Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2017; 9(3); 395-399

DOI number: 10.25258/phyto.v9i2.8091

ISSN: 0975-4873

Research Article

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

Arun Satyadev Siddhanadham[1*], Rajendra Prasad Yejella[1], Rajkumar Prava[1], Jayapal Reddy Sama[2], Aparna Koduru[1]

[1]AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India.
[2] Department of pharmacognosy, Manipal college of pharmaceutical sciences, Manipal university, Manipal, India

Received: 21st Feb, 17; Revised: 15th March, 17; Accepted: 20th March, 17 Available Online: 25th March, 2017

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 H^1 NMR spectra. Compound Za-04 was found to be N-(3^1,4^1-Methylenedioxyphenyl ethyl)-3,4-methylenedioxycinnamoyl amide and Compound Za-05 was found to be N-(3^1,4^1-dimethoxyphenylethyl)-3,4-methylenedioxydihydrocinnamoyl 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 histocompatability, 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 capitates, fruit is schizocarp subglobose[19]. A temperate and subtropical genus, comprising 20-30 species distributed in E.Asia, Phillipinese. Malasiya and N.America. Most *Zanthoxylum* species produce pungent alkamides derived from polyunsaturated carboxylic acids, which are stored in the pericarp (fruit wall, shell) but not in the seeds. The exact nature of these alkamides may vary from species to species[20].

Zanthoxylum armatum DC [syn. Z. alatum Roxb.] (*Rutaceae)* is extensively used in the Indian system of medicines as a carminative, stomachic, and anthelminthic[21]. 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 roundworms[22]. 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

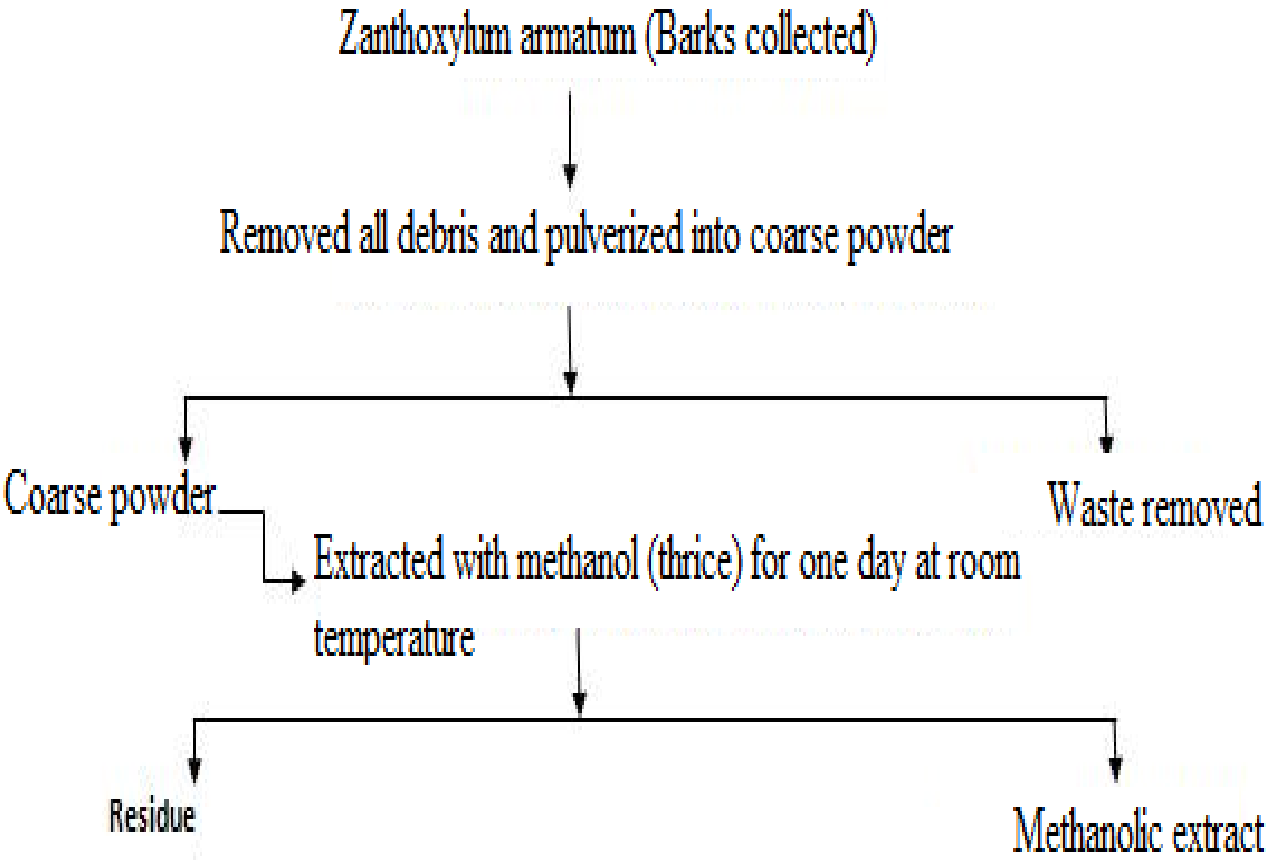
The aim of the current research is to carryout the extraction process of bark of *Zanthoxylum armatum* with methanol and to isolate different components from the extract with the help of column chromatography and to screen antimicrobial and antimycobacterial activity of the two lignans isolated.

