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Chemical constituents and antifeedant potential of *boenninghausenia albiflora*

Rajnikant Sharma[1], Nisha Negi[1], Simon Gibbons[2], Hideaki Otsuka[3], Devendra S.Negi[1]*

[1]Department of Chemistry, PO Box No 75, HNB Garhwal University, Srinagar Garhwal, Uttarakhand-246 174, (INDIA)

[2]Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, (U.K)

[3]Department of Pharmacognosy, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, (JAPAN)

Tell : 911370-267994 ; Fax : 911346- 252174

E-mail : devendra_negi@yahoo.com

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ABSTRACT

Hexane and methanolic extract of *Boenninghausenia albiflora* leaves were evaluated against polyphagous pest *Spodoptera litura* L. The bioactive guided fractionation led to isolation of 2-Octadecanone(1), palmitic acid(2), p-methoxy-methyl cinnamate(3) and 3-(3',4'dihydroxyphenyl) propyl hexadecanoate(4) identified by employing GC-MS. Two known coumarins, murralongin(5) and albiflorin-3(6) were also isolated as antifeedant from methanol soluble fraction.

KEYWORDS

Boenninghausenia albiflora: Rutaceae; Antifeedant: Phenolic esters; Coumarin; *Spodoptera litura*.

INTRODUCTION

Boenninghausenia genus is known to be a rich source of acridone alkaloids, coumarins and essential oils[1-7]. *Boenninghausenia albiflora*(Rutaceae) commonly known as "*Uparnaya ghas*" is used traditionally for various diseases and as an insecticide[8]. A insecticidal coumarin have been previously reported from *B.albiflora*[9]. As a part of studies on the Rutaceae family of the Garhwal Himalayan flora for insecticidal/pesticidal potential we examined hexane and methanolic extracts of *B.Albiflora* for antifeedant assay and found it active against *Spodoptera litura* L(Lepidoptera). Bioactive fraction subjected to capillary Gas Chromatography-Mass Spectroscopy GC-MS and 3-(3',4'-dihydroxyphenyl) propyl hexadecanoate along with 2-octadecanone, palmitic acid and *p*-methoxy-methyl cinnamate were identified. Two coumarins were also characterized from *Boenninghausenia albiflora* with antifeedant activity. This paper is concerned with antifeedant potential of hexane and methanolic extracts and isolated compounds along with structural determination by GC-MS and spectroscopic techniques

EXPERIMENTAL

1. General

Mp uncorrected. UV spectra were recorded on Perkin Elmer lambda 15 using spectral methanol. IR spectra were recorded on a Shimadzu 8201 PC spectrophotometer. ^{1}H & ^{13}C NMR spectra were recorded on Bruker AVANCE 500MHz spectrophotometer at 500, 400 and 125MHz using $CDCl_3$ with TMS as internal standard. EIMS were recorded on Micro mass

Note

Quattro II at 70 eV. The mixtures were analyzed using GC(30 m DB-1 WOT i.d. 320μm; On column-injection at 50^0C, temperature programming initial hold at a initial temperature 50^0C for 2 minute and then at a rate of 40^0Cmin^{-1} to 200^0C, 2 minute at 200^0C, 48min at 5 kPa, 4 kPa min^{-1} to 18 kPa,. wis MS detection(70eV m/z 0-600). Silica gel(60-120 mesh, Merck) and silica gel-G(Merck) were used for CC and TLC.

2. Plant material

Plant material was collected from the Nagdev region, Pauri, Uttarankhand India at a height of 2200mtr and identified by taxonomist of botany department. A voucher specimen is deposited in the Herbarium of Department of Chemistry, HNB Garhwal University, Uttarakhand.

2.3. Extraction and isolation

Air dried powdered leaves of *B.albiflora*(4kg) were extracted with 90% EtOH for 60 hours in a soxhlet apparatus. The ethanol extract was concentrated under reduced pressure. The extract(124g) thus obtained was partitioned with hexane and MeOH to afford a hexane and methanol soluble fractions. The hexane soluble fraction(30g) was charged to gross chromatography over a Column of silica gel(500g) and eluted with C_6H_{12}: EtOAc in sequence of increasing polarity and finally eluted with EtOAc. Two fractions were collected. Fraction F001 showed the presence of several spot on TLC and was subjected to capillary gas chromatography with mass spectroscopy. GC-MS led to indication of three compounds identified as **(1-3)**. F002 employing GCMS indicated for **(4)**. Fraction from gross CC of methanol soluble fraction(94:6, $CHCl_3$: MeOH) rechromatographed using C_6H_{12}: EtOAc solvent system yielded **(5)**(30mg) and **(6)(**42mg).

