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Antibacterial Capacity of *Plumeria alba* Petals

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***Abstract*—**Antibacterial activity of *Plumeria alba* (Frangipani) petals methanolic extracts were evaluated against *Escherichia coli, Proteus vulgaris,Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus saprophyticus, Enterococcus faecalis* and *Serratia marcescens* by using disk diffusion method. Concentration extracts (80 %) showed the highest inhibition zone towards *Escherichia coli* (14.3 mm). Frangipani extract also showed high antibacterial activity *against Staphylococcus saprophyticus, Proteus vulgaris* and *Serratia marcescens*, but not more than the zones of the positive control used. Comparison between two broad specrum antibiotics to frangipani extracts showed that the 80 % concentration extracts produce the same zone of inhibition as Streptomycin. Frangipani extracts showed no bacterial activity towards *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*. There are differences in the sensitivity of different bacteria to frangipani extracts, suggesting that frangipani's potency varies between these bacteria. The present results indicate that frangipani showed significant antibacterial activity especially to *Escherichia coli.*

***Keywords*—**Frangipani, *Plumeria alba*, anti microbial, *Escherichia coli*

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I. Introduction

WHITE frangipani (*Plumeria alba)* are from the family of *Apocynaceae.* The flower of the plant is white with yellow centers. Frangipani is well-known for its intensely fragrance and spiral-shaped blooms [1].

The plant is mainly grown for its ornamental and fragrant flowers. Methanolic extract of this flower has showed antimicrobial activity against *Bacillus anthracis* and *Pseudomonas aeruginosa* [2]. Species of *Plumeria* include *P. rubra, P. acutifolia, P. obtusa, P. obtusifolia, P. alba, P. bicolor, P. tricolour and P. jamesoni*. The bioactive compounds prepared from *P. rubra* having molluscicidal, cytotoxic and anti-bacterial activities. The plant is reported as medicinal which contains amyrinacetate, mixture of amyrins, ß-sitosterol, scopotetin, the iriddoids isoplumericin, plumieride, plumieride coumerate and plumieride coumerate glucoside [9]. Bioactive richness of these active constituents was present in the plant. The active ingredients in plants are produce as secondary metabolites, which may not only be developmental stage-specific but also organ and tissue specific [3]. The flower petals which provide physical protection to the reproductive components can be expected to synthesize potent bioactive compounds. Interestingly the symptoms of most plant disease of bacterial or fungal origin have been reported mostly on the leaves, stem, roots, and seldom on petals.

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Study shows that the floral petals of many angiosperm plant species contain antibiotic substances. The study open up the area for further detailed characterisation of higher plants, using a wide spectrum of biological screen including plant, as well as animal and pathogenic bacteria. The rapidity of this screening procedure by direct testing of the petals may allow large-scale screening to identify the petals of specific plant species as sources of new antibiotics, drugs or agrochemical [25].

Extracts of the flowers of *Plumeria* have also been used as fragrances in cosmetics. The present inventor has discovered that extracts from different parts of *Plumeria* also have therapeutic properties and can be used in the prevention or treatment of skin cancers, fungal infections, and viral infections, various skin defects, anti filarial and other afflictions [35].

Different part of the plant was believed, have been useful in variety of diseases. Namely the diseases of Malaria, Leprosy, Rheumatism and abdominal tumors. However, little study have been done to determine the antibacterial property of this species of frangipani. The development of bacterial resistance against synthetic antimicrobial agents encourages an alternative insight on another source of bacterial infection treatment.

II. Materials And Methods

The frangipani flowers were collected fresh in Malaysia. The frangipani was identified as *Plumeria alba* species by comparing with the standard description of the species. The study was done to determine frangipani petals ability in inhibiting bacteria, mainly which is involved in urinary tract infection or which act as human pahogens.

Plumeria alba petals were air-dried for 3 weeks at room temperature. The air-dried samples were ground to a mesh size of 1mm. A 67.5 g sample of the powdered materials was soaked in 300 ml of a mixture of methanol and water (4:1) for 96 hr. These were filtered and concentrated to a small volume to remove the entire methanol using rotary evaporator at 400rpm/50°C. 25 ml of gummy extraction were obtained upon evaporation. The gummy extract was kept in the fridge at 8°C for further studies.

