Research Article

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PHYTOCHEMICAL AND BIOLOGICAL EXAMINATION OF THE ROOT EXTRACT OF *ARTOCARPUS HIRSUTA*, LAM.

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Abstract: The pantropical genus *Artocarpus* have been extensively investigated and is well-known to be rich in isoprenylated phenolic secondary metabolites. In the present study the chemical examination of *Artocarpus hirsuta* roots, on conventional extraction and various chromatographic methods led to the isolaton of stigmasterol, lupeol, cyclomorusin, cycloartomunin and betulinic acid. All the compounds were characterized by 2D NMR and MS spectral data and comparison with published data for the known compounds. The compound cyclomorusin is new to the genus *Artocarpus* and all the compounds were reported for the first time from the roots of this species. The work was further extended to test the crude extracts for antibacterial and antifungal activities. The results from the present study have shown that the species have considerable activity against selected bacterial and fungal strains which can be attributed to the presence of steroidal and phenolic compounds in the crude extracts of *Artocarpus hirsuta*.

Keywords: Prenylated flavonoids, *Artocarpus hirsuta*, Antibacterial activity, Antifungal activity

INTRODUCTION

Species of the family Moraceae have a long story of use in traditional medicine, agriculture, and industry (lumber and fabrics), especially in Asia. This plant family, which is distributed in the tropical and subtropical regions of Asia, comprises of around 1400 species divided among 60 genera (1,2). The genus *Artocarpus,* which comprises about 50 species, is native to South and South-East Asia, New Guinea, and the southern Pacific, distributed in Sri Lanka, India, Pakistan and Indo-China. (3), and are been used for their edible and succulent fruits. Many species are also being used as a source of good and durable timber and some of them are also used in traditional medicine preparations for the treatment of various diseases (4,5,6,7). *Artocarpus* species have been extensively investigated and are well-known to be rich sources of the isoprenylated phenolic secondary metabolites, including flavones, isoflavones, chalcones, xanthones, 2-arylbenzofurans, and stilbenes (8,9,10,11,12,13,14,15). Further many species of *Artocarpus* have been reported to exhibit activities like carcinogenic, anti-inflammatory, antioxidant; anti-bacterial, anti-fungal; anti-viral, anti-malarial, and pesticidal activities (16,17,18,19,20,21,22,23).

Artocarpus hirsuta Lam., is a very large ever green tree growing in the West Coast forests from sea level to 3,500 feet, extending from Annamali to Travancore (India). In the recent years, an abiding interest has been developed in these classes of natural products, as they possess an attractive combination of structural uniqueness, simplicity, and important biological activities. As part of an intensive and systematic study of the bioactive metabolites from moraceous plants, the present work has been carried out in *Artocarpus hirsuta*, since no previous phytochemical and antimicrobial investigation had been reported in the roots of this species.

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MATERIALS AND METHODS

Plant Material:

The roots of *Artocarpus hirsuta* were collected and authenticated by Prof. T. Pulliah, Taxonomist, Department of Botany, Sri Krishnadevaraya University, Anantapur, India. A voucher specimen has been deposited at the Herbarium, Department of Botany, Andhra University, Visakhapatnam, India. All chemicals and solvents used were of analytical grade and obtained from Ranbaxy Fine Chemicals and Merck Ltd., Mumbai.