2.3.1.Octadecanone

$C_{18}H_{36}O$: GC-EIMS(70eV rel. int.) m/z: 268[M]$^+$, 251 (3), 250(13), 235(4), 225(3), 210(4), 195(2), 179(6), 123(18), 109(34), 95(36), 85(36), 58(100).

2.3.2. Palmitic acid

$C_{16}H_{32}O_2$: GC-EIMS(rel. int.) m/z: 256[M]$^+$, 258 [M+2H]$^+$(100), 229(23), 211[M-45]$^+$(28), 199(44), 187(49), 185(23), 171(32), 157(9), 155(13), 143(6), 141(11), 129(23), 115(29), 87(4), 60(18), 45(8), 43(7). 175, 171, 143, 136, 115, 102, 74, 60, 43.

2.3.3. p-methoxy methyl cinnamate

$C_{11}H_{12}O_3$: GC-EIMS(rel. int.) m/z: 192[M]$^+$(2), 191 [M-H]$^+$(5),177[M-CH_3]$^+$(4), 162(100), 161[M-OCH_3]$^+$(91), 135(13), 134[OCH_3-Ph-CH=CH_2] $^+$(87), 133[M-$OOCH_3$]$^+$(38), 119(3), 105(20), 89(4), 78(24), 77(18).

2.3.4. (3',4'dihydroxyphenyl) propyl hexade canoate

$C_{25}H_{42}O_4$: GC-EIMS(rel. int.) m/z: 406[M]$^+$(4), 405[M-H]$^+$(11), 391[M-Me]$^+$(2), 390(3), 331(11), 282(6), 281(18), 269(9), 253(16), 1208(10), 207(45), 195(5), 153(6), 151[$(OH)_2$-Ph-CH_2-CH=CH_2]$^+$(3), 139(9), 125(9), 124(6), 123[$(OH)_2$-Ph-CH_2]$^+$(5), 111 (38), 97(73), 85(39), 83(79), 71(57), 69(71), 57 (100), 55(81), 43(8), 41(61), 28(55).

2.3.5. Murralongin

white solid : $C_{15}H_{14}O_4$
mp : 128-129^0C
UV(methanol) : λ_{max} nm: 223, 228, 322
IR(KBr) νcm^{-1} : 1742 (C-O), 1662 (conjugatedcarb onyl), 1585(aromatic moiety)
^{1}H NMR($CDCl_3$ 400 MHz) δppm: 6.12(1H, d, J=9Hz, H-3), 7.68(1H, d, J=9Hz, H-4), 7.45(1H, d, J=9Hz, H-5), 6.55(1H, d, J=9Hz, H-6), 3.82(bs, methoxy), 1.62(3H, s, methyl), 2.25(3H, s, methyl), 10.42(CHO)
^{13}C-NMR($CDCl_3$ 100MHz)δppm: 160.7(C-2), 113.5 (C-3), 143.5(C-4), 128.5(C-5), 107.5(C-6), 159.4 (C-7), 113(C-8), 112.2(C-4a), 160(C-8a), 152.4(C-1'), 129.5(C-2'), 19.4(C-3'), 24.8(C-4'),57.2 (methoxy), 188.7(CHO) HRMS: 258.2218 cal.for 258.0892

2.3.6. Albiflorin-3

White solid, : $C_6H_{12}O_6$
Mp : 144-145^0C
UV(methanol) : λ_{max} nm: 210, 254, 323
IR(KBr) ν_{max} cm^{-1} : 3470 (tertiaryhydroxyl), 1750, 1596(unsaturated lactone)
^{1}H NMR($CDCl_3$ 500MHz) δppm: 6.28(1H, d, J=9Hz, H-3), 7.46(1H, d, J=9Hz, H-4), 7.61(1H, d, J=9Hz, H-5), 6.86(1H, d, J=9Hz, H-6), 4.90(1H, d, J=9Hz, H-1'), 4.72(1H, d, J=9Hz, H-2'), 4.64-4.53(2H, m, H-4'),3.91(bs, methoxy-7), 3.42(bs, methoxy-1'), 1.69(3H, s, methyl) ^{13}C NMR($CDCl_3$ 100 MHz) δppm: 160(C-2), 113.4(C-3), 143.6(C-4), 128.8(C-5),

Note

107.5(C-6), 162.0(C-7), 113.6(C-8), 112.8(C-4a), 153.4(C-8a), 76.2(C-1'), 78.1(C-2'), 142.9(C-3'), 114.2(C-4'), 57.1(methoxy-7), 57.1(methoxy-1'), 17.2(methyl) HRMS: 290.3316 cal. for 290.1154

2.4 Antifeedant activity

2.4.1. Pest

Field collected larvae of *S. litura* L cultured on castor leaves(*Ricinus communis* L) in the laboratory at 25±2^{0}C. Second generation larvae(third instar) from the laboratory culture were used for antifeedant assay.