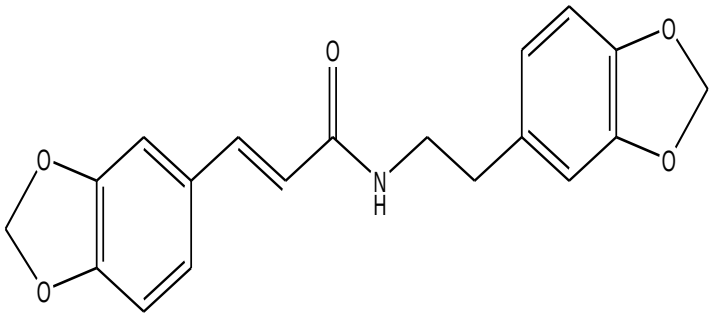
MATERIALS AND METHODS

Collection and Authentication of Plant Material

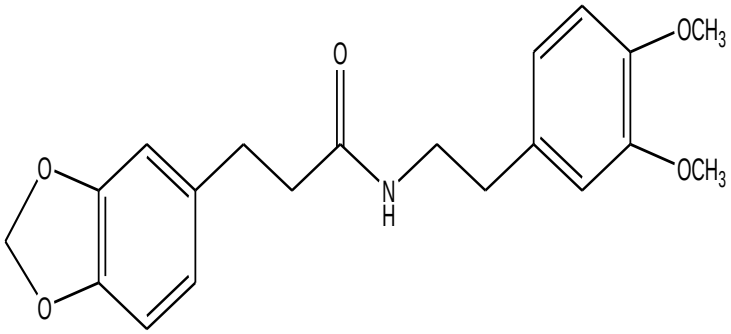
The bark of the plant, *Zanthoxylum armatum* growing in

**Author for Correspondence: arun.satyadev673@gmail.com.*





N-(3[1],4[1]-Methylene dioxy phenyl ethyl)-3,4-methylene dioxy cinnamoyl amide.



N-(3[1],4[1]-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 4^0c until used for evaluation.

Characterization of the two isolated lignans

N-(3^1,4^1-Methylenedioxyphenylethyl)-3,4-methylenedioxycinnamoyl 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 $C_{19}H_{18}NO_5$. 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 CH_2 of Methylene dioxy), 1250 (C-O-C) and 1606 (C=O). In the ^{1}HNMR spectrum(400 MHz,$CDCl_3$) 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 C_7 and C_8 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-(3^1,4^1-Methylenedioxyphenylethyl)-3,4-methylenedioxycinnamoyl amide. The structure is well illustrated in figure I .

N-(3^1,4^1-dimethoxyphenylethyl)-3,4-methylenedioxydihydrocinnamoyl 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 $C_{20}H_{23}NO_5$. 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 CH_2 of Methylene dioxy), 1243 (C-O-C) and 1741 (C=O). In the ^{1}HNMR spectrum (400 MHz,$CDCl_3$) 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 C_7 and C_8 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-(3^1,4^1-dimethoxy phenyl ethyl)-3,4-methylenedioxydihydrocinnamoyl amide. The structure is well illustrated in figure 2.

