Journal of Pharmacognosy
ISSN: 0976-884X & E-ISSN: 0976-8858, Volume 3, Issue 2, 2012, pp.-51-54.
Available online at http://www.bioinfo.in/contents.php?id=70

ANTIBACTERIAL AND ANTIFUNGAL STUDIES OF PRENYLATED ISOFLAVONES AND PRENYLATED 3-ARYL COUMARINS ISOLATED FROM *Derris scandens* BENTH.

SRIDHAR RAO A.[1,2]*, RAMCHANDER MERUGU[1] AND MADHUSUDANA RAO J.[2]

[1]Mahatma Gandhi University, Nalgonda-508001, AP, India.
[2]Indian Institute of Chemical Technology, Uppal Road, Hyderabad-500 607, India.
*Corresponding Author: Email- asrao@gmail.com

Received: March 21, 2012; Accepted: April 09, 2012

Abstract- A bioassay-guided fractionation and chemical investigation of the petroleum ether and chloroform extracts of the stem of *Derris scandens* resulted in the isolation of fourteen prenylated isoflavones, prenylated 3-aryl coumarins (1-14). In isolated compounds 4'-O-methyl osajin (1), osajin (2) scandenone (5), scandinone (6), Derrisisoflavone (10), scandenin A (12) and scandenin (13) displayed good antibacterial activity. The results of the present work are discussed in the light of existing literature.
Keywords- *Derris scandens*, *Leguminoceae*; antibacterial; antifungal activity, prenylated isoflavones, prenylated 3-aryl coumarins

Citation: Sridhar Rao A., Ramchander Merugu and Madhusudana Rao J. (2012) Antibacterial and antifungal studies of prenylated isoflavones and prenylated 3-aryl coumarins isolated from *Derris scandens* Benth. Journal of Pharmacognosy, ISSN: 0976-884X & E-ISSN: 0976 -8858, Volume 3, Issue 2, pp.-51-54.

Introduction

D. scandens Benth. (Leguminaceae), is a well known asian medicinal plant. It is a large climbing shrub with drooping branches and is distributed in the sub himalayan tract from Oudh eastwards to Assam, and in central and south India to Andamans. It is cultivated in gardens for its graceful recaemes from seeds[1]. This plant is reported to posses' antiinflammatory, antihypertensive, and imunomodulatory and anti-HIV properties[2-4]. *D. scandens* dried stem has been used as an expectorant, anti-tussive, diuretic and anti-dysentery agent and for the treatment of muscle aches and pains[5]. A hydro alcoholic extract of the stem was reported to have both antimicrobial[6] and immunostimulating activities[7]. In pharmacological study, the polar fraction when applied resulted in a marked decrease in blood pressure and heart rate[8]. Coumarins and isoflavones and isoflavone glycosides have also been previously reported as chemical constituents of the stem of *D. scandens*[9-15]. We have isolated several isoflavones and 3-phenyl coumarins from the petroleum ether and chloroform extracts of *D. scandens*. Antibacterial and antifungal activity of some of the petroleum ether extracted and chloroform extracted compounds were investigated as they have not been investigated earlier.

The whole plant *D. scandens* was sequentially extracted with petroleum ether and chloroform. The petroleum ether extract was repeatedly chromatographed over silica gel using increasing polarities of hexane and ethyl acetate to yield four compounds, 4'-O-methyl osajin (1), osajin (2), 4'-O-methylscandinone (3) and 4', 5, 7-trihydroxy biphenyl isoflavone (4), scandenone (5), scandinone (6), isoscandinone (7) lupalbigenin (8), derrisisoflavone A (9), derrisisoflavone C (10), 4', 4-0-dimethylscandenin (11), scandenin A (12), scandenin (13), and scandenin B (14)[16-18] (Fig. 1).

