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In vitro antibacterial screening of fatty acid from *Aegiceras corniculatum* against human pathogens

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Abstract
This present study aimed to investigate the antibacterial screening of fatty acids (FAs) from *Aegiceras corniculatum* against human pathogens *viz. Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Staphylococcus aureus.* The leaf, stem, flower and pneumatophore of the species were used for the study. FAs were isolated as fatty acid methyl esters from the total extracts of *A. Corniculatum* and characterized using GC-FID for their compositions. Palmitic acid (C16:0) found to a chief constituent in the crude extracts of leaf, stem and flower. Whereas, In pneumatophore, methyl-cis-5,8,11,14- Eicosotetraenoic acid (PUFA, C20:4) was found to be predominant. Potential of fatty acid methyl ester against human pathogens were accessed using disc diffusion assay. Among the four pathogens investigated, fatty acid showed significant activity against Gram-negative *K. pneumonia* and moderate activity against Gram-positive *S. aureus.* In conclusion, FAs derived from *A. corniculatum* needs further evaluation as possible new agents to treat infections caused by these pathogens, especially in synergistic combinations with antimicrobial agents already used clinically.

Keywords: Fatty acids, mangroves, *Aegiceras corniculatum*, anti-bacterial

1. Introduction
Aegiceras corniculatum is a mangrove plant, commonly known as Black Mangrove, River Mangrove or Khalsi, exists in coastlines of tropical and subtropical regions. The plants belonging to the genus *Aegiceras* are used in the treatment of ulcers and liver injuries in the form of decoctions and macerates. *A. corniculatum* have been reported to act as antidiabetic [1], antifungal [2], antiplasmodial [3], hepatoprotective, anti-inflammatory [4] and antinociceptive [5]. But, the studies exhibiting the spectrum of activities shown by fatty acid fractions of this mangrove are limited.

Fatty acids (FAs) are potential therapeutic antimicrobial agents due to their potency, broad spectrum of activity and lack of classical resistance mechanism against the actions of these compounds [6]. In particular, various long chain polyunsaturated FAs, which are found naturally at high levels in many marine organisms, have been shown to exert highly potent activity against Gram-positive bacteria, including eicosapentanoic acid (EPA; C20:5*n*-3) [7] docosahexanoic acid (DHA; C22:6*n*-3) [8], γ- linoleic acid (GLA; C18:3*n*-6) and dihomo- γ -linolenic acid (DGLA: C20:3*n*-6). Similar to many other PUFAs, eicosapentaenoic acid (EPA; C20:5 n-3) exerts potent effects against Gram-positive species, including human pathogens *Bacillus cereus* and *S. aureus*.

Thus, the aim of this present study was to investigate the antimicrobial effects of fatty acid extracts from *A. corniculatum* against human pathogens, to evaluate the potential of these compounds for treating topical infections caused by these pathogens.

2. Materials and Methods
2.1. Sample collection and Preparation of crude extract
The mangrove used in the study was *A. corniculatum* (family *Myrisine)* freshly collected from the mangrove forest of Kunjimangalam, Kannur, Kerala, India. Samples were shade dried, (leaves, stem, flower and pneumatophore) powdered (50g each) and extracted with Methanol: Dicholromethane (1:2).

2.2. Extraction of fatty acids
The method described by Harvey [9] followed for the fatty acid extraction of the present study.

2.3. Fatty acid methyl esters were analysed by GC-FID
Dried fatty acid sample converted into fatty acid methyl esters (FAME) and analyzed in Gas chromatography.

The FAME components in the extract matrices were identified using the retention time established using reference standard for FAs and percentage of individual FAME was made in relation to total area of the chromatogram (FAME mix C4-C24). Total run time was 61 min. The nitrogen was the carrier gas at a flow rate of 1.2 mL/min. The split ratio was 1:10.

2.4. Bacterial culture

The test organisms included the clinical human pathogens Gram-positive *S. aureus*, and Gram-negative *E. coli*, *K. pneumonia*, *P. aeruginosa*, were used in the study. The bacteria were grown in the nutrient broth at 37 °C and maintained on nutrient agar slants at 4 °C.

2.5. Assay for antimicrobial effect of fatty acid fractions through disc diffusion

General disc diffusion method was designed to evaluate antimicrobial activity [10].

