ANTI-MICROBIAL ACTIVITIES OF FATTY ACIDS AND ITS METHYL-ESTER (FAME) COMPOUNDS DERIVED FROM *CAESALPINIA BONDUCELLA* (L.)

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ABSTRACT

Fatty acids are widely occurring natural fats and dietary oils and are known to have antimicrobial properties. However, little is known on the antibacterial and antifungal properties of the *Caesalpinia bonducella*. The present study determines the antibacterial and antifungal activities of fatty acid ester compound plants found in India and more than 500 species of herbaceous plants are recorded from costal area in southern India. The dominant species belonging to genus Caesalpinia is *Caesalpinia pulcherrima.* Gram negative bacteria were resistant to Caesalpinia extract when compared to Gram positive bacteria.

Key words: Antimicrobial activity, Caesalpinia, Fatty Acid Methyl Ester.

INTRODUCTION

Fatty acids are widely occurring in natural fats and dietary oils and are known to have antimicrobial properties (Agoramoorthy et al., 2007). The *Caesalpinia bonducella* is a typical species that occur along the coastal area of southern India. This perennial tree has religious significance for the local community as well. Different parts of this plant have been used in traditional medicine for the treatment of leprosy and also as an aphrodisiac. (Kirtikar and Basu, 1999). A novel phorbol ester, an anti-HIV principle has also been isolated from the leaves and stem of this plant (Karali et al., 1994).

Fatty acids are widely occurring in natural fats and dietary oils which play an important role as nutritious substances and metabolites in various living organisms (Kakir, 2004). Many fatty acids are known to have antibacterial and antifungal properties as well (Scidel and Taylor, 2004). However, little is known on the antibacterial and antifungal properties of *Caesalpinia bonducella*. An attempt is being made in the present investigation, on the fatty acid methyl-

ester of leaves of *Caesalpinia bonducella* to evaluate its antibacterial and antifungal properties. The seeds of the plant have been reported to possess in-vitro as well as in-vivo antibacterial activity.

MATERIALS AND METHODS

Oil seed leaves of *Caesalpinia bonducella* we collected during a routine field trip conducted during November 2008. The leaves were washed with running water, later the surface was sterilized with 10% Sodium Hypochlorite solution and revised with distilled water as per the standard method prescribed by Venkatesalu et al., (2003). The seeds were roasted and ground at room temperature. Twenty grams of powder were refluxed with mixture of methanol and benzene (200:100) for 3 hours. The filtrate was transferred to a separating funnel and 60-80 CC of double distilled water was added. A small amount of benzene was added and pooled. The hexane fraction was separated into two layers and the bottom layer was removed. The upper layer was then washed with 0.8% Sodium Chloride solution. The upper layer was then passed through Sodium Sulphate and the extract obtained was evaporated (Venkatesalu et al., 2004). The residue was dissolved in benzene and analysed by gas chromatography (Varian, Inc, USA).

The test solution was prepared with known weight of crude extracts dissolved in Dimethyl *Sulphoxide* (5%) and filtered using sterile filter paper discs (Whatman No. 1:6 mm) were impregnated with 20 ml of the extract and allowed to dry at room temperature. Four strains of Gram-positive bacteria (*Bacillus subtilis*, *B. pamilus*, *Micrococcus luteus* and *Staphylococcus spp.*) and three strains of Gram-negative bacteria (*Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli*) were obtained from the natural collection of industrial microorganisms, Biochemical Science of National Chemical Laboratory at Pune (India). The stock cultures were maintained on nutrient agar medium at 5° C. *Candida albicans,*

C Krusei were obtained from the Rajah Muthiah Medical College and Hospital, Annamalai University India.

Antibacterial activity was determined in-vitro using Mueller Hinton agar and Mueller Hinton broth method. In-Vitro antifungal activity was determined by using antifungal assay agar, Sabourauds dextrose agar and yeast nitrogen base (obtained from Himedia Ltd. Mumbai, India). The selected bacteria and yeast of 24 hour culture were mixed with physiological saline and the turbidity was adjusted to a Mac Farland turbidity standard of 0.5 by adding sterile physiological saline. The agar diffusion method was used for antibacterial and antifungal tests (Ozeelik et al., 2005) plates were prepared by pouring freshly prepared Hinton M agar for bacteria and antibacterial assay agar for fungi into petriplates and allowed to solidify to which 0.1 ml standardized inoculum suspension was poured and uniformly spread. The plates were dried after decanting the inoculum. The discs were placed on the inoculated agar. Ciprofloxacin and Amphotericin (100 units/disc) were used as positive control and 5% DMSO was used as negative control. The inoculated plates were incubated at 37° C for 23 hr (Bacteria) and 28°C for 47 hr (Yeast).