Extraction and Isolation:

The roots of *A. hirsuta* (2kg) were air dried, powdered in a Willey mill and was later extracted with chloroform and subsequently concentrated under reduced pressure to get their corresponding residue (15gm.) under vaccum. The crude extract gave positive colour reactions with Ferric chloride solution (green colour) and Shinoda's test (orange color) indicating the presence of phenolic constituents especially flavonoids. It also gave Lieberman-Burchard reaction

for triterpenes and sterols. Hence, the extract (15gm.) was column chromatographed over silica gel (>100gm), eluting with solvents petroleum ether, benzene, chloroform and methanol mixtures in the order of polarity. Fractions of 250 ml each were collected and monitored on the TLC (Acme-Silica gel was used for thin layer chromatography) and the spots on chromatogram were detected under UV light (254 and 365 nm) and by spraying with 5% H_2SO_4 in methanol. The compounds stigmasterol, lupeol, cyclomorusin, cycloartomunin and betulinic acid were isolated and identified by chemical tests and spectral analysis.
Characterization of the compounds:

Stigmasterol:

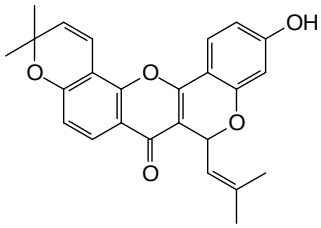
It was crystallized as colorless feathery needles from alcohol, m.p. 169-170, $[\alpha]_D^{30}$ (chloroform). It gave a play of colors in Lieberman-Burchard reaction characteristic of sterols. It was analysed for the formula C_{29} H_{48} O (M+412). The IR spectrum showed absorption bands at: 3450 (OH), 1170, 1132, 1074, 991, 971 cm (Trans double bond). It formed a monoacetate, C_{31} $H_{50}O_2$, m.p. 136-138°, $[\alpha]_D^{30}$-39.2 (chloroform) and a mono benzoate, C36 H_{52} O_2, m.p. 149-153, (α) -39.2 (chloroform). Thus the evidence obtained through IR and mass spectral data, pointed out that the sterol may be stigmasterol and its identity was confirmed by comparison with an authentic sample and through super imposable IR.

Lupeol:

It was crystallized from chloroform- methanol as needles, m.p. 212-213°, $[\alpha]_D^{30}$ +24° (chloroform) and analyzed for the formula $C_{30}H_{50}O$. It gave pink color in Lieberman Burchard reaction and yellow color with tetranitromethane. The IR spectra showed bands at 3540 (-OH), 1380and 1390(gem dimethyl) and at 890 cm^{-1} (venylmethlenes). It formed a monoacetate, m.p.214°, $[\alpha]_D^{30}$ +26°(chloroform), ^{1}HNMR exhibited signals at (90 MHz, CDCl3, ó), o. .78, 0.80, 0.83, 0.90 and 1.02 (18H, s, 6/Me) 1.63 (3H, s, CH3-c'=CH2), 2.25 (1H, *d*, 19-), 3.15(IH, *m*, 3α-H), 4.51(2H, *d*, CH2). The above data was in good agreement with that of lupeol and the identity was confirmed by comparison with an authentic lupeol (mmp and co-TLC).

Cyclomorusin:

It was obtained as yellow crystals from methanol, m.p 242-244°C, $[\alpha]_{30}^{D}$ +20° (C= 0.01 in methanol). The compound showed UV absorptions at 223 (4.4), 225 (4.29), 283(4.42), 383 (4.18) nm. It showed IR bands at 3500, 1660, 1620 and 1590 cm^{-1}. The ^{1}HNMR in $(CD_3)_2$ Co,90 MH_z) showed signals at δ 1.59 (64,S, c-14-$(CH_3)_2$), 1.72, 1.98 (each 3H,S,C-H,CH_3 x2),5.49(1H *d*,*J*=10H_2, C-104), 5.78 (1H,*d*, *J*= 10 H_2, C-13H), 6.17 (1H, *s*,C-64),6.24(1H,*d*,*J*=10H_2,C-9H), 6.45(1H,*d*,*J*=2H_2, C-3'H), 6.67 (1H,*dd*, *J*=2 and 9H_2,C-5'H), 6.95(1H, *d*, *J*=10H_2,C-12H), 7.82(1H,*d*,*J* =9H_2,C-6'H). This data suggested that the compound was cyclomorusin, It was further confirmed by comparison with the authentic sample, Co.TLC and m.m.p.