Prior swabbing on the agar plate the bacteria was standardized to McFarland standards. A 0.5 McFarland standard is prepared by mixing 0.05 ml of 1.175% barium chloride dihydrate ($BaCl_2 \bullet 2H_2O$), with 9.95 mL of 1% sulfuric acid (H_2SO_4). A 0.5 McFarland standard is comparable to a bacterial suspension of 10^8 cfu/ml.

Pure frangipani extract obtained was subjected into serial dilution. The gummy extract is diluted into serial 80%, 60%, 40% and 20% concentration using sterile distilled water. The antibiotic positive control of this test was Gentamicin and

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Streptomycin. Sterile disc diffused with each extracts was impregnated and place firmly on the inoculated bacteria lawn and subjected to incubation for 24 hours at 37°C. The dishes are taken out for visual analysis of inhibition diameter, upon incubation.

III. Results And Discussion

Data collected was compared with a positive control and the standard inhibition diameter measurement was done. Eight species of bacteria commonly related to urinary tract infection, were screened. Observation showed an intermediate capacity zone in anti microbial activity of the extract towards *Staphylococcus saprophyticus*, *Proteus vulgaris* and *Serratia marcescens (Table 4-9)*, having zones less than the positive control. High concentrations of the extract produce an inhibitory zone towards the *Escherichia coli* resembling the antibiotic, Streptomycin. Gentamicin however gives a smaller zone difference of 10 mm towards *Escherichia coli.* No zone was formed towards lawn of *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterococcus faecalis.*

The antibacterial inhibition of *Escherichia coli* were shown in Table 2 and Table 3, with the average measurement of minimum inhibition excluding other inhibition data which is completely negative. Fig. 1 and Figure 2 summarise the mean inhibitory zones of the bacteria tested with the extracts, using both chosen antibiotics, respectively.

TABLE 1
STANDARD INHIBITION MEASUREMENT

Measurement Value (mm)	Result
5mm and below	Resistant
5 to 11mm	Intermediate
12mm and above	Sensitive

TABLE II
Escherichia coli antibacterial inhibition comparison between extract and Gentamicin

Petri Dishes	Gentamicin	Extract (80%)	Extract (60%)	Extract (40%)	Extract (20%)	Methanol 80%
1	23	19	12	10	8	Negative
2	24	15	13	13	8	Negative
3	22	9	9	8	6	Negative
mean	23	14.33	11.33	10.33	7.33	

Table II: Gentamicin recorded the highest reading of 24mm and the lowest of 22mm. 80% extract recorded the highest reading of 19mm and the lowest of 9mm. Meanwhile in 60% extract, the highest reading is 13mm and the lowest is 9mm. 40% extract scores the highest reading of 13mm and the lowest is 8mm. 20% extract shows the highest of 8mm and the lowest of 6mm. 80% methanol posted negative result. The average of each are as follows; Gentamicin- 23mm (sensitive), 80% extract- 14.33mm (sensitive), 60% extract – 11.33mm (intermediate), 40% extract- 10.33mm (intermediate), 20% extract- 7.33mm (intermediate). 80% methanol posted negative result.

TABLE III
Escherichia coli antibacterial inhibition comparison between extract and Streptomycin

Petri Dishes	Streptomycin	Extract (80%)	Extract (60%)	Extract (40%)	Extract (20%)	Methanol 80%
1	13	14	12	10	8	Negative
2	14	13	12	11	8	Negative
3	13	13	10	10	8	Negative
mean	13.33	13.33	11.33	10.33	8	

Table III shows the result obtained from *Escherichia coli* to compare with extracts and Streptomycin. Streptomycin recorded the highest reading of 14mm and the lowest of 13mm. 80% extract recorded the highest reading of 14mm and the lowest of 13mm. Meanwhile in 60% extract, the highest reading is 12mm and the lowest is 10mm. 40% extract scores the highest of 11mm and the lowest of 10mm. 20% extracts shows the flat reading of 8mm. 80% methanol posted negative result. The average of each are as follows; Streptomycin- 13.33mm (sensitive), 80% extract- 13.33mm (sensitive), 60% extract – 11.33mm (intermediate), 40% extract- 10.33mm (intermediate), 20% extract- 8mm (intermediate). 80% methanol posted negative result.

