

Antibacterial Activity of the Fatty Acid Methyl Esters from Synthesis of Sesbania Rostrata Seed by in-Situ Transesterification Reaction

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Summary

The antibacterial activity of the fatty acid methyl esters from synthesis of Sesbania rostrata Seed by In-Situ Transesterification reaction was evaluated at two different concentrations by the agar well diffusion method. The synthesis of fatty acid methyl esters Sesbania rostrata Seed was most active against *Pseudomonas pseudoalcaligenes*. The synthesis of fatty acid methyl esters Sesbania rostrata Seed was more active against Gram-negative bacteria as compared to Gram-positive. The results obtained indicated that fatty acid methyl esters Sesbania rostrata Seed different antimicrobial activity.

Keywords: fatty acid methyl esters; Sesbania rostrata Seed; Gram-negative, Gram-positive; antibacterial activity

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Introduction

Microbial promoted infectious diseases are a major public health issue worldwide. Among other, these include infection by antibiotic-resistant microorganisms, which are considered very problematic since only few antibiotics currently available are effective against resistant microbial strains [1-3]. One of the most promising classes of antibiotics is inhibitors of bacteria cell wall synthesis, which are largely based on different classes of synthetic products [4]. Therefore, there is great interest in finding new classes of synthetic products that may be effective against antibiotic-resistant bacteria. Although extremely effective, antibiotics are able to induce resistance in bacteria. For 450 years, bacterial resistance has been the main factor responsible for the increase of morbidity, mortality and health care costs of bacterial infections. This bacterial defense mechanism is widely present in bacteria (e.g. *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Acinetobacter*, *Salmonella*, *Staphylococcus*, *Enterococcus* and *Streptococcus*) and became a world

health problem worsened by developments in human, animal and plant transportation (9). Also, tuberculosis promoted by *Mycobacterium tuberculosis* is a major cause of mortality. It is estimated that about 2 billion people are currently infected with *M. tuberculosis*. The number of infections by *M. tuberculosis* appear to be increasing, due to co-infection with HIV and the emergence of multiple drug resistant *Mycobacterium* strains [5, 6]. Although several drugs for the treatment of tuberculosis are available, it is a long-term therapy (6 months), which very frequently present side effects. Therefore, there is an urgent need for new anti-tuberculosis drugs that must be effective, less toxic and promote a short period of treatment [7,8].

This paper reports the first attempt to study the antimicrobial activity of fatty acid methyl esters from synthesis of *Sesbania rostrata* Seed by In-Situ Transesterification reaction was evaluated for the potential antibacterial property. The selection of these fatty acid methyl esters from synthesis of *Sesbania rostrata* Seed for evaluation was based on its traditional usage. Fatty acid methyl esters from synthesis of *Sesbania rostrata* Seed by In-situ transesterification seed powder is extracted with alcohol where alcohol acts as a solvent as well as reactant. This process reduces the cost of final product as this process has less number of unit operations. The study synthesis of fatty acid methyl esters *Sesbania rostrata* Seed was more active against Gram-negative bacteria as compared to Gram-positive was evaluated at two different concentrations by the agar well diffusion method.

Materials and Methods

Chemicals

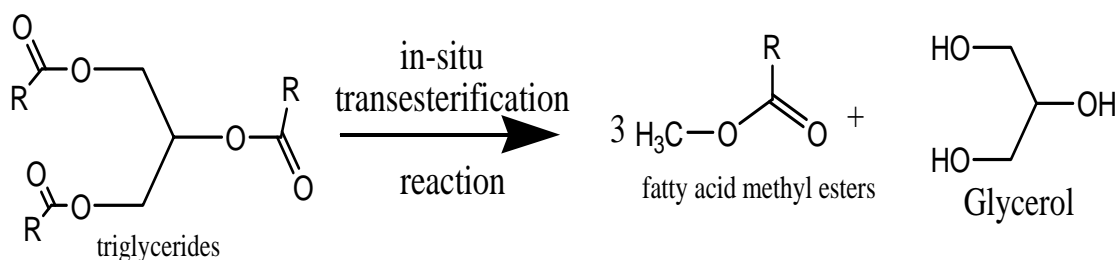
Methanol, ethanol, KOH, *Sesbania rostrata* Seed, Fatty acid methyl ester, etc.