Cyclomorusin

Cycloartomunin:

It was obtained as yellow needles from methanol m.p.278-280°. It showed olive green color with Ferric chlonride and positive (orange color) with Shinoda's test. The UV spectrum showed absorptions at UV_{max}^{MeOH} nm (logЄ):211 (4.72), 280(4, 28), 400 (3.65). The ^{1}HNMR showed two chromenmetho groups at δ1.45 and two doublets at 85.58 and δ5.76(J=10Hz) indicated the presence of a 2.2- diamethylchromen moiety. Two vinyl methyls at δ1.69 and 1.97 together with two doublets (I=9Hz) at δ5.46 (H-12) and 5.21 (H-11) suggested that 2^1 – hydroxyl group of the 'B'ring in the flavonoid has been oxidativelyclyclized with the allylic methylene of a prenyl chain in the 3- position. The spectrum also showed three singlets at δ6.24, 6.49 and 7.27 assigned to the protons located at C-C, C-3^1 and C-C^1, a methoxyl signal at δ3.91 and a chelated hydroxyl at δ12.80. Based on the data together with the presence of a bathochromic shift with $AlCl_3$ and the absence of bathochromic shift with NAOAc-H_3 BO3 and NAO Me indicated that the compound was cycloartomunin.

Betulinic Acid:

It was crystallized from chloroform-methanol as shining silky needles, m.p. 294-296°, $[\alpha]_D^{30}$ +8.5° (chloroform) and analyzed for the formula CHO. It gave pink colour in L.B. test. IR spectrum showed absorptions at 3460(-OH), 1690 (carbonyl of COOH), 1640 (double bond), 1380 and 1390 cm (gem. dimethyl). It formed a mono acetate, m.p. 287- 290°, $[\alpha]_{30}^{D}$ +12.2 (chloroform), a methyl ester acetate, with AC O/Py, m.p. 195- 198°, (α) +14.2 (chloroform). The HNMR data of the methyl ester acetate is given in the following Table 1.

Table.1: H^1 NMR data of the methyl ester acetate

Chemical Shift(δ)	Proton integration	Multiplicity	Assignment
0.83-1.60	15H	S	5/CH 3
1.70	3H	S	C=C-CH3
1.96	3H	S	OCOCH3
3.60	3H	S	COOCH3
4.65	2H	D J=8H2	Vinyl protons

The above data agreed well with that of betulinic acid and the identity was further confirmed by comparison with an authentic sample, (mmp and co-TLCo).

Biological Studies:

Some of the *Artocarpus* species were found to contain antibacterial and antifungal activities. Species of *Artocarpus, A. heterophyllus, A. communis, A. lakoocha, A. nobilis, A., champedon and A. rotunda* (24, 25) exhibited proven antimicrobial activity. Hence, the author has attempted to study the antimicrobial activity of the chloroform extracts of the roots of *A. hirsuta*.

Antibacterial activity:

Test Samples: Antimicrobial activity was carried out by the cup- plate agar diffusion method (26). The chloroform extract of the roots of *A. hirsuta* were used in two dose levels of 100mg/ml and 300mg/ml.

Test Organisms: For testing the antibacterial activity, the following *Gram (+) ve* (*Bacillus subtilis, Bacillus pumilis, Streptococcus pyogenes, Micrococcus luteus*) and *Gram (-) ve* (*Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Enterococcus faecalis*) bacterial strains were selected.

Procedure: The Nutrient medium prepared was inoculated with 18 hours old cultures of the above mentioned test organisms and were transferred into sterile 15cm diameter petridishes. The medium in the plates were allowed to set at room temperature for about 10 minutes and allowed to solidify in a refrigerator for about 30 minutes. 5 cups of 5mm diameter were made in each plate at equal distance. Stock solutions of the test residual extract were prepared in concentrations of 100mg/ml and 300mg/ml. 100µg/ml of each concentration were placed in the cups by means of sterile pipettes. In each plate one cup was used for control and standard. Antibiotic Benzyl penicillin (100µg/ml) was used as standard. The petridishes thus prepared were incubated for 16hrs at 30°C and were later examined by measuring the zones of inhibition. The experiments were run in duplicate and the average diameter of the zones of inhibition was recorded and the results were tabulated in the table.2.