Materials and Methods

The shade dried plant material (2 kg) was powdered and extracted with hexane in a Soxhlet apparatus for 24 h. The solvent was evaporated under reduced pressure in a rotary flash evaporator to obtain a residue (20 g). The residue was adsorbed on silica gel and subjected to column chromatography over silica gel (100 cm×40 mm, diametre). The column was subjected to elution with hexane first followed by mixture containing increasing amounts of acetone. The fractions eluted at 2% (1.5 L), 4% (3.75 L), 6% (1.5 L) and 10% (3.0 L) were collected separately and concentrated to obtain residues A (0.310 g), B (13.10 g), C (0.645 g) and D (4.72 g). The residue A (0.31 g) was loaded on a silica gel column (60-

120 mesh, 50 cm×10 mm, diametre) and eluted with 2% ethyl acetate in hexane to obtain compound 1 (0.20 g). The residue B (13.10 g) was loaded on a silica gel column (60-120 mesh, 100 cm×25 mm, diametre) and eluted with 3% ethyl acetate in hexane to find compound 2 (12.0 g). The residue C (0.645 g) was loaded on a silica gel column (60-120 mesh, 30 cm×10 mm, diametre) and eluted with 5% ethyl acetate in hexane to get compound 3 (0.490 g). The residue D (4.72 g) was loaded on a silica gel column (60-120 mesh, 60 cm×20 mm, diametre) and eluted with 6% ethyl acetate in hexane to attain compound 4 (3.50 g). On completion of the hexane extraction, the powdered plant material was extracted with chloroform to obtain 100 g of residue. The extract was dissolved in a 1:1 mixture of chloroform and methanol. The residue was adsorbed on silica gel and subjected to column chromatography over silica gel (60-120 mesh, 150 cm×40 cm, diametre). The column was subjected to elution with hexane first followed by increasing polarities of hexane/acetone. The fractions eluted at 15% (4.5 L), 30% (2.75 L), 40% (1.5 L) and 50% (5.0 L) were collected separately and concentrated to obtain residues E (15.90 g), F (6.25 g), G (0.78 g) and H (23.0 g). The residue E (15.90 g) was loaded on a silica gel column (60-120 mesh, 100 cm×35 mm, diametre) and eluted with 10% ethyl acetate in hexane to yield compound 5 (0.80 g), elution with 12% ethyl acetate afforded compound 6 (6.0 g) and elution with 15% afforded compound 7 (7.0 g). The residue F (6.25 g) was loaded on a silica gel column (60-120 mesh, 50 cm×20 mm, diametre) and eluted with 15% ethyl acetate in hexane to get compound 8 (2.0 g) and compound 9 (1.5 g). Further elution of the column with 17% ethyl acetate afforded compound 10 (0.50 g). The residue G (0.78 g) was loaded on a silica gel column (60-120 mesh, 30 cm×15 mm, diametre) and eluted with 18% ethyl acetate in hexane to give compound 11 (0.295 g). Further elution of the column with 22% ethyl acetate led to the isolation of compound 12 (0.185). The residue H (23.0 g) was loaded on a silica gel column (60-120 mesh, 150 cm×40 mm, diametre) and eluted with 25% ethyl acetate in hexane to lead compound 13 (20.0 g). Further elution of the column with 35% afforded compound 14 (0.245 g).

Six test organisms, *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeruginosa* (MTCC 741), *Klebsiella aerogenes* (MTCC 39), *B. sphaericus* (MTCC 511) and *Chromobacterium violaceum* (MTCC 2656) were obtained from the Institute of Microbial Technology, Chandigarh. Cultures of test organisms were maintained on nutrient agar slants and were subcultured in Petri dishes prior to testing.

Two test organisms *Rhizopus oryzae* (MTCC 262) and *Aspergillus niger* (MTCC 281) were obtained from the Institute of Microbial Technology, Chandigarh. Cultures of test organisms were maintained on potato dextrose agar slants and were subcultured in Petri dishes prior to testing. The nutrient agar and the potato dextrose agar media were procured from M/S Himedia, Mumbai, India. Anti bacterial assay of compounds was done in the ready made nutrient agar medium (23 g) which was suspended in distilled water (1000 ml) and heated to boiling until it dissolved completely. The medium and the petri dishes were autoclaved at a pressure of 15 lb/ inc for 20 min. Stock solutions were prepared by dissolving plant extract in DMSO and different concentrations were made (30 mg and 100 mg). Agar cup bioassay was employed for testing antibacterial activity of plant extract. The medium was poured in to Petri dishes under aseptic conditions in a laminar flow chamber. When the medium in the plates solidified, 0.5 ml of 24 h old culture of test organism was inoculated. After inoculation, cups were scooped out with 6mm sterile cork borer and the lids of the dishes were replaced. To each cup different concentrations of test solutions (30, 100 mg) were added. Controls were maintained with DMSO and penicillin G (for gram positive bacteria) and Streptomycin (for gram negative bacteria). The treated and the controls were kept in an incubator at 37^0C for 24 h to 48 h. Inhibition zones were measured and diameter was calculated. Three to four replicates were maintained for each treatment. 3.9? Anti fungal assay of compounds 1, 3 and 4 The method followed for anti fungal bioassay is similar to that followed for anti bacterial assay where in the medium is potato dextrose agar 39 g/l and the control is clotrimazole. Also, the treated and the controls were kept at RT for 24-96 h and inhibition zones were measured and diameter was calculated.