Synthesis of *Sesbania rostrata* fatty acid methyl ester (SRFAME):-

The seeds of *Sesbania rostrata* collected, cleaned and dried. The seeds are then grinded to fine powder by using heavy duty electric mixer of high rpm. Ten grams of seed powder was used as a starting material. It was mixed with mixture of Methanol and Ethanol. The in-situ transesterification with continuous stirring was carried out by adjusting 300 rpm oscillations. The heat is given by hot plate by keeping at 80⁰ C for about 45 minutes. The solid cake and mother liquor were separated by vacuums filtration. A rotary evaporator was used for separation of solvent. The oil fraction separates at 80⁰C. The oil content was preserved in airtight containers and used for further analysis. The moisture content of dry seed powder and oil extracted by reactive extraction was obtained by Karl Fischer Titrator, **µaquacal₁₀₀**, manufactured by Analab Scientific Instruments Pvt. Ltd. During in-situ transesterification various concentrations of potassium hydroxide were used as a catalyst along with the mixture of alcohols. The reaction time was finalized for optimum yield is 45 minutes. The reaction was carried out at different temperatures. The temperature 80⁰C will give maximum yield where as the oil was also separated at 80⁰C by rotary evaporator. The water quantity will also affect the rate of reaction considerably. Increase of aqueous medium will reduce the yield of reactive extraction. The observed yield is maximum without addition of water the separation of various components was

studied by Thin layer Chromatography. The best solvent for separation was found to be Acetic Acid, Pet. Ether and Ethanol with volume ratio 0.75: 7.25: 2.00. The silica gel suspended in chloroform and a pinch of plaster of Paris was used for preparation of Chromatography plate. The spots were observed on chromatogram by keeping dry developed plate in iodine chamber. The areas of pick were calculated by using gas chromatograms Capillary column (DB wax 70) by chemito (GC 1000SIMDIS) instruments. The present (%) monoglycerides, Fatty acid methyl ester, diglycerides Triglycerides, free fatty acids compounds was determined percent (%) yield

Reaction:



Microorganisms Tested

The bacterial strains used to assess the antibacterial properties of fatty acid methyl esters *Sesbania rostrata* Seed of included six Gram-positive and nine Gram-negative bacteria (Table 1). The investigated microbial strains were obtained from standard Laboratory, Pune, India. The organisms were maintained on nutrient agar (Hi Media, India) slope at 4°C and sub-cultured before use. The bacteria studied are clinically important ones causing several infections and it is essential to overcome them through some active therapeutic agents.

Determination of Antibacterial Assay

Antibacterial Activity of the fatty acid methyl esters from synthesis of *Sesbania rostrata* Seed was studied against ten bacterial strains by the agar well diffusion method (15). Mueller Hinton agar no. 2 (Hi Media, India) was used as the bacteriological medium. The fatty acid methyl esters from synthesis of *Sesbania rostrata* Seed were diluted in 100% dimethylsulphoxide (DMSO) at the concentrations of 5 mg/mL and 2.5 mg/mL. The antibacterial activity was evaluated at two different concentrations viz. 500 µg/ well and 250 µg/ well. The Mueller Hinton agar was melted and cooled to 48 - 50°C and a standardized inoculum (1.5×10⁸ CFU/mL, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile Petri dishes to give a solid plate. Wells were prepared in the seeded agar plates. The test compound (100 µl) was introduced in the well (8.5 mm). The plates were incubated overnight at 37°C. The antimicrobial spectrum of the extract was determined for the bacterial species in terms of zone sizes around each well. The experiment was performed three times to minimize the error and the mean values are presented.

