

Isolation, Characterization and Biological Evaluation of Two New Lignans from Methanolic Extract of Bark of *Zanthoxylum armatum*

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ABSTRACT

Two new amides (Za-04, Za-05) were isolated from methanolic extract of bark of *Zanthoxylum armatum* along with Lupeol, Campesterol, β -Sitosterol, Stigmasterol. The structures of these new compounds were elucidated through a variety of spectroscopic techniques such as IR spectroscopy, Mass spectroscopy, and ¹H NMR spectra. Compound Za-04 was found to be N-(3¹,4¹-Methylenedioxyphenyl ethyl)-3,4-methylenedioxy-cinnamoyl amide and Compound Za-05 was found to be N-(3¹,4¹-dimethoxyphenylethyl)-3,4-methylenedioxy-dihydrocinnamoyl amide. The compounds were identified as lignans, as they gave positive result for labat test. The two compounds were screened for antimicrobial and antimycobacterial activity. The compounds exhibited moderate to potent activity when compared to their respective standards.

Keywords: *Zanthoxylum armatum*

INTRODUCTION

Since time immemorial the extracts of plants have been recognized to possess important medicinal activities. *Ayurveda*, the most ancient medicinal system contributed by our country, includes in its "Materia Medica" drugs from plant and animal sources even today plants are the almost exclusive source of drugs for the majority of the world's population^{1,2,3}. "Herbal drugs" are gaining increasing importance over chemotherapeutic agents because of their histocompatibility, less toxic and fewer side effects.^{4,5} Pure compounds are generally employed when the active principles of a medicinal plant exhibit strong, specific activity and/or have a small therapeutic index, requiring accurate and reproducible dosage. Herbal drugs and the active constituents extracted from them are used as anticancer,^{6,7} antimalarial,^{8,9} antiviral^{10,11} cardiovascular^{12,13} and contraceptive agents.^{14,15} There are several constituents isolated from the plant extracts such as phenols, flavonoids, alkaloids, glycosides etc^{16,17}.

The plant of the genus *Zanthoxylum* belongs to the family Rutaceae and is known as Toothache tree in English, Tejphal in Hindi and Tumboonalari in Malayalam.¹⁸ The plants are often shrubs or trees. Leaves are alternate, imparipinnate, glandular-punctate. Flowers are mostly unequal, in axillary or terminal cymes. calyx are 3-8 lobed or absent. Petals 3-5, occasionally absent. Stamens are 3-8, rudimentary in the female flowers. Ovary 1-5-locular, style sub lateral, free or united above, stigma capitate, fruit is schizocarp subglobose¹⁹. A temperate and subtropical genus, comprising 20-30 species distributed in E.Asia, Phillipinese. Malasiya and N.America. Most *Zanthoxylum* species produce pungent alkalimides derived

from polyunsaturated carboxylic acids, which are stored in the pericarp (fruit wall, shell) but not in the seeds. The exact nature of these alkalimides may vary from species to species²⁰.

Zanthoxylum armatum DC [syn. *Z. alatum* Roxb.] (*Rutaceae*) is extensively used in the Indian system of medicines as a carminative, stomachic, and anthelmintic²¹. The bark is pungent, and sticks prepared from it are used for preventing toothache. The fruits and seeds are employed as an aromatic tonic in fever, dyspepsia, and expelling roundworms²². Clinical trials were also conducted for hepatoprotective action and Anti malarial action. Here, in this present experimental design we illustrate the isolation, characterization and biological activity of two new amides. The known compounds are identified as Lupeol, campesterol, β -sitosterol, stigmasterol from the hexane extract. The two new amides are obtained from different compositions of hexane: ethylacetate fractions. The known compounds were confirmed with the help of previous literature and published spectral data.