Table.2: Antibacterial activity of chloroform extract of the roots of *Artocarpus hirsuta*

Extract/Compound	Gram +ve				Gram-ve			
	B.S.	*B.P.*	*S.P.*	*M.L.*	*E.C.*	*K.P.*	*P.V.*	*E.F*
AHR (100mg/ml)	13	20	15	15	21	16	15	14
AHR (300mg/ml)	13	20	15	15	21	16	16	16
AHR (0.5µg/ml)	-	-	-	-	-	7	7	-
AHR (1.0µg/ml)	-	-	11	11	-	10	7	-
Benzyl pencillin (19µg/ml)	36	32	34	32	36	36	34	38

(Cup diameter = 6mm, **AHR**-*Artocarpus hirsuta* root, ***B.S***-*Bacillus subtilis*, ***B.P***- *Bacillus pumilis*, ***S.P***-*Streptococcus pyogenes*, ***M.L.*** *Micrococcus luteus*, ***E.C***- *Eschericia coli*, ***K.P***- *Klebsiella pneumoniae*, ***P.V***- *Proteus vulgaris*, ***E.F***- *Enterococcus faecalis*), Zone of inhibition (mm)

Antifungal activity:

Test samples: The antifungal activity was assayed using Agar cup plate method. The chloroform extracts of the roots of *A. hirsuta* were used in two dose levels of 100mg/ml and 300mg/ml.

Test organisms: For testing antifungal activity the following fungal strains *Aspergillus fumigans, Pencillium excelsa, Pencillium chrysogenum, Saccharomyces ceriviseae* were used.

Procedure: The Nutrient PDA medium (Hi-media) was prepared and inoculated with 0.5 ml of aqueous suspension of the above mentioned test organisms, which were prepared from 48 hour cultures, are thus transferred into sterile petridishes. The medium in the plates were allowed to set at room temperature for about 10 minutes. 5 cups of 5mm diameter were made in each plate at equal distances. Stock solutions of the test residual extract were prepared in concentrations of 100mg/ml and 300mg/ml. 100µg/ml of each of the above stock concentrations were placed in the cups by means of sterile pipettes. In each plate one cup was used for control and standard. Nystatin (100µg/ml) is used as reference standard. The petridishes thus prepared were incubated for 48 hours at 37° C and were later examined by measuring the zones of inhibition. The experiments were run in duplicate and the average diameter of the zones of inhibition was recorded and the results were tabulated in the Table 3.

Table.3: Antifungal activity of chloroform extract of the roots of *A. hirsuta*

S.NO	Extracts	Zones of inhibition (mm)			
		A.F	*S.C*	*P.E*	*P.C*
1.	AHR-Chloroform extract (100mg/ml)	-	18	-	8
2.	AHR-Chloroform extract (300mg/ml)	-	22	-	8
3.	AHR- (0.5ug/ml)	-	10	-	-
4	AHR- (1.0ug/ml)	-	12	-	-
5	AHR- (0.5ug/ml)				
7	Nystatin (10µg/ml)	19	19	17	15

(Cup diameter: 6mm, ***AHR:*** *A.hirsuta* root extract, ***A.F***, *Aspergillus fumigans*, ***P.E*** *Pencillium excelsa*, ***P.C*** *Pencillium chrysogenum*, ***S.C***-*Saccharomyces ceriviseae*)