Results

Table 1. Antibacterial activity of fatty acid methyl esters from synthesis of *Sesbania rostrata* Seed by agar well diffusion method.

Microorganisms	Inhibition Zone (mm)*	
	FAME of <i>Sesbania rostrata</i> Seed (5 mg/mL)	(2.5 mg/mL)
<i>Bacillus cereus</i> (ATCC 11778)	14	12
<i>Enterobacter aerogenes</i> (ATCC 13048)	18	15
<i>Staphylococcus aureus</i> (ATCC 25923)	14	11
<i>Alcaligenes fecalis</i> (ATCC 8750)	19	16
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	13	10
<i>Micrococcus flavus</i> (ATCC 10240)	-	-
<i>Pseudomonas pseudoalcaligenes</i> (ATCC 17440)	22	20
<i>Staphylococcus epidermidis</i> (ATCC 12228)	14	11
<i>Salmonella typhimurium</i> (ATCC 23564)	16	12
<i>Staphylococcus subflava</i> (NCIM 2178)	10	8
<i>Bacillus subtilis</i> (ATCC 6633)	-	-
<i>Klebsiella pneumoniae</i> (NCIM 2719)	18	16
<i>Proteus vulgaris</i> (NCTC 8313)	18	15
<i>Proteus mirabilis</i> (NCIM 2241)	9	-
<i>Pseudomonas testosteroni</i> (NCIM 5098)	17	14

*: values include cup borer diameter (8.5 mm) and are mean of three replicates.

Discussion

The antibacterial activity of the fatty acid methyl esters from synthesis of *Sesbania rostrata* Seed was determined against fifteen bacterial strains which is reported in Table1. The antibacterial activity was observed to be in dose dependent manner i.e. 5 mg/mL showed more level of activity than 2.5 mg/mL against all the tested microorganisms. Gram-positive bacteria, *B. subtilis* and *M. flavus* were most resistant strains. Gram-negative bacteria *P. mirabilis* showed antibacterial activity at only one concentration i.e. 5 mg/mL. The Antibacterial activity of fatty acid methyl esters from synthesis of *Sesbania rostrata* Seed was most active against *P. pseudoalcaligenes* in comparison to all the microorganisms tested. Gram-negative bacteria were more susceptible to the Antibacterial activity of fatty acid methyl esters from than Gram-positive bacteria which contradict the previous reports that fatty acid methyl esters are more active against Gram-positive bacteria than Gram-negative bacteria. It is therefore theorized that Gram-positive bacteria are more susceptible than Gram-negative bacteria due to the differences in their cell wall structure. Gram-negative organisms are considered to be more resistant due to their outer membrane acting as a barrier to many environmental substances, including antibiotics.

However, the results from this study reveals that the fatty acid methyl esters from synthesis of *Sesbania rostrata* Seed with significant antibacterial property which enables the fatty acid methyl esters from synthesis of *Sesbania rostrata* Seed to overcome the barrier in Gram-negative cell wall. In addition, these results form a good basis for selection of the fatty acid methyl esters from synthesis of *Sesbania rostrata* Seed for further pharmacological investigation. The results of the present study supports the folkloric usage of the studied fatty acid methyl esters from synthesis of *Sesbania rostrata* Seed and suggests that the fatty acid methyl esters from synthesis of *Sesbania rostrata* Seed with antibacterial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens.