Aim and Objectives

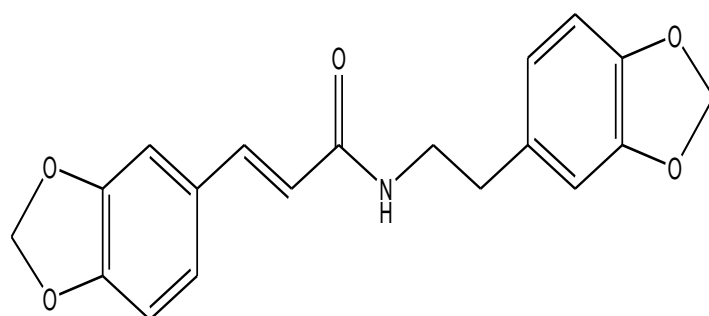
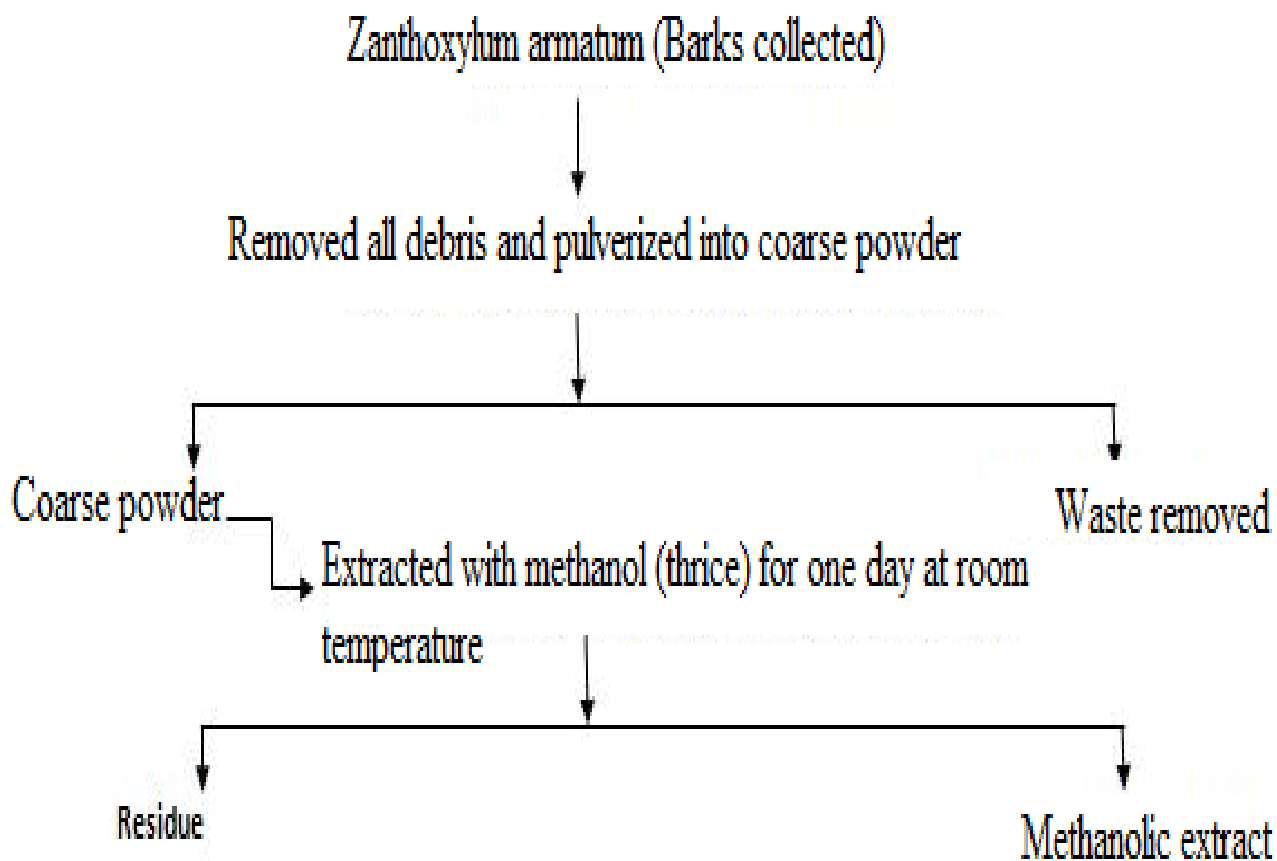
The aim of the current research is to carryout the extraction process of bark of *Zanthoxylum armatum* with methanol and to isolate different components from the extract with the help of column chromatography and to screen antimicrobial and antimycobacterial activity of the two lignans isolated.