Isoscandinone (**7**); Pale yellow solid; Mp. 142^oC; IR (KBr) gmax: 3250, 1650, 1610, 1380, 1360 cm^{-1}; FABMS: *m/z* 419.0 [M^++H]; (Found: C, 71.82; H, 5.93; $C_{26}H_{26}O_5$ requires: C, 71.75; H, 5.91%). UV λ^{MeOH}_{max} nm 270 & 320 nm: ^{1}H NMR (200 MHz, $CDCl_3$): d13.20 (1H, s, Ar-OH), 5.60 (1H, s, 4'- OH), 7.80 (1H, s, H-2), 7.50 (2H, d, *J* = 9 .0 Hz, H-2', 6'), 7.20 (2H, d, *J* = 9.0 Hz, H - 3', 5'), 6.75 (1H, d, *J* = 12.0 Hz, H -4''), 5.65 (1H,d, *J* = 12.0 Hz, H-3''), 3.45 (2H, d, *J* = 7 Hz, CH2-a), 5.35 (1H, t-like, *J* = 7.2 Hz, H-b), 1.45 (6H, OMeX2, 2''), 1.85 (3H, s, Me) and 1.70 (3H, s, Me).

Scandenin A (**12**); A white amorphous solid; M. P. 189^0C; IR (KBr) Ymax 3298, 1688, 1605, 1340 and 1310 cm^{-1}; FABMS: *m/z* 449.0 [M^++H]; ^{1}H NMR (200 MHz, $CDCl_3$): d 10.0 (1H, s, Ar-OH), 7.50 (2H, d, *J* = 8.4 Hz, H-2', 6'), 7.00 (2H, d, *J* = 8.4 Hz, H- 3', 5'), 6.50 (1H, d, *J* = 9.8 Hz, H-1'''), 5.78 (1H, d, *J* = 9.8Hz, H-2'''), 5.23 (1H, t-like, *J* = 7.2 Hz, H-2''), 3.98 (3H, s, C5-OMe), 3.82 (3H, s, OMe), 3.48 (2H, d, *J* = 7.4 Hz, H-1''), 1.84 (3H, s,), 1.64 (3H, s,) and 1.50 (6H, s, 2Me).

Scandenin B (**13**); Pale yellow amorphous solid; M. P. 129^OC; IR (KBr) γ_{max} 3410, 3250, 2926, 1682, 1515, 1219 and 834 cm^{-1}; FABMS: *m/z* 451.0 [M^++H]; $[a]_D^{25}$ -20.4 [c=0.1, chloroform]; ^{1}H NMR (200 MHz, $CDCl_3$): d 10.0 (1H, s, Ar-OH), 7.38 (2H, d, *J* = 8.4 Hz, H-2', 6'), 6.80 (2H, d, *J* = 8.4 Hz, H- 3', 5'), 6.50 (1H, d, *J* = 9.8 Hz, H-1'''), 5.80 (1H, d, *J* = 9.8 Hz, H-2'''), 4.98 (1H, s), 4.82 (1H, s, H-5'''), 4.32 (1H, dd, *J* = 8.3 Hz and 2.6 Hz, H-2''), 4.95 (3H, s, 5-OMe), 3.10 (1H, dd, *J* = 14.6 Hz and 8.3 Hz, H-1a''), 3.01 (1H, dd, *J* = 14.6 Hz and 2.6 Hz, H-1b''), 1.89 (3H, s,) and1.44 (6H, s, Me)

