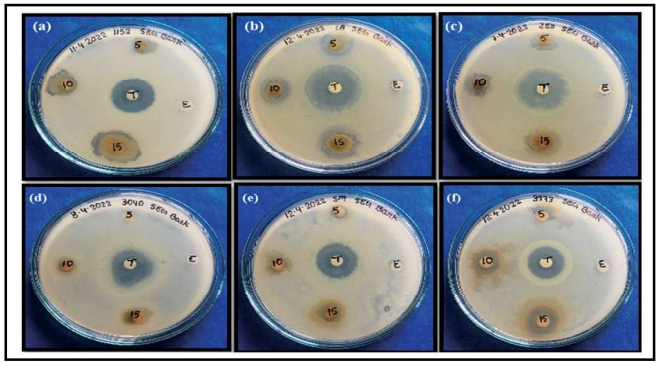


Figure 2. Antibiotic capacity of ethanol bark excerpts against a- MCC 1152 (p-value: 6.28*10^{-5}), b- MCC LA (p-value: 0.410), c- MCC 2511(p-value: 0.259), d- MCC 3040(p-value: 0.0014), e- MCC SM (p-value: 0.0011) and f- MCC 3973 (p-value: 2.38*10^{-5}).

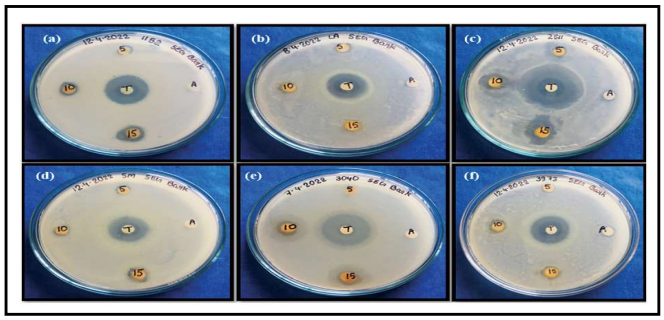
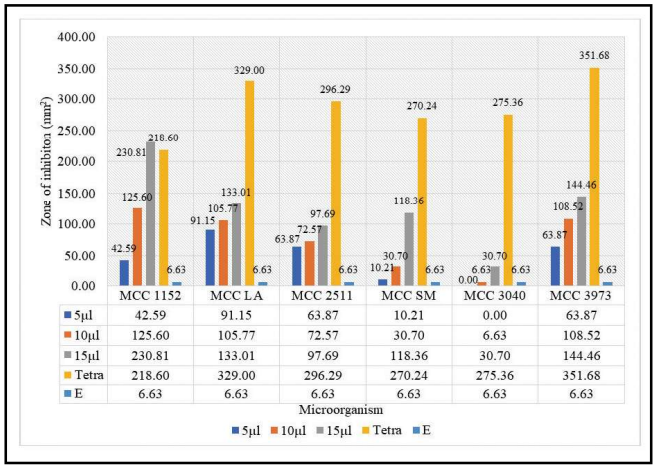
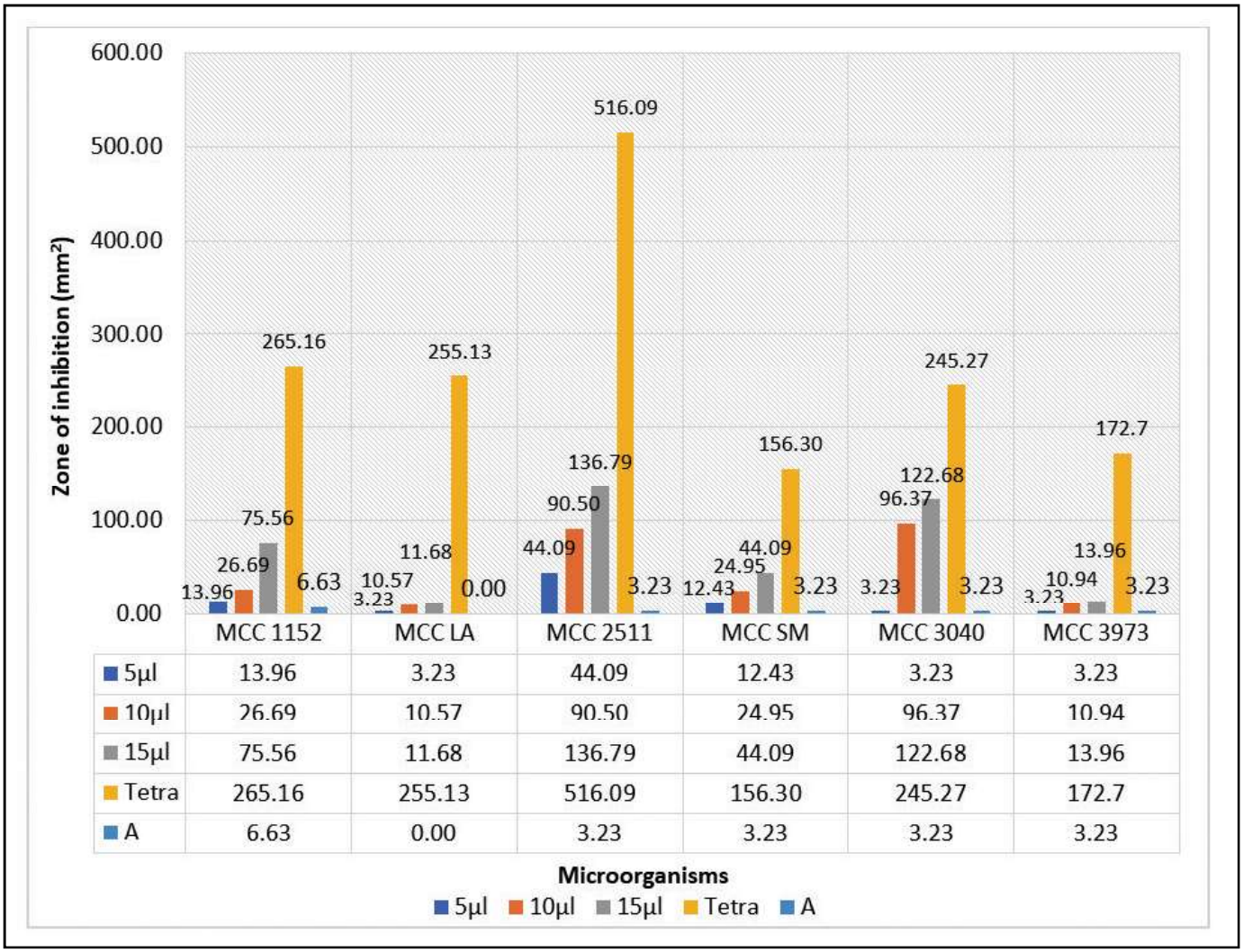