TABLE IV
Staphylococcus saprophyticus antibacterial inhibition comparison between extract and Gentamicin

Petri Dishes	Gentamicin	Extract (80%)	Extract (60%)	Extract (40%)	Extract (20%)	Methanol 80%
1	23	10	7	7	6	Negative
2	20	14	12	13	10	Negative
3	23	15	13	10	10	Negative
mean	22	13	10.67	10	8.67	

Table IV shows the result obtained from *Staphylococcus saprophyticus* to compare with extract and Gentamicin. The highest reading still comes from the antibiotic gentamicin, 22mm (sensitive). Next goes to 80% extract in which the reading is 13mm (sensitive). The reading gradually decrease as the concentration decrease as shown for 60% extract, 40% extract and 20% extract recorded 10.67mm (intermediate), 10mm (intermediate) and 8.67mm (intermediate) respectively.

TABLE V
Staphylococcus saprophyticus antibacterial inhibition comparison between extract and Streptomycin

Petri Dishes	Streptomycin	Extract (80%)	Extract (60%)	Extract (40%)	Extract (20%)	Methanol 80%
1	20	10	9	Negative	Negative	Negative
2	20	11	9	Negative	Negative	Negative
3	19	10	7	Negative	Negative	Negative
mean	19.67	10.33	8.33			

Table V shows the result obtained from *Staphylococcus saprophyticus* to compare with extracst and Streptomycin. The streptomycin scores the highest reading of 19.67mm (sensitive). While the 80% extract and 60% extract scores 10.33mm (intermediate) and 8.33mm (intermediate) respectively. Negative result posted by 40% extract, 20 % extract and 80% methanol. The negative result of 40% extract and 20% extract were deviated from those in the comparison with gentamicin.

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TABLE VI
PROTEUS VULGARIS ANTIBACTERIAL INHIBITION COMPARISON BETWEEN EXTRACT AND GENTAMICIN

Petri Dishes	Gentamicin	Extract (80%)	Extract (60%)	Extract (40%)	Extract (20%)	Methanol 80%
1	20	12	10	10	8	Negative
2	20	12	11	8	10	Negative
3	20	12	10	8	7	Negative
mean	20	12	10.33	8.67	8.33	

Table VI shows the result obtained from *Proteus vulgaris* to compare with extracts and Gentamicin. Gentamicin scores 20mm (sensitive) while the 80% extract scores 12mm (sensitive). The rest of the concentration show gradually decrease in which 60% extract, 40% extract and 20% extract recorded 10.33mm (intermediate), 8.67mm (intermediate) and 8.33mm (intermediate) respectively.

TABLE VII
PROTEUS VULGARIS ANTIBACTERIAL INHIBITION COMPARISON BETWEEN EXTRACT AND STREPTOMYCIN

Petri Dishes	Streptomycin	Extract (80%)	Extract (60%)	Extract (40%)	Extract (20%)	Methanol 80%
1	13	12	11	9	8	Negative
2	12	13	12	10	9	Negative
3	20	12	11	12	10	Negative
mean	15	12.33	11.33	10.33	9	

Table VII reveals the result obtained from *Proteus vulgaris* to compare with extracts and Streptomycin. Streptomycin records a high 20 mm and low 12mm readings. Meanwhile 80% extract manages a maximum of 13mm and minimum of 12mm. 60% shows the highest of 12mm and the lowest of 11mm. 40% extract records the high reading of 12mm and low reading of 9mm. on the other hand the 20% extract shows the highest reading of 10mm and the lowest of 8mm. In average, streptomycin come out the highest with 15mm (sensitive), 80% extract 12.33mm (sensitive), 11.33mm (intermediate),10.33mm (intermediate) and 20% extract, 9mm(intermediate). 80% methanol remains negative result as it serves as a negative control.