2.4.2. Determination of antifeedant activity

The dual choice leaf disc method was performed. Field collected *Ricinus communis* were cut in to circular discs(180cm^2) with the median vein as the marker between two equal halves. Extract and compounds were disssolved in acetone and water, which was sprayed with the help of fine sprayer on the right half of the circular leaf disc to have the concentration of 2.5 and 50μg/cm^2 leaf area. The left half of the leaf was treated with one ml of acetone as control. After air drying each leaf disc was placed in a petridish 15cm. Dia. Five freshly moulted Inset third instar larvae of *S.litura(*from the same egg mass having similar weight) were placed in the center of the leaf and left to feed for 24h. For each(hexane extract, **5** and **6**), five replicates were maintained. After 24hr the larvae were removed and unfed area in the treated and control halves wee measured using ΔT area measurement meter. Percent Feeding Index(PFI) was calculated as:

$$PFI = \frac{\text{\%area fed in treated}}{(\text{\%area fed in treated} + \text{\%area fed in control})} \times 100$$

RESULT AND DISCUSSION

Hexane extract of fresh leaves of *B.albiflora* was subjected to gross CC and isolated bioactive fractions employing capillary gas chromatography with mass spectrometric detector(GC-MS) led to isolation of four compounds**(1-4)** according to their retention indices and mass spectra. **(1)** and **(2)** were detected as 2-octade canone and palmitic acid by direct comparison of their mass spectra with that of data reported and Co-TLC with authentic sample[10-11].

TABLE 1 : Antifeedant activity of hexane, methanol extracts and isolated compound of *B.albiflora*

Particular	Percent feeding index(PFI)
Hexane extract*	40.45±2.74
Methanol extract*	14.02±11.05
Murralongin** 5	50.24±1.08
Albiflorin-3** 6	41.10±2.24

Values are mean ± SD; n=5 ; *2.5μm/cm^2 ; **50μm/cm^2

Compound**(3)** showed molecular ion peak at 192m/z from its EIMS. Its other fragments at 177[M-CH_3]$^+$, 161[M-OCH_3]$^+$ and 133[M-$COOCH_3$]$^+$ m/z indicated the presence of ester function. Other prominent fragments were suggested for methoxy cinnamic acid moiety. Thus**(3)** was identified as p-methoxy-methyl cinnamate by comparison of its mass spectral data with reported data and Co-TLC with authentic sample[12].

Compound **(4)** showed molecular ion peak at 406 m/z along with two series of fragment 55, 69, 83 etc and 57, 71, 85m/z etc indicated the presence of fatty acid moiety. Other prominent peaks at 123 m/z suggested for dihydroxy benzyl moiety, 391 due to methyl loss [M-CH_3]$^+$, 151[$(OH)_2$-Ph-CH_2-CH=CH_2]$^+$ and 123[$(OH)_2$-Ph-CH_2]$^+$ described for 3-(3',4'-dihydroxyphenyl)-propyl ester[13]. So**(4)** was identified as 3-(3',4'dihydroxyphenyl)propyl hexadecanoate.

Column chromatography of bioactive guided fractionation of methanol soluble fraction led to isolation of two known coumarin named as murralongin and albiflorin-3 identified by direct comparison of its NMR data with that of reported in literature[14].

Hexane extract and methanolic extracts of *B.albiflora* leaves along with**(5)** and**(6)** were tested for antifeedant activity against *S.litura* by dual choice leaf disc method and Percent Feeding Index(PFI) was measured at a concentration of 2.5 and 50g/cm^2. Hexane extract showed 40.45± 2.74 PFI whereas methanolic extract showed significant PFI 14.02± 11.05 against *S.litura*. Compound **(5)** and **(6)** exhibited 50.24±1.08 and 41.10±2.24 PFI given in TABLE 1.

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Note

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