Inhibitory concentration value of FAME extract was tested in Mueller Hinton broth for bacteria and yeast nitrogen base for yeast by serial dilution method. The test extract was dissolved in 5% DMSO to get 4 mg stock solution. 0.5 mg of stock solution was incorporated into 0.5 ml of Mueller Hinton broth for bacteria and yeast nitrogen base for yeast to get a concentration of 2, 1.5, 0.5, 0.25, 0.125 and 0.06 mg^{-1}. 50 µl of standardized suspension of the test organism were transferred to each tube. A control tube containing the micro-organism was also prepared. The culture tubes were incubated at 37° C for 23 hr (Bacteria) and 28°C for 47 hr (Yeast). The MIC was defined as the lowest concertation of the extracts that did not show any growth of the tested micro-organisms after microscopic evaluation. The minimum bactericide concentration (MBC) and minimum fungicide concentration (MFC) of the extracts

were determined by plating 100 µl samples from each MIC assay tube with inhibition into Mueller Hinton agar for bacteria and Sabourand dextrose agar for yeast plates. The MBC and MFC were recorded as the lowest concertation of the extract that did not permit any visible bacterial and fungal colony growth on the agar plates after the period of incubation.

RESULTS AND DISCUSSION

The analysis of FAME Extract of *Caesalpinia bonducella* by Gas Chromatography revealed that higher amount of Saturated fatty acids than unsaturated fatty acids (Figure-1). Among the Fatty acids, Palmitic acid (54.05%) and Lauric acid (16.11%) were recorded in higher quantity (Table-1). Among the Saturated fatty acids, higher amount of Myristic acid (3.81%) followed by Stearic acid (2.50%), Pentadecanoic acid (2.42%), Heptadecanoic acid (0.09%) and lower amount of Tridecanoic acid (0.80%), Behenic acid (0.80%), Arachidic acid (0.13%) and Nonadecanoic acid (0.02%) were recorded. The presence of Myristic, Stearic, Heptadecanoic and Arachidic acids were reported previously in the roots, shoots and seeds of *Caesalpinia bonducella*. Besides, the occurrence of Myristic acid and Pentadecanoic acid have been reported in some species. Among unsaturated acids, higher percentage of Linolenic acid (6.20%.) followed by Linoleic acid (2.74%) and Oleic acid (2.72%) were recorded.

The FAME extracts processed antibacterial and antifungal activities against a total of 11 micro-organisms (7 bacteria and 4 yeasts) are depicted in Table-2. The mean zone of inhibition of the extract, assayed against the test organisms ranged between 7.3 and 16.6 mm. The Ciprofloxacin (5 µg /disc) antibacterial positive control produced zones of inhibition that ranged from 31 To 36 mm.

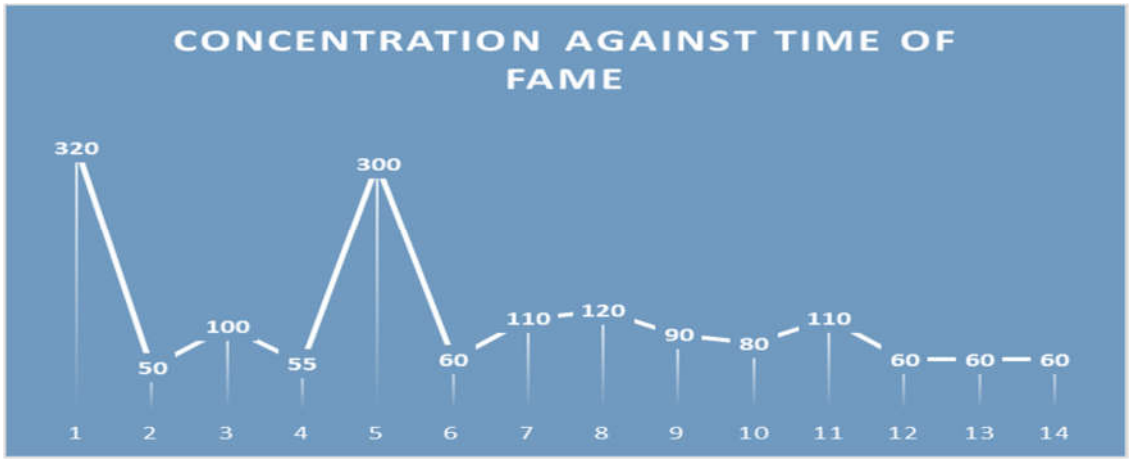

Figure 1: A Chromatogram of the Fatty Acid Methyl-Ester (FAME) of the leaves of *Caesalpinia* (8, 9 and 10 unsaturated fatty acids)