RESULTS AND DISCUSSION

The Phytochemical examination of *Artocarpus hirsuta* roots, on conventional extraction and various chromatographic methods yielded five compounds: stigmasterol, lupeol, cyclomorusin, cycloartomunin and betulinic acid. All the compounds were isolated for the first time from this species *Artocarpus hirsuta* and the Isolation of cyclomorusin is the first time report from the genus *Artocarpus*. The isolation of the above bioactive chemical constituents renders the medicinal significance of the species *Artocarpus hirsuta*, which are presently been the subject of human curiosity and need. Further the compounds such as morusin, morin, cyclomorusin were earlier reported from *Morus* species and the isolation of these compounds from *A. hirusta* is quite interesting and supports chemo taxonomically the relationship between *Morus* and *Artocarpus* belonging to the family Moraceae.

The root chloroform extracts of *A. hirusta* showed good activity against all the tested bacterial organisms. They exhibited maximum activity against *Bacillus pumilis* and *E. coli* when compared to other test organisms. They displayed promising activity against *Saccharomyces Cerevisiae* and showed no activity against rest of the fungal organisms. The activity was enhanced with increased dosage levels. The antimicrobial properties exhibited by the plant could be due to the presence of flavonoids and sterols which are conspicuous constituents of the species belonging to the Moraceae family.