Figure 3. Antibiotic capacity of acetone bark excerpts against a- MCC 1152 (p-value: 2.7*10^{-5}), b- MCC LA (p-value: 0.055), c- MCC 2511(p-value: 7.62*10^{-5}), d- MCC 3040 (p-value: 0.0008), e- MCC SM (p-value: 1.62*10^{-5}) and f- MCC 3973 (p-value: 0.096).



	MCC 1152	MCC LA	MCC 2511	MCC SM	MCC 3040	MCC 3973
5µl	42.59	91.15	63.87	10.21	0.00	63.87
10µl	125.60	105.77	72.57	30.70	6.63	108.52
15µl	230.81	133.01	97.69	118.36	30.70	144.46
Tetra	218.60	329.00	296.29	270.24	275.36	351.68
E	6.63	6.63	6.63	6.63	6.63	6.63

Table 1. Zone of inhibition of ethanol solvent bark excerpts



	MCC 1152	MCC LA	MCC 2511	MCC SM	MCC 3040	MCC 3973
5µl	13.96	3.23	44.09	12.43	3.23	3.23
10µl	26.69	10.57	90.50	24.95	96.37	10.94
15µl	75.56	11.68	136.79	44.09	122.68	13.96
Tetra	265.16	255.13	516.09	156.30	245.27	172.7
A	6.63	0.00	3.23	3.23	3.23	3.23