MATERIALS AND METHODS

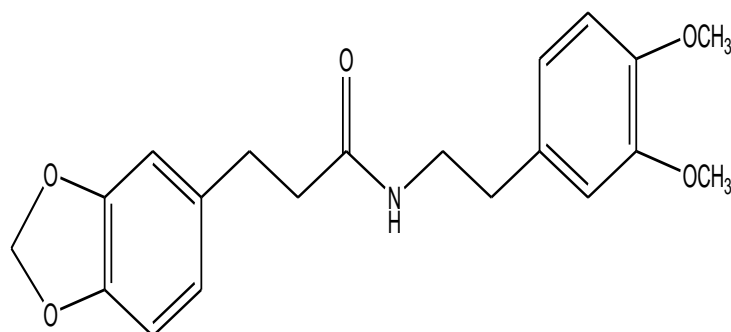
Collection and Authentication of Plant Material

The bark of the plant, *Zanthoxylum armatum* growing in

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N-(3',4'-Methylene dioxy phenyl ethyl)-3,4-methylene dioxy cinnamoyl amide.



N-(3',4'-dimethoxy phenyl ethyl)-3,4-methylene dioxy dihydro cinnamoyl amide.

the local areas of Visakhapatnam of Andhra Pradesh state were collected during the month of October. It was identified and authenticated by Dr. Venkaiah, Dept. of Botany, Andhra University and Sample specimen was kept in our laboratory for future reference. The bark was washed thoroughly, initially with tap water and then with distilled water to remove any debris or dust particles and then allowed to dry in shade. The dried plant material was ground to fine powder and stored at room temperature in air tight container until used further.

Preparation of Plant Extract

To 500g of *Zanthoxylum armatum* bark powder, 1500ml of each solvent, viz. methanol was added for preparing the extract. Extraction with the solvent was done for 24 hrs at room temperature, after which the supernatant of each solvent was recovered by filtering through whatmann filter paper. This process was repeated thrice and the respective solvent from the supernatant was evaporated in a rotavapor to obtain crude extracts which are to be stored at 4⁰c until used for evaluation.

Characterization of the two isolated lignans

N-(3¹,4¹-Methylenedioxyphenylethyl)-3,4-methylenedioxy-cinnamoyl amide, is a white crystalline compound, which was obtained from hexane: ethylacetate fraction (10% polarity) from methanol extract of bark. The compound was analyzed for the molecular formula of C₁₉H₁₈NO₅. gave a molecular ion at (m/z) 339 Consistent with the molecular formula. The compound displayed characteristic absorption bands in IR spectrum at 3446(N-H), 2850-2902 (Doublet due to CH₂ of Methylene dioxy), 1250 (C-O-C) and 1606 (C=O). In the ¹HNMR spectrum(400 MHz,CDCl₃) showed a characteristic signals at δ 5.93(S,4H,J=12) integrating for four protons, characteristic for two methylenedioxy groups. The presence of methylene dioxy groups in the compound is further supported by a positive Labat test (Green colour).Two Doublets at δ 4.65(d,1H,J=4) and δ 4.20(d,1H,J=20) each integrating for one proton attributed to the protons present at C₇ and C₈ respectively. Two multiplets were also observed. one at δ 3.0(m,2H,J=4.8) and the other at δ 3.80(m,2H,J=14.4) each again integrating for two protons which can be attributed to the two methylene groups adjacent to the nitrogen. The spectrum also showed the aromatic region in between δ 6.70- δ 6.90(s, 6H, J=15.5) integrating for six protons

which accounts for the six aromatic protons present in the compound.

Based on the structural information obtained and also based on the literature data the compound was identified as:

N-(3¹,4¹-Methylenedioxyphenylethyl)-3,4-methylenedioxy-cinnamoyl amide. The structure is well illustrated in figure I.

N-(3¹,4¹-dimethoxyphenylethyl)-3,4-methylenedioxy-dihydrocinnamoyl amide is a white crystalline compound, which was obtained from hexane:ethylacetate fraction (25% polarity) from methanol extract of bark. The compound was analyzed for the molecular formula of C₂₀H₂₃NO₅. and gave a molecular ion at (m/z) 357 Consistent with the molecular formula. The compound displayed characteristic absorption bands in IR spectrum at 3434(N-H), 2854-2924 (Doublet due to CH₂ of Methylene dioxy), 1243 (C-O-C) and 1741 (C=O). In the ¹HNMR spectrum (400 MHz,CDCl₃) showed a characteristic signals at δ 5.95(S,2H,J=12) integrating for two protons, characteristic for methylenedioxy groups. The presence of methylene dioxy groups in the compound is further supported by a positive Labat test(Green colour). The spectrum also revealed two methoxyls one at δ 3.90 and the other at δ 3.83.A triplet and multiplet at δ 4.65(t,2H,J=4) and δ 4.20(d,2H,J=20) each integrating for two proton attributed to the protons present at C₇ and C₈ respectively. Two multiplets were also observed,one at δ 3.0(m,2H,J=4.8) and the other at δ 3.82(m,2H,J=14.40) each again integrating for two protons which can be attributed to the two methylene groups adjacent to the nitrogen. The spectrum also showed the aromatic region in between δ 6.70- δ 6.90(s,6H,J=15.5) integrating for six protons which accounts for the six aromatic protons present in the compound. Based on the structural information obtained and also based on the literature data the compound was identified as: N-(3¹,4¹-dimethoxy phenyl ethyl)-3,4-methylenedioxy-dihydrocinnamoyl amide. The structure is well illustrated in figure 2.