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References

1. Andersson, D. I. Persistence of antibiotic resistant bacteria. *Curr. Opin. Microbiol.* **2003**, *6*, 452–456.
2. DaSilva, E.; M. Iaccarino, M. Emerging diseases: a global threat. *Biotech. Adv.* **1999**, *17*, 363–384.
3. Projan, S. J. Why is big Pharma getting out of antibacterial drug discovery? *Curr. Opin. Microbiol.* **2003**, *6*, 427–430.
4. Silver, L. L. Novel inhibitors of bacterial cell wall synthesis. *Curr. Opin. Microbiol.* **2003**, *6*, 431–438.
5. Tripathi R. P.; Tewari, N.; Dwivedi, N.; Tiwari, V. K. Fighting tuberculosis: an old disease with new challenges. *Med. Res. Rev.* **2005**, *25*, 93–131.
6. Lawn S. D., Bekker, L.G.; Miller, R. F. Immune reconstitution disease associated with mycobacterial infections in HIV-infected individuals receiving antiretrovirals. *Lancet Inf. Dis.* **2005**, *5*, 361–373.
7. Ballell, L.; Field, R. A.; Duncan, K.; Young, R. J. New small-molecule synthetic antimycobacterials. *Antimic. Agent. Chemoth.* **2005**, *49*, 2153–2163.
8. Zhang, Y. The magic bullets and tuberculosis drug targets. *Ann. Rev. Pharm. Tox.* **2005**, *45*, 529–64.
9. Deivy Clementino de Lima, Paula Alvarez Abreu, Cí' cero Carlos de Freitas, Dilvani Oliveira Santos, Rodrigo Oliveira Borges, Tereza Cristina dos Santos, et al. Snake venom: any clue for antibiotics and CAM? *Evid Based Complement Alternat Med* **2005**; *2*:39–47.
10. Anjaria, J.; Parabia, M.; Dwivedi, S.; (2002). *Ethnovet heritage Indian ethnoveterinary medicine – An Overview*; Pathik Enterprise, Ahmedabad (Gujarat), India.

11. Barbour, E.K.; Al Sharif, M.; Sagherian, V.K.; Habre, A.N.; Talhouk, R.S.; Talhouk, S.N.; (2004). Screening of selected indigenous plants of Lebanon for antimicrobial activity. *J. Ethnopharmacol.*, 93, 1-7.
12. Motsei, M.L.; Lindsey, K.L.; van Staden, J.; Jaeger, A.K.; (2003). Screening of traditionally used South African plants for antifungal activity against *Candida albicans*. *J. Ethnopharmacol.*, 86, 235-241.
13. Shahidi, G.H.; Karimi Nik, A. (2004). Antibacterial activity of some medicinal plants of Iran against *Pseudomonas aeruginosa* and *P. fluorescens*. *Asian J. Plant Sci.*, 3, 61-64.
14. Machado, T.B.; Pinto, A.V.; Pinto, M.C.F.R.; Leal, I.C.R.; Silva, M.G.; Amaral, A.C.F.; Kuster, R.M.; Netto-dosSantos, K.R.; (2003). *In vitro* activity of Brazilian medicinal plants, naturally occurring naphthoquinones and their analogues, against methicillin-resistant *Staphylococcus aureus*. *Int. J. Antimicrob.*, 21, 279-284.
15. Perez, C.; Paul, M.; Bazerque, P.; (1990). An antibiotic assay by the agar-well diffusion method. *Acta Biol. Med. Exp.*, 15, 113-115.
16. Iyer KM, Pillay PP. A chemical examination of *Emblica Officinalis*, Gaertn. *Curr Sci* 1958;27:266-7.
17. Bhattacharya SK, Bhattacharya D, Muruganandam AV. Effect of *Emblica Officinalis* tannoids on a rat model of tardive dyskinesia. *Indian J Exp Biol* 2000;38:945-7.
18. Jamwal KS, Sharma IP, Chopra CL. Pharmacological investigation of the fruit of *Emblica Officinalis*. *J Sci Ind Res* 1959;18:180-1.
19. Reddy BM, Rao NK, Ramesh M, Appa Rao Avn, Lin LJ, Lin LZ, et al. Chemical investigation of the fruits of *Terminalia Chebula*. *Int J Pharmacog* 1994;32:352-6.
20. Grampurohit ND. Gallic acid from myrobalans. *Indian J Nat Prod* 1986;2:10-11.