Table 2. Zone of inhibiton of acetone solvent bark exerpts

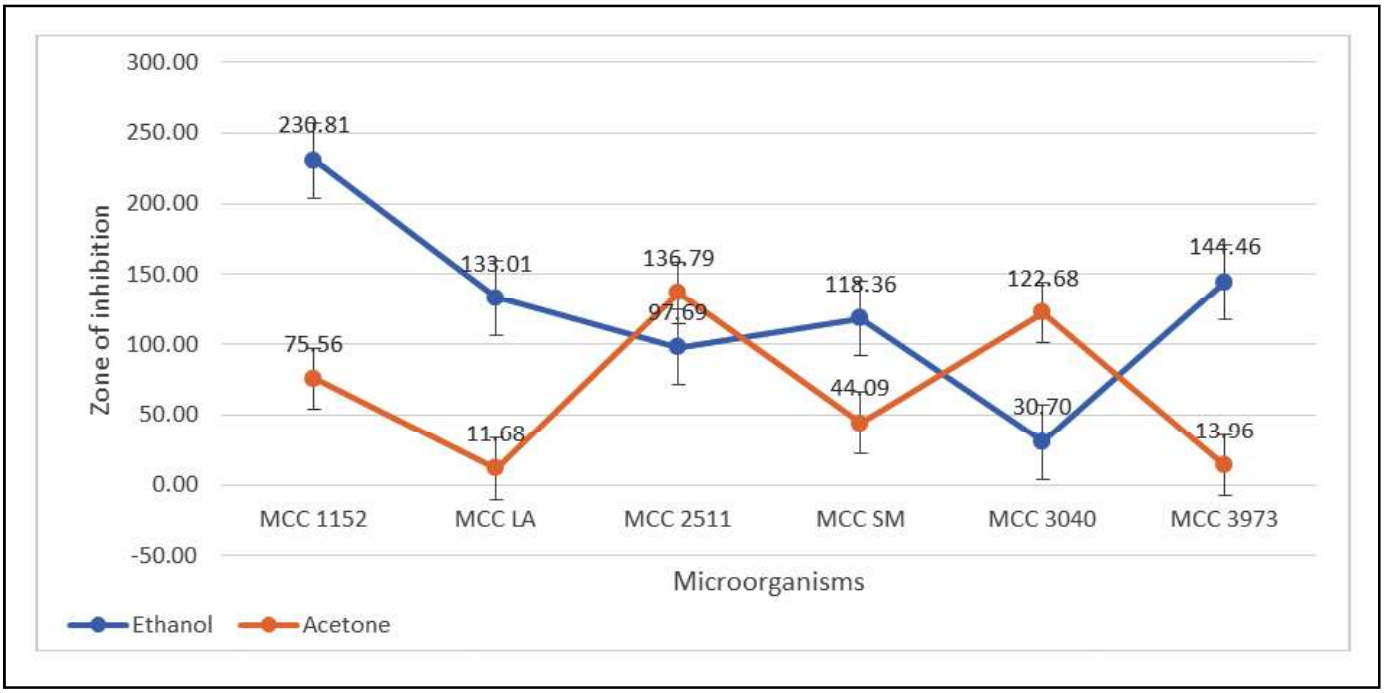


Table 3. Comparison of the zone of inhibition of ethanol and acetone solvent bark excerpts

Table 4. % inhibition of ethanol-solvent bark excerpts

Table 5. % inhibition of acetone-solvent bark excerpts

3.2 MIC and MBC

The MIC and MBC values were determined for *Sesbania grandiflora* bark extracts at concentrations ranging from 10 to 200 µg.

In ethanol extracts, the maximum MIC was observed against MCC SM, while the minimum was against MCC LA. Similarly, in acetone extracts, the maximum MIC was observed against MCC 2511, while the minimum was against MCC SM (refer to Table 6). Additionally, the maximum MBC value for ethanol extracts was reported against MCC SM, while the minimum was against MCC LA. In acetone extracts, the maximum MBC value was observed against MCC 2511, while the minimum was against MCC SM.

Tetracycline was used as the standard antibiotic for comparison.

Table 6. MIC and MBC value of bark excerpts against diferent microoganisms.

S. NO.	Microorganisms	Concentration: 5mg/ml			
		Volume (µl)			
		MIC		MBC	
		Ethanol	Acetone	Ethanol	Acetone
1	MCC 1152	11	18	21	24
2	MCC LA	10	20	18	28
3	MCC 2511	16	24	22	30
4	MCC SM	20	14	28	22
5	MCC 3040	18	18	24	26
6	MCC 3973	14	22	26	28

3.3 DPPH free Radical Scavenging Activity

The DPPH free radical scavenging activity assay was conducted to assess the antioxidant potential of *Sesbania grandiflora* ethanol and acetone bark extracts, a method commonly employed to evaluate the ability of plant extracts to counteract free radicals.

In the ethanol extracts, the highest scavenging activity was observed at a concentration of 30 µg/ml, demonstrating a percentage inhibition of 19.50 %, while the lowest activity was recorded at a concentration of 5 µg/ml, with a percentage inhibition of 1.96 %. Similarly, in the acetone extracts, the maximum scavenging activity was noted at a concentration of 30 µg/ml, exhibiting a percentage inhibition of 15.60 %, while the minimum activity occurred at a concentration of 5 µg/ml, with a percentage inhibition of 1.96 % (see Table 7).