Biological Evaluation

Antibacterial activity²³

Antibacterial activity was carried out on Gram positive micro organisms *Staphylococcus aureus* and Gram negative micro organisms *Escherichia coli*. The results depicted that the two compounds (Za-04 & Za-05) have shown better inhibition on gram positive rather than gram negative microorganisms. Za-05 has shown better anti

Table 1: Antimicrobial activity of the two lignans isolated from bark extract of *zanthoxylum armatum*.

S. No	COMPOUND (Code)	Zone Of Inhibition in mm			
		Bacteria <i>Staphylococcus</i> (Gram +ve)	<i>aureus</i>	<i>Escherichia</i> (Gram -ve)	Fungi <i>coli</i> <i>Aspergillus niger</i>
1	Za-04	12		12	09
2	Za-05	13		15	12
3	Benzyl (100µg/ml)	20	penicillin	22	--
4	Fluconazole (100µg/ml)	--		--	25

All the figures given in the table are inhibition zone diameters measured in mm.

Table 2: Antitubercular activity of the two lignans isolated from bark extract of *zanthoxylum armatum*.

S No	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1	Za-04	S	S	S	R	R	R	R	R
2	Za-05	S	S	S	S	R	R	R	R
3	Pyrazinamide	S	S	S	S	S	S	R	R
4	Streptomycin	S	S	S	S	S	R	R	R
5	Ciprofloxacin	S	S	S	S	S	S	R	R

bacterial activity when compared to Za-04. the results of antifungal activity revealed that the two compounds have shown significant anti fungal activity when compared to standard Ketoconazole. Among the two compounds Za-05 was found to have better antifungal activity shown by zone of inhibition.

Antifungal activity²⁴

The above compounds tested for antibacterial activity, were also studied for their antifungal activity. The antifungal activity was also tested by the same procedure describe above using yeast extract and malt extract (YEME) agar medium. The test organism used was *Aspergillus niger* (AN) and Ketoconazole was used as standard.

The results of antibacterial and anti fungal are well illustrated in Table 1.

Antitubercular activity³²

Micro Plate Alamar Blue Assay (MABA).

The antitubercular activity of the synthesized compounds was determined using the MABA method as analytical method. Briefly, 200µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middle brook 7H9 broth and serial dilutions of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml. plates were covered and sealed with Para film and incubated at 37°C for five days. After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from pink to blue. The efficacy of the compounds was compared by repeating the procedure with the standard first line drugs. The results were well illustrated in Table 2.

Strain used: *M.tuberculosis*(H37 RV strain)

Standard values for the Anti-Tb test which was performed.

Pyrazinamide- 3.125µg/ml

Streptomycin- 6.25µg/ml

Ciprofloxacin-3.125µg/ml

RESULTS AND DISCUSSIONS

The Extract shows Significant inhibition on gram positive organism *Staphylococcus aureus* and shows moderate inhibition on gram negative organism *Escherishia coli*. The bark extract of *zanthoxylum armatum* shows more inhibition zone on *Staphylococcus aureus*, when compared to remaining test organism, but the inhibition zones of extract on different organisms was less when compared

with standard inhibition zone. The methanolic extract of bark of *zanthoxylum armatum* shows significant antifungal activity against *Aspergillus niger*. The chemical components of bark extract of *Z.armatum* inhibits the mycelial growth of test fungus. organisms. The inhibition zones of extract on fungal mycelia was less when compared with standard inhibition zone.

Anti tubercular activity of the two compounds also showed significant results when compared with that of the standard. The two compounds showed resistance at concentrations of 25µg/ml and 12.5µg/ml against *mycobacterium* respectively. But the two compounds showed less activity than that of standard which are used for screening.

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