REFERENCES

1. Jarrett FM, *Studies in Artocarpus and allied genera, IV. A Revision of Artocarpus subgenus Pseudojaca*, *J. Arnold Arbo*., 1960, 73–320.
2. Kochummen KM, Go R, Artocarpus JR. & G. Forster. In: Soepadmo E, Saw LG (eds) Tree Flora flora of Sabah and Sarawak, vol 3. Sabah Forestry Department, Forest Research, Institute Malaysia and Sarawak Forestry Department, Kuala Lumpur, Malaysia, 2000, p 187.
3. Mabberley DJ, The Plant-book, second ed. Cambridge University Press, Cambridge, 1997, 58.
4. Nomura T, *Phenolic compounds of the mulberry tree and related plants*, *Fortschr. Chem. Org. Naturst.*, 1988, 64, 87–201.
5. Nomura T, and Hano Y, *Isoprenoid-substituted phenolic compounds of moraceous plants*, *Nat. Prod. Rep*., 1994, 11, 205–218.
6. Hakim EH, Achmad SA, Juliawaty LD, Makmur L, SyahYM, Aimi N, Kitajima M, Takayama H, Ghisalberti EL, Prenylated flavonoids and related compounds of Indonesian *Artocarpus* (Moraceae). *J Nat Med*, 2006, 60,161–184.
7. Wang Y, Xu K, Lin L, Pan Y, Zheng X, Geranyl flavonoids from leaves of *Artocarpus altilis, Phytochemistry*, 2007, 68, 1300–1306.
8. Boonlakshmi C, Oonant W, Kongsaree P, Kittakoop P, Tanticharoen M, Thebtaranonth Y, An antimalarial stilbene from *Artocarpus integer, Phytochemistry*, 2000, 54 (4), 415-417.
9. Achmad SA, Emilo NA, Ghisalberti L, Hakim EH, Kitajima M, Makur L, Mujahidin D, Syah YM, Takayama H, Molecular diversity and biological activity of natural products from Indonesian Moraceous plants, *J. of the chem.soc.of Pakistan*, 2004, 26 (3), 316-332.
10. Jayasinghe ULB, Samarakoon TB, Kumarihamy BMM, Hara N, Fujimoto Y , Four new prenylated flavonoids and xanthones from the root bark of *Artocarpus nobilis, Fitoterapia* ,2008,79,37–41.
11. Syah YM, Achmad SA, Ghisalberti EL, Hakim EH, Mujahidin D, Two new cytotoxic isoprenylated flavones, artoindonesianins U and V, from the heartwood of *Artocarpus champeden, Fitoterapia,* 2004, 75,134–140.
12. Nomura T, The chemistry and biosynthesis of isoprenylated flavonoids from moraceous plants, *Pure Appl Chem*, 1999, 71, 1115–1118.
13. Jagtap UB, Bapa, VA, *J. Ethnopharmcol*., 2010, 129, 142.
14. Hashim N, Rahmani M, Sukari MA, Ali AM, Alitheen NB, Go R,Ismail HBM, Two new xanthones from *Artocarpus obtusus, J Asian Nat Prod Res*, 2010, 12, 106–112.
15. Shireen Shaharina Shamaun, Mawardi Rahmani, Najihah Mohd Hashim, Hazar Bebe Mohd Ismail, Mohd Aspollah Sukari, Gwendoline Ee Cheng Lian, Rusea Go, Prenylated flavones from *Artocarpus altilis* *J Nat Med*, 2010, 64,478–481.
16. Ko FN, Cheng ZJ, Lin CN, Teng CM, Scavenger andantioxidant properties of prenylflavones isolated from *Artocarpus heterophyllus*. *Free Radic Biol Med*, 1998, 25, 160–168.
17. Chung Mei- Ing, Ko Horng-Huey, Yen Ming-Hong, Lin Chun-Nan, Yang Sheng-Zehu, Tsao Lo-Ti, Wang Jih-Pyang, Artocarpol A, a novel constituent with potent anti-inflammatory effect, isolated from *A.rigida, Helvitica Chimica Acta,* 2000 ,83 (b), 1200-1204.
18. Cidade Honorina M, Nasimento Maria Sao Jose, Pinto Madalena MM, Kijjoa Anake Silva, Artur MS, Herz Werner, Artelastocarpin and carpelastofuran, two new flavones from *Artocarpus elasticus* against three cancer cell lines, *Planta Medica,* 2001, 67, (9), 867-870.
19. Hakim EH, Asnizar Y, Aimi N, Kitajima M, Takayama H, Artoindonesianin P, A new prenylated flavone with cytotoxic activity from *Artocarpus lanceifolius*. *Fitoterapia,* 2002, 73, 668–673.
20. Hakim EH, Makmur L, Achmad SA, Aimi N, Ghisalberti EL, Kitajima M, Mujahidin D, Syah YM, Takayama H, *Recent studies on biologically active natural products from Artocarpus species of Indonesian rainforest*, In Atta-ur-Rahman, M.I. Chouidhary and K.M. Khan (Eds.), Natural Product Chemistry at the Turn of the Century, Prints Arts, Karachi, 2002, pp. 331–338.
21. Wang YH, Hou AJ, Chen L, Chen DF, Sun HD, Zhao QS, Bastow KF, Nakanishi Y, Wang XH, Lee KH, New isoprenylated flavones, artochamins A-E, and cytotoxic principles From Artocarpus chama, *J Nat Prod,* 2004 , 67,757–761.
22. Wei Bai-Luh, Weng Jing- Ru, Chin Pao-Hui, Hung Chi-Feng, Wang Jih-Pyang, Lin Chun-Nan, Anti-inflammatory Flavonoids from *Artocarpus heterophyllus* and *A. communis, J. of Agri. and Food Chemistry*, 2005, 53(10), 3867- 3871.
23. Arung ET, Muladi S, Sukaton E, Shimizu K, Kondo R , Artocarpin, a promising compound as whitening agents and anti skin cancer, *J Trop Wood Sci Technol*, 2008, 6,33–36.
24. Khan MR, Omoloso AD, Kihara M, Antibacterial activity of *Artocarpus* heterophyllus, *Fitoterapia*, 2003, 74, 501-503.
25. Seo Eun- Kyouhg, Lee Daugho, Shin Young Genn, Bioactive prenylated flavonoids from the stem bark of *A. kemendo, Archives of pharmacal* Research, 2003, 26 (2), 124-127.
26. Kavanagh F., Analytical Microbiology-II, Academic Press, New York, 1992, 241-243.

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