Results and Discussions

Fourteen compounds were assayed for the antibacterial activity against gram positive bacterial strains, *Bacillus subtilis*, *Bacillus sphaericus* and *Staphylococcus aureus* and gram negative bacterial, *Pseudomonas aeruginosa*, *Klebsiella aerogenes* and *Chromobacterium violaceum* in vitro. Among all 4'-O-methyl osajin (1), osajin (2), scandenone (5), scandinone (6), lupalbigenin (8), Derrisisoflavone C (10), scandenin A (12) and scandenin (13) showed a high degree of antibacterial activity against gram positive and gram negative bacterial strains (Table-1). Analysis of antibacterial

results of compounds (1-14) and their structural features makes it possible to infer some regular patterns of SAR activity relationship. Prenylated 3-aryl coumarins displayed better activity than prenylated isoflavones. Among the prenylated 3-aryl coumarins, compounds with more number of hydroxyl groups on the aromatic ring along with prenyl group exhibited good activity. Among bi-prenylated isoflavones, compounds in which one of the prenyl groups which have undergone cyclization leaving another free prenyl group exhibited better activity than compounds where in both prenyl groups are free.

Table 1- Antibacterial activity levels and MIC values of compounds isolated from D. scandens

Compounds	Microorganisms					
	gram positive			gram negative		
	Bacillus subtilis	*Bacillus sphaericus*	*Staphylococcus aureus*	*Pseudo. aeruginosa*	*Klebsiella aerogenes*	*Chromobacterium violence*
4'-0-methyl osajin (1)	12.5	12.5	25	--	12.5	12.5
Osajin (2)	25	12.5	12.5	--	25	12.5
4-0-methyl scandinone (3)	50	25	25	--	25	12.5
4, 5,7-trihydroxy biphenyl isoflavone (4)	50	25	25	--	25	25
Scandenone (5)	12.5	12.5	25	--	12.5	12.5
Scandinone (6)	12.5	12.5	25	--	12.5	12.5
Isoscandinone (7)	50	25	25	--	25	25
Lupalbigenin (8)	12.5	12.5	25	--	25	25
Derrisisoflavone A (9)	50	25	25	--	25	25
Derrisisiflavone C (10)	12.5	12.5	25	--	25	25
4, 4-0-dimethyl scandenin(11)	50	25	12.5	--	25	12.5
Scandenin A (12)	12.5	12.5	6.25	--	12.5	12.5
Scandenin (13)	6.25	6.25	12.5	--	25	6.25
Scandenin B (14)	50	25	25	--	25	12.5
Streptomycin (std-1)	6.25	12.5	6.25	--	1.562	3.125
Penicillin (std-2)	1.562	3.125	1.562		6.25	12.5

**Negative control Acetone - No activity Concentration in mg/ml*

All the isolated compounds showed moderate degree of antifungal activities (Table-2).

Table 2- Antifungal activity levels of compounds isolated from D. scandens.

Compounds	*Aspergillus niger*		*Rhizopus oryzae*	
	30 µg	100 µg	30 µg	100 µg
4-O-methyl osajin (1)	5	11	7	11
Osajin (2)	8	12	8	11
4-O-methyl scandinone (3)	8	12	8	12
4, 5,7-trihydroxy biphenyl isoflavone (4)	9	12	8	12
Scandenone (5)	7	9	7	10
Scandinone (6)	6	9	6	8
Isoscandinone (7)	7	11	8	12
Lupalbigenin (8)	9	12	7	11
Derrisisoflavone A (9)	6	9	6	9
4, 4-O-methyl scandenin (11)	6	9	6	9
Scandenin A (12)	8	11	6	8
Scandenin (13)	6	9	8	11
Scandenin B (14)	7	10	7	9
Clotrimazole (Std-1)	-	26		23

Negative control (Acetone) - No activity
Zone of inhibition values are indicated in millimeter