The IC_{50} value, representing the concentration of the extract required to scavenge 50% of the free radicals, was higher for acetone extracts (93.60 µg/ml) compared to ethanol extracts (60.35 µg/ml) (refer to Table 7).

3.4 CUPRAC Antioxidant Assay

The CUPRAC antioxidant assay was utilized to assess the free radical scavenging activity of Sesbania grandiflora ethanol and acetone bark extracts, employing a standard calibration curve (refer to Table 8).

In both ethanol and acetone extracts, the highest free radical scavenging activity was observed at a concentration of 100 µg/ml. Specifically, ethanol extracts displayed a percentage scavenging activity of 8 %, while acetone extracts exhibited a percentage scavenging activity of 2.28 %. Conversely, the lowest scavenging activity was noted at a concentration of 2 µg/ml for both ethanol and acetone extracts. Ethanol extracts demonstrated a scavenging activity of 0.31 %, while acetone extracts exhibited a scavenging activity of 0.25 % (refer to Table 9).

Table 7. DPPH free radical scavenging activity

Free radical (DPPH antioxidant assay) scavenging activity						
Concentrations (µg/ml)	**Ethanol excerpts**		**Acetone excerpts**		**Ascorbic acid**	
	Mean SD (±)	**% Inhibition**	**Mean SD (±)**	**% Inhibition**	**Mean SD (±)**	**% Inhibition**
5	1.85±0.028	1.96	1.85±0.028	1.96	0.82±0.002	38.88
10	1.82±0.025	3.68	1.83±0.022	3.33	0.72±0.002	46.07
15	1.75±0.02	7.32	1.80±0.00	5.01	0.63±0.00	52.96
20	1.63±0.014	13.86	1.73±0.012	8.51	0.53±0.004	60.57
25	1.57±0.017	16.83	1.67±0.017	11.55	0.46±0.003	65.24
30	1.52±0.02	19.50	1.60±0.00	15.60	0.38±0.02	71.54
p-value	$8.7*10^{-6}$		$1*10^{-5}$		$2.2*10^{-12}$	
IC_{50} value	60.35		93.60		11.59	

Table 8. Standard curve (Asorbic acid) for CUPRAC free radical scacenging activity