Biological Evalution

Antibacterial activity[23]

Antibacterial activity was carried out on Gram positive micro organisms *Straphylococcus aureus* and Gram negative micro organisms *Escherechia 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		Fungi
		Staphylococcus aureus (Gram +ve)	*Escherichia coli* (Gram -ve)	*Aspergillus niger*
1	Za-04	12	12	09
2	Za-05	13	15	12
3	Benzyl penicillin (100µg/ml)	20	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 activity[24]

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 activity[32]

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 deionzed 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 37ºC 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.

ACKNOWLEDGEMENT

The first author grateful to Prof. Y.RAJENDRA PRASAD, Dept.of pharmaceutical chemistry Andhra University for his valuable guidance, also Dr.M.Murali Krishna and Dr.Girija sastry for their constant encouragement and support. The author is thankful to Dr. M. Venkaiah Dept. of Botany, Andhra University for the identification of plant *zanthoxylum armatum.*

REFERENCES

1. ACS *Symposium. Series*, Washington DC 1993, 534 P-2.
2. Neil, M.O, and lewis, J.A. In "Human Medicinal Agents from plants" Ed by kinghorn, A.D. and Balandrim, M.F., ACS *symposium series.*, Washington, D.C 1993, 534 P-48.
3. Hostettmann, K. Bull, *Soc. Frib. Sc. Nat*, 1987, 76, 51.
4. Farnworth, N.R. Akerela, O.Bingel, A.S. Soejar to, D.D. and Guo. Z. Bull, W.H.O, 1989, 63, 965.
5. Cragg, G.M., Boyd, M.R., Cardellina, J. H., Grever, M.In: "Human Medicianl Agents from plants" Ed by kinghorn, A.D. and Balandrim. M.F., ACS *Symposium series*, Washington, D.C, 1993, 534, P-80.
6. Jardine, I. In; "Anticancer Agents based on natural product Models" Ed by Cassady, JM and Douros, J.D., Academic press, New York, 1980, P-319.
7. Kingston, D.G. I. In: "Human Medicinal Agents from Plants" Ed by Kinghorn A.D and Balandrim M.F., ACS *Syonposium series*, Washington; DC. 1993 534, P – 48.
8. Khan, H.A., Chandrasekharan, I., Ghanin A., *Phytochemistry*, 1986, 25, 767-768.
9. Verkov J & Sedmera P. *Photochemistry* 1972, 11, 2647.
10. Chibbar, S.S., Butt, S.K., Sharma, R.P. and Sharma, A., *Indian J. Chem*, 1981, 20 B, 626.

11. Smalberger, T.M., Vleggar, R. and Weber, J.C., *Tetrahedron*, 1974 30, 3927.
12. Balandrin, M.F., Kinghorn, A.D & Farmsworth, N.R., In: "Human Medicinal Agents from Plants" Ed By kinghorn, A.D & Balandrin, M.F., Carter, S.K. and Livingston, R.B. *Cancer Treat rep* 1976, 60, 1141.
13. Shirley. J. Morris and R.H. Thompson, *Tetrahedron* letters, 1963, 45, 264-265.
14. J.B. Harbone, "Comparative Biochemistry of Flavonoids", *Academic press*, Londan, 1967.
15. C.H. Ile and S.H. Wender , *J. American, Chemical Society* 1953, 75, 117.
16. Hussain, S. F.; Gozler, B.; Shamma, M.; Gozler, T. Phytochemistry 1982, 22, 2979-2980.
17. Simeray, J.; Chanmont, J. P.; Bevalot, E.; Vaquette, J. *Phytochemistry*1985, *24*, 2720-2721.
18. Horhammer, L and Hansel, R. Arch. Pharm, 1953, 286, 425.
19. C.W, Wilson, *J. American, Chemical Society* 1936, 61, 2303.
20. Shah NC (1991): Chemical composition of the pericarps oil of Zanthoxylum armatum DC. J Essent Oil Res 3: 467-468.)
21. L.R. Row, eft.al., *Proc. Indian Academic Science* 1945, 22A, 215.
22. Dillard, H.R., A.C. CobandJ.S.Lombay.1998. Plant Disease, An International Journal of Applied Plant Pathology: 153-157.
23. J. Shinoda, *J. Pharm. Society* Japan 1928, 48, 214.
24. J.C. Pew, *J. Am Chem*. 1948, 90, 3031.