TABLE VIII
SERRATIA MARCESCENS ANTIBACTERIAL INHIBITION COMPARISON BETWEEN EXTRACT AND GENTAMICIN

Petri Dishes	Gentamicin	Extract (80%)	Extract (60%)	Extract (40%)	Extract (20%)	Methanol 80%
1	19	10	8	7	6	Negative
2	18	10	8	7	6	Negative
3	19	10	9	7	6	Negative
mean	18.67	10	8.33	7	6	

Table VIII shows the result obtained from *Serratia marcescens* to compare with the extracts and Gentamicin. Gentamicin shows the highest average with 18.67mm (sensitive) while the extract shows a gradually decrease of reading as the concentration decrease. 80% extract scores the average of 10mm (intermediate). 60% average records 8.33mm (intermediate). 40% and 20% extract shows a low reading with 7mm (intermediate) and 6mm (intermediate) respectivey. 80% methanol posted negative result.

TABLE IX
SERRATIA MARCESCENS ANTIBACTERIAL INHIBITION COMPARISON BETWEEN EXTRACT AND STREPTOMYCIN

Petri Dishes	Streptomycin	Extract (80%)	Extract (60%)	Extract (40%)	Extract (20%)	Methanol 80%
1	17	11	10	11	9	Negative
2	17	11	9	8	7	Negative
3	18	11	10	10	7	Negative
mean	17.33	11	9.67	9.67	7.67	

Table IX shows the result obtained from *Serratia marcescens* to compare with extracst and Streptomycin. In average Streptomycin comes out with 17.33mm (sensitive). 80% extract records 11mm (intermediate) while 60% and 40% extract scores the same readings with 9.67mm (intermediate). 20% extracts records 7.67mm (intermediate). 80% methanol serves as negative control.

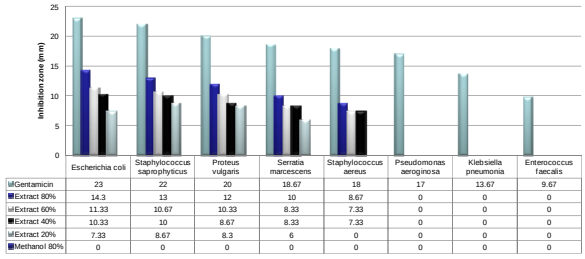


Fig. 1 Gentamicin versus Frangipani extract

Fig. 1 shows *Escherichia coli* came out on top of other bacteria tested with frangipani extract. An average of 14.3 achieved by *Escherichia coli* and the lowest recorded by other three bacteria namely *Klebsiella pneumonia, Pseudomonas aeruginosa* and *Enterococcus faecalis*, which recorded null inhibition. In the case of the extract concentration, extract 80% shows the highest inhibition activity against the five other bacteria. As the concentration decrease the inhibition zone gradually decrease.

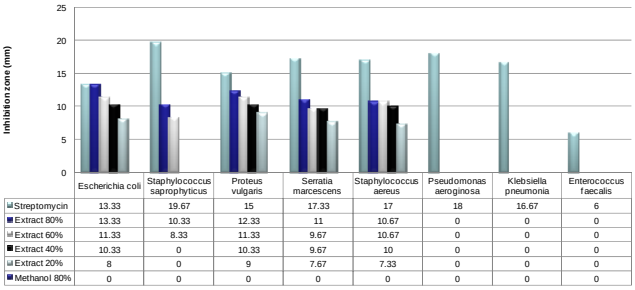


Fig. 2 Streptomycin versus Frangipani extract

Fig. 2 shows the average comparison between Streptomycin and extracts 80%, 60%, 40% and 20%. *Escherichia coli* came on top of other bacteria tested in comparison between Streptomycin and extract. Extract 80% recorded the highest inhibition activity against the five bacteria that are sensitive to the extract. As it is show in the figure, Streptomycin and extract 80% has recorded the same mean value of inhibition zone. This is a positive indicator that the frangipani has the potential of behaving as an antibiotic producer agent.

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IV. Conclusion

Plumeria alba appears to have significant antimicrobial capacity resembling a broad spectrum antibiotic against the common uro-gastro pathogenic *Escherichia coli*, one of the common bacteria with pathogenic strains and are relatively resistant towards synthetic drugs. This aromatic plant can be a potential source of evolving newer anti microbial compound and as a non toxic antibiotic producer agent. The extracts of frangipani have a potential as a natural anti toxic antibiotic producer especially against Escherichia coli.

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