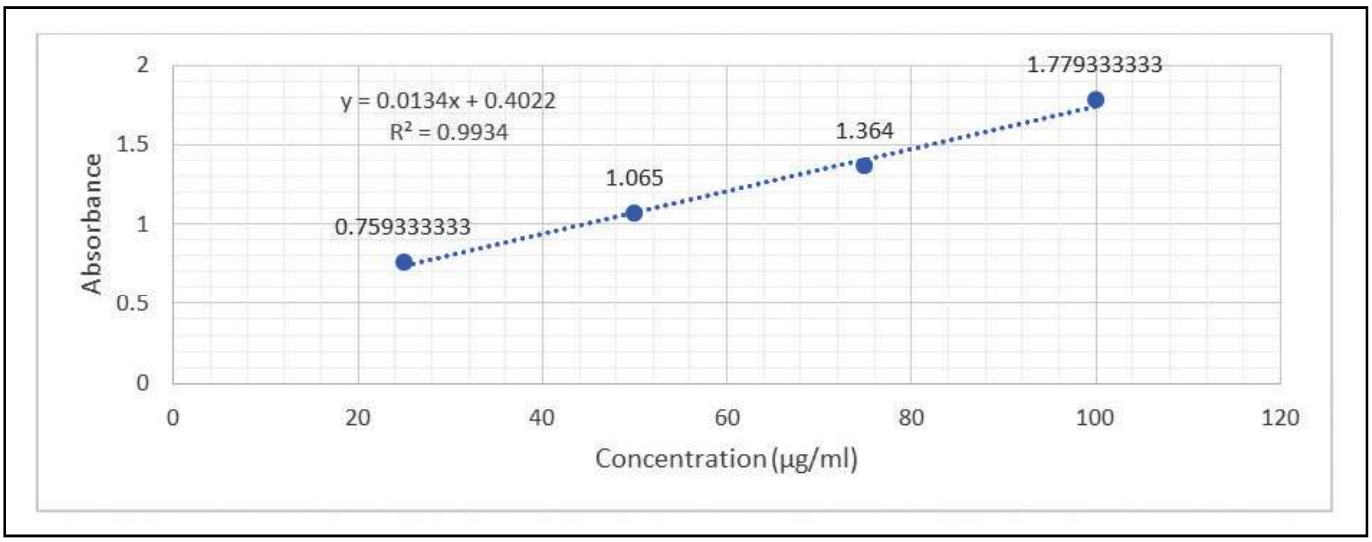


Table 9. CPPRAC free radical scavenging activity

CPPRAC free radical scavenging activity (%)													
Ascorbic acid equivalence antioxidant capacity (mg AAE/g)													
Concentration (µg/ml)													
2		5		10		20		40		80		100	
E	A	E	A	E	A	E	A	E	A	E	A	E	A
0.31 ± 0.02	0.25 ± 0.03	0.84 ± 0.02	0.29 ± 0.04	2.26 ± 0.04	0.60 ± 0.02	3.47 ± 0.03	1.07 ± 0.02	4.49 ± 0.04	1.35 ± 0.02	6.72 ± 0.02	1.74 ± 0.03	8.00	2.28 ± 0.07

***E= Ethanol and A= Acetone**

4. DISCUSSION AND CONCLUSIONS

Sesbania grandiflora is abundant in both non-enzymatic and enzymatic antioxidants, effectively scavenging free radicals[26]. The Minimum Bactericidal Concentration (MBC) value of its bark extracts is lower than the Minimum Inhibitory Concentration (MIC) value for gram-negative bacteria such as *S. sonnei & S. typhi,* and for gram-positive bacteria including *B. cereus, S. epidermidis, E. faecalis, & S. aureus,* where the MIC value closely matches the MBC value.[27] Ethyl acetate extracts of this bark demonstrate efficacy against *Staphylococcus aureus* (methicillin-resistant), *enterococci* (vancomycin-resistant), *Pseudomonas aeruginosa* & *Escherichia coli*[28]. Moreover, bark extracts of the Agastya plant exhibit promising antibiotic activity against *Candida albicans, Staphylococcus aureus, Escherichia coli* & *Pseudomonas aeruginosa,* with Amphotericin-B for fungus and Streptomycin for bacteria serving as standards.[29] The present study also highlights effectiveness against *Pseudomonas aeruginosa & Candida albicans,* while antibiotic potential against *Lactobacillus acidophilus, Bacillus subtilis, & Streptococcus mutans* remains unexplored.

In addition to its antimicrobial properties, *Sesbania grandiflora* bark extracts demonstrate significant antioxidant activity. Antioxidants have garnered scientific interest due to their numerous health benefits, including anti-aging, anti-inflammatory, and anticancer effects. Our study contributes to the existing body of evidence supporting the role of plant-based antioxidants in promoting health and reducing the risk of various diseases.[30] Research suggests that a higher intake of dietary antioxidants is associated with reduced mortality from all causes and cardiovascular diseases among adults with diabetes.[31] Furthermore, individuals who consume antioxidants have a 21 % lower mortality risk from respiratory diseases compared to non-users.[32] This finding, derived from a study involving 62,063 participants from the Singapore Chinese Health Study, underscores the importance of a diet rich in antioxidant nutrients. Public health recommendations advocate increased consumption of plant-based foods abundant in antioxidants.[33]

Plant-based antioxidants offer several advantages, including reliability, cost-effectiveness, renewability, and easy availability. The consistent effectiveness of *Sesbania grandiflora* bark, supported by numerous pieces of literature, underscores its reliability. The bark of this plant is rich in glycosides, alkaloids, flavonoids, tannins, and saponins. The total phenolic content in methanolic and aqueous extracts varies between 54.83, 58.06, 33.87, 54.19 µg/ml respectively, expressed in gallic acid equivalents (GAE).

The high phenol content in the bark extract explains its potent free radical scavenging activity.[34]

Results of DPPH scavenging activity indicate that the IC^{50} value of the ethanol extract (60.35 µg/mL) is significantly lower than that of the acetone extract (93.60 µg/mL). This difference may be attributed to the strong hydrogen donating ability of polyphenols present in the ethanol extract, leading to the reduction of DPPH, compared to the weaker abilities observed in the acetone extracts. The present results reflect the high polyphenol content present in the bark of this plant. While the antioxidant potential of this plant bark remains underexplored, our study demonstrates that bark extracts in both solvents efficiently inhibit free radicals. Overall, our findings suggest that *Sesbania grandiflora* bark extracts possess promising antimicrobial and antioxidant properties, warranting further investigation in animal models infected with drug-resistant bacteria. Further research is necessary to explore the therapeutic potential of this plant in combating infectious diseases and oxidative stress-related conditions.

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