Peak No.	*Retention time (Min)*	*Fatty acids*	*No. of Carbon atoms*	*Relative Percentage*
1	2.783	Lauric acid	C12:0	18.21
2	2.746	Tridecanoic acid	C13:0	0.70
3	4.247	Myristic acid	C14:0	3.16
4	5.606	Pendecanoic acid	C15:0	2.55
5	7.304	Palmitic acid	C16:0	56.02
6	9.726	Heptadecanoic acid	C17:0	1.03
7	12.714	Steric acid	C18:0	2.08
8	13.315	Oleic acid	C18:1	1.07
9	14.519	Linoleic acid	C18:2	3.13
10	15.874	Nonadecanoic acid	C19:0	0.01
11	18.330	Linolenic acid	C18:3	7.20
12	23.71	Arachidic acid	C20:0	0.12
13	33.524	Heneicosanoic acid	C21:0	ND
14	44.272	Behenic	C22:0	0.79

Table-1: Fatty acid composition of leaves of *Caesalpinia*

ND = Could not be detected; Saturated fatty acids-83.99; Unsaturated Fatty acids-14.04; Unidentified acids-1.97, Total-100.00

Amphotericin-B (100 units/disc) antifungal control produced Zones of inhibition that ranged from 17 to 18mm. The MIC of the FAME extracts were 0.124 mg/ml for *Bacillus subtilis* and *Staphylococcus aureus*; 0.5 mg for *Bacillus pumilus*, *Micrococcus luteus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Candida albicans, C. krusei and C. parapsilosis* and 1.2

mg for *Escherichia coli* and *Candida tropicalis.* These differences could be due to the nature and level of the antimicrobial agents present in the extract and their mode of action on different test micro-organisms (Barbour et al., 2004).

The highest mean zone of inhibition of 16 mm and the lowest MIC value of 0.125 mg and MBC valves of 0.24mg were produced by FAME extract of *Caesalpinia bonducella* against *Bacillus subtilis* and *Staphylococcus aureus*. We have reported a similar observation previously with the extract of certain marine algae against *Bacillus subtilis*, *Micrococcus luteus, Staphylococcus aureus, Salmonella tryhimurium, Pseudomonas aeruginosa, Klebsiella pneumonia and Escherichia coli*. More over a previous study of lipophilic extracts derived from 15 different plant parts showed activity against *Escherichia coli* and *Enterococcus faecalis*, similarly linoleic acid isolated from other plant displayed antibacterial activity against *Bacillus subtilis, Staphylococcus aureus* and *Escherichia coli,* Similar observations were made by Mac Gaw et al., (2002).

Sl No	Microorganisms	Mean zone of inhibition			FAME Extract			
		FAME extract concentration of the disc			CIP 5 mg / disc	AMP 100 units / disc	MIC mg	MBC / MFC mg
		200	100	50				
1	*Bacillus subtilise*	15.6	13.0	11.6	32.0	NT	0.12	0.22
2	*Bacillus pumilus*	12.0	09.0	07.8	36.0	NT	0.40	1.00
3	*Micrococcus luteus*	13.0	12.0	10.0	31.0	NT	0.50	1.00
4	*Staphylococcus aureus*	15.0	14.0	12.0	32.0	NT	0.125	0.25
5	*Pseudomonas aeruginosa*	11.0	09.0	06.6	33.0	NT	0.50	1.00
6	*Klebsiella pneumoniae*	12.0	10.0	07.3	34.0	NT	0.50	1.00
7	*Escherichia coli*	09.6	09.0	08.2	30.0	NT	1.00	2.00
8	*Candida albicans*	12.5	10.9	08.8	NT	18.0	0.50	1.00
9	*Candida tropicalis*	11.2	09.8	08.0	NT	18.2	0.50	1.00

Table 2: Antibacterial and antifungal activity of Fatty acids and Methylester compound (FAME) Extracts of *Caesalpinia bonducella.* CIP – Ciprofloxacin antibacterial standard. AMP – Amphoterein antifungal standard, NT – not tested.

Linoleic acid was reported to be active against *Mycobacterium smegmatis* and *M. fortuitum.* Gram-positive bacteria were more susceptible than the Gram-negative bacteria in the current study. Similar results were obtained with FAME extracts of various plant parts of plant that is leaves of *Ipomoea pescaprae* and lipophilic extracts of plant parts of *Pistacia vera* (4.12). These differences in the fatty acids sensitivities between Gram-positive and Gram-negative bacteria may result from the impermeability of the outer membrane of Gram-negative bacteria since the outer membrane of Gram-negative bacteria is an effective barrier against hydrophobic substances. In fact, Gram-negative bacteria are more resistant to inactivation by medium and long chain fatty acids that has potential antibacterial and antifungal principle for clinical application.

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