3. Result

3.1. Fatty acid profiling

Fatty acid composition of *A. corniculatum* (leaves, stem, flower and pneumatophore) has shown in Table 1.In leaf, Palmiitic acid (C16:0), α - Linolenic acid (C18:3) and Linolenic acid Palmitoleic acid (C16:1), Stearic acid (C18:0), Oleic acid (C18:1), and Heptadecanoic acid (C17:0) were estimated. The stem and flower of *A. corniculatum* shows a similar fatty acid profile, dominant fatty acids in both were Palmitic acid (C16:0), α - Linolenic acid (C18:3) and Linolenic acid (C18:3), Oleic acid (C18:1) and Stearic acid (C18:0). Whereas in pneumatophore, Methyl cis-5, 8, 11, 14-Eicosatrienoic acid (C20:4) was the major component followed by Linoleic acid (C18:2), Palmitic acid (C16:0), Lignoceric acid (C24:0), Cis-10 heptadecanoic acid (C17:1), Stearic acid (C18:0), Oleic acid (C18:1) and Palmitoleic acid (C16:1).

Table 1: Fatty acid composition of *Aegiceras corniculatum* Relative %

S No:	Fatty acid	Leaf	Stem	Flower	Pneumatophore
1	C8	0.02	-	-	-
2	C14	0.18	-	-	-
3	C15:1	-	-	-	-
4	C16	43.27	87.85	87.86	0.07
5	C16:1	0.233	-	-	0.05
6	C17	0.3	0	0	-
7	C17:1	-	-	-	11.48
8	C18	11.29	11.58	12.33	0.16
9	C18:1	13.78	0.34	0.35	0.25
10	C18:2	-	-	-	2.00
11	C18:3	0.34	0.22	0.24	0
12	C18:3	30.57	0.01	0.001	0.5
13	C20:3	-	-	-	-
14	C20:4	-	-	-	82.96
15	C22	0	-	-	0
16	C23	-	-	-	-
17	C24	-	0	0	0.23

3.2. Antibacterial assay

FAs isolated from numerous plants have shown antibacterial activity using bioassay-guided fractionation. FAs derived from leaf, stem, flower and pneumatophore of *A. corniculatum* has shown similar inhibition pattern against all the pathogens selected for the study (Table 2). Among all the clinical pathogens, higher inhibition observed against Gram-negative *K. pneumonia* and moderate inhibition observed against gram positive *S. aureus*. Slight activity has shown against *P. Aeruginosa* and it was inactive against *E. coli*. Compared to leaf, stem and flower fatty acid derived from pneumatophore has shown higher growth inhibition activity against *K. pneumonia.* Antibiogram results (Fig.2) which compare the free fatty acids (FFAs) and antibiotic (Fig.1) clearly shows the potential of FFAs especially from pneumatophore, which is dominant in PUFAs, to suppress the activity of *K. pneumonia.*

Table 2: Antimicrobial activity of fatty acid fractions against clinical human pathogen

S. No	Pathogen	Leaf	Stem	Flower	Pneumatophore
1	*Escherichia coli*	--	--	--	--
2	*Klebsiella pneumonia*	10 ++	8 mm +	8 mm +	13 mm +++
3	*Pseudomonas aeruginosa*	7 mm +	7mm +	8mm +	11 mm ++
4	*Staphylococcus aureus*	6 mm +	6 mm +	8 mm +	10 mm ++

+ Slightly active, ++ Moderate active, +++Highly active, -- No active

Fig 1: Antibiogram comparing the antibiotic activity (T & F) against fatty acid extract from pneumatophore (P & G)

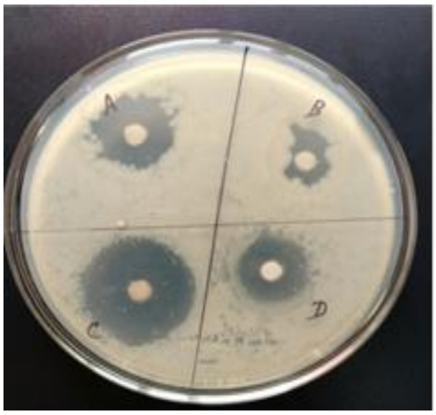

Fig 2: Antibiogram of *Klebsiella pneumonia* incubated with fatty acid fractions

4. Discussion

New treatments are required for topical infections caused by human pathogens like *E. coli*, *K. pneumonia*, *P. aeruginosa*, and *S. aureus*. The efficacy of many treatments has reduced due to drug resistance and undesirable side effects can also cause problems for patients. In the present investigation, antimicrobial effects of FAs from *A. corniculatum* have studied. All the FAs derived from leaf, stem, flower and pneumatophore of *A. corniculatum* inhibited the growth of four selected pathogens *in vitro*.