The minimum inhibitory concentrations (MIC) were determined by the agar micro dilution method. Test samples were dissolved in dimethyl sulfoxide (DMSO, Merck). Serial 2-fold dilutions of the test samples were mixed with melted Mueller Hinton agar (Difco) in the ratio of 1:100 in microtiter plates (Nunc). *S. aureus* ATCC 25923 and MRSA SK1 isolated from clinical specimen were used as test strains. Ten m1 of inoculum suspensions (104 cfu) were incubated at 35^{0}C for 16-18 hr. MICs were recorded by reading the lowest concentration that inhibited visible growth. The test was performed in triplicates. Growth controls were performed on agar containing DMSO of isoscandinone (7), scandenin A (13) and scandenin B (14) and eleven known compounds were obtained against three representative gram positive organisms viz *Bacillus subtilis* (MTCC 441), *Bacillus sphaericus* (MTCC 11), *Staphylococcus aureus* (MTCC 96) and three gram negative organisms viz Chromobacterium (MTCC 2656), *Klebseilla aerogenes* (MTCC 39), *Pseudomonas aeruginosa* (MTCC 741) by broth dilution method recommended by National Committee for Clinical Laboratory (NCCL) standards[19]. Standard antibacterial agents like Streptomycin (std-1) and Penicillin (std-2) were also screened under identical conditions for comparision. The minimum inhibitory concentrations were represented in table-1. It has been observed that the test compounds exhibited interesting biological activity however, with a degree of variation. The antifungal testing was carried out according to the micro dilution method using potato dextrose agar 39g/L. Test samples obtained from the plants were dissolved in DMSO or saline (10, 1, 0.1 and 0.01 mg/ml) an assayed in various concentrations at 270C for 24-96 hr. MICs were defined as the lowest concentration showing the complete inhition of growth. Clotrimazole was used as a reference standard (std-1) (1, 0.1, 0.01 and 0.001 mg/ml).

Conclusion

Fourteen compounds were isolated from the whole plant of *D. scandens*. All the isolated compounds were tested for their antibacterial and antifungal activities. 4'-O-methyl osajin (1), osajin (2) scandenone (5), scandinone (6), Derrisisoflavone (10), scandenin A (12) and scandenin (13) displayed significant activity against a panel of antibacterial strains. Antibacterial activity of compounds scandenin (13) and scandenin A (12) were equal to the standard of Streptomycin. Anti-bacterial test results of the compounds (1-14) and their structural features exhibited some regular pattern of structure activity relationship (SAR).

Acknowledgements

The authors thank Mahatma Gandhi University, Nalgonda & IICT, Hyderabad for providing necessary infrastructural facilities.

References

[1] Sastri B.N. (1952) *The wealth of India: Raw Materials*, III, 38-39.
[2] Lawpattarakasem P., Houghton P.J., Hoult J.R.S. (2004) *Planta Med.*, 70, 496.
[3] Mahabusarakam W., Deachathai S., Phangpaic S., et al. (2004) *Phytochemistry*, 45, 1185.
[4] Sriwanthana B., Chavalittumrong P.J. (2001) Ethanopharmacol., 76, 125.
[5] Chavalittumrong P., Chivapat S., Chuthaputti A., et al. (1999)

J. Sci. Technol., 21, 425.
[6] Dhawan B.N., Patnaik G.K., Rastogi R.P., et al. (1977) *Indian J. Exp. Biol.*, 15, 208.
[7] Chuthaputti A., Chavalittumrong P., Thai (1998) *J. Pharm. Sci.*, 22, 137.
[8] Jansakul C., Srichanbarn A., Saelee A. (1997) *J. Sci. Soc.*, 23, 323.
[9] Chuankamnerdkarn M., Sutthivaijakit S., Thasana N., et al. (2002) *Heterocycles*, 7, 1901.
[10] Ruckachaisirikul V., Sukpondma Y., Janasakul C., et al. (2002)*Phytochemistry*, 60, 827.
[11] Sekien T., Inagaki M., Ikegami F., et al. (1999) *Phytochemistry*, 52, 87.
[12] Falshaw C.P., Harmer R.A., Ollis W.D., et al. (1969) *J. Chem Soc. C. Org.*, 3, 374.
[13] Johnson A.P., Pelter A., Stainton P. (1966) *J. Chem. Soc. C. Org.,* 2, 192.
[14] Pelter A., Stainton P. (1966) *J. Chem. Soc. C. Org.,* 7, 701.
[15] Johson A.P., Pelter A. (1966) *J. Chem. Soc. C. Org.*, 6, 606.
[16] Mizuno M., Matsuura N., Iinuma M., et al. (1990) *Phytochemistry*., 29, 2675.
[17] Sridhar A.R., Srinivas P.V., Tiwari A.K., et al. (2007) *J. Chromatogr. B.*, 15, 166-172.
[18] Ravikumar K., Sridhar B., Rao A.S., et al. (2005) *Acta Crystallographica*, 61(3), 0596-0598.
[19] National Committee for Clinical Laboratory Standards (NCCLS) (1982) Nat.Comm. Clini. Lab. Stands, 242.