FAs isolated from numerous plants have shown antibacterial activity using bioassay-guided fractionation. Cerdeiras *et al.*

[11] identified 11-O-(6'-O-acetyl-β-D-glucopyranosyl)- Stearic acid as the main antibacterial component of aerial parts of Ibicellalutea. This fatty acid derivative showed an interesting antibacterial activity, being active against *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus* with the minimal inhibitory concentration value of 9 µg mL-1 against *S. aureus*. Dilika *et al*. [12] described the antibacterial activity of linoleic and oleic acids isolated from the leaves of *Helichrysum pedunculatum*. Previous studies shown that, linoleic and oleic acids inhibited the growth of Gram-positive *B. subtilis*, *Micrococcus kristinae* and *S. aureus.* And it was also showed activity against *B. cereus* and *Bacillus pumilis*. Both acids displayed any activity against Gram-negative *Enterobacter cloacae*, *E. coli, K. pneumoniae, P. aeruginosa and Serratia marcescens*. The present study also reveals the similar trend, fatty acid fractions of *A. corniculatum* doesn't show growth inhibition activity against *E. coli.*

Additionally, long-chain unsaturated FAs are bactericidal to important pathogenic microorganisms, including Methicillin-resistant *S. aureus* [13] *Helicobacter pylori* [14], and *Mycobacteria*. In recent years, it has been reported that eicosapentaenoic acid (EPA) also exhibited antimicrobial activity against *B. subtilis*, *Listeria monocytogenes*, *S. aureus* and *P. aeruginosa* [15].These antibacterial actions of FAs are usually attributed to long-chain unsaturated FAs including oleic acid, linoleic acid, and linolenic acid, whereas long-chain saturated FAs, including palmitic acid and stearic acid, are less active [14].

This might be the reason for highest antibacterial activity shown by fatty acid derived from pneumatophore of *A. corniculatum* against *K. pneumonia*, which is characterised by highest unsaturation index compared to leaf and stem under study. The greater percentage of polyunsaturated FAs (PUFAs) along with long chain FAs (C18:1, C18:2 and C18:3) may be increased rate of inhibition of fatty acid fraction derived from pneumatophore. Some PUFAs like oleic acid, linolenic acid, and EPA have been demonstrated to be bactericidal to important pathogenic microorganisms including antibiotic-resistant *S. aureus* [16].

Despite the wide literature concerning the influence of FAs, and particularly unsaturated FAs, on bacterial and fungal growth dynamics, including growth delay, death or stimulation, the mechanism by which FAs affect cellular functions is still not totally understood. A number of studies have suggested that the toxicity of PUFAs against microorganisms may be due to their oxidation products derived from metabolic processes.

The broad spectrum of activity and non-specific mode of action of at least some FAs make them attractive as antibacterial agents for several applications in medicine, agriculture, food preservation and the formulation of cosmetics or nutraceuticals, especially where the use of conventional antibiotics is undesirable or forbidden. By and large, the evolution of inducible FA-resistant phenotypes is less problematic than with conventional antibiotics [14]. A further problem may be the perceived lack of patentable intellectual property concerning these ubiquitous antibacterial compounds. However, these problems can be overcome, and the usefulness of FAs from the mangrove *A. corniculatum* in antibacterial applications should not be lay off.

5. Conclusion

Our study undoubtedly confirms that the fatty acid extracted from pneumatophore of *A. corniculatum* contains a higher relative percentage of the PUFAS that has potential anti-bacterial principle for clinical application. Bactericidal activities shown by the fatty acid fractions were dependent on the abundance of long-chain unsaturated fatty acids. The present study demonstrates that these natural compounds deserve evaluation for the treatment of infections caused by these Human pathogens and fatty acids could be applied in combination with some currently available treatments to enhance therapeutic efficacy. Considering the high concentration of methyl cis -5,8,11,14 eicosatetraenoic acid (82.96 %), pneumatophore of *A. corniculatum* could be harvested as potential source for its isolation for developing therapeutically active formulation.

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7. References

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