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Research Article

ISOLATION AND CHARECTERISATION OF PHENOLICS FROM THE ROOTS OF *COTONEASTER ACUMINATUS* AND DETERMINATION OF THEIR ANTIMICROBIAL ACTIVITY

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Abstract

Two new phenolic glycoside 3β, 7, 3' trihydroxy 4' methyl flavan 5-O-β-D glucopyranoside and 3', 4'dihydroxy 6-methyl 7-O-α-L rhamnopyranoside was isolated from the alcoholic extract of roots of *Cotoneaster acuminatus* along with the known compounds epicatechin and apigenin 7-0-β-D glucoside .

INTRODUCTION

Cotoneaster acuminatus vern. Ruins belongs to family *Rosaceae*, is a deciduous shrub or small tree,upto 8 m.high, commonly found in open scrub of upper reaches of montane zones of Himalayas or under growth of Oak and Rhododenderon forests. The decoction of leaves of the plant is used as external remedy in different conditions of scabies and rheumatic arthritis [1]. Most of the plants of the *Rosaceae* are known for different phenolic and flavonoidal constituents of important and diverse biological activities [2].

The present paper deals with isolation and characterisation of new phenolic glycoside 3β, 7, 3' trihydroxy 4' methyl flavan 5-O-β-D glucopyranoside together with epicatechin, apigenin 7-0-β-D glucoside and 3', 4'dihydroxy 6-methyl 7-O-α-L rhamnopyranoside. The characterization of isolated compounds was done by using different spectroscopic techniques.

MATERIAL AND METHODS

General experimental procedure

MPs were incorrected; UV spectra were taken in MeOH using $AlCl_3$ as shift reagent. IR recorded in KBr on a Perkin Elmer FT-IR Spectrophotometer. ^{1}H-NMR were run at 300MHz using TMS as internal standerd and C_5D_5N and CD_3OD as solvent 13C-NMR recorded in 90MHz, using CD_3OD as solvent, FAB-MS on JOEL. JMS700 Mstaion Spectrophotometer.

Plant material

The plant material for study were collected from Sainti Ghat, Chamoli, Uttarakhand, India and identified from Ethanobotanical Plant Identification Laboratory, Department of Botany, H. N. B. Garhwal University Srinagar Garhwal. A voucher specimen was deposited in herbarium of the Department.

Extraction and isolation

The roots (3Kg) of *Cotoneaster acuminatus* were exhaustively extracted with petroleum ether followed by extraction with EtOH. A waxy substance is obtained when the petroleum extract is concentrated. The waxy substances were saponified with ethanolic KOH (100 %) and the hydrolysate so obtained was extracted with ether. Ether soluble and ether insoluble parts are obtained. From ether soluble (non-saponifiable) part of the hydrolysate, solvent was removed under pressure, when yellowish brown solid was obtained. The solid on chromatography over silica gel afforded compounds 1 and 2. Ethanolic solution on concentration under reduced pressure afforded a yellowish green mass which was further subjected to column chromatography over silica gel (eluent $CHCl_3$: MeOH) resulted in the isolation of compounds 3 and compound 4 respectively.

RESULTS AND DISCUSSION

Characterization of compound 1

It was crystallized from methanol as yellow crystallize solid:

M. P	240-242^{0}C
Molecular Formula	$C_{22}H_{26}O_{10}$
Molecular weight	450 amu
IR (λ_{max} ^{KBr}) cm^{-1}	3410, 1650, 1600, 1525, 1430.

FABMS (m/z)

489[M+K] $^+$, 450[M+H] $^+$, 307 (M+3H-146+H_2O), 273(M+3H-146+2H_2O), 242[M-(146+2OH+OCH_3)]

^{1}H-NMR (C_5D_5N) δppm

6.06 (d,J=1.2H_z, H-3),7.7(S, H-5), 7.1 (d,J=3.2H_z, H-5'), 6.9(d,J=3.2H_z, H-6'), 7.3(S-H-5), 6.38(s,H-2"), 1.72(rhamnose 3H) 2.16(S,OCH_3) (03H), 3.32-3.43 (sugar multiplet), 4.2 (5, rhamnose H-1')

^{13}C-NMR (CD_3OD, δ ppm)

146(C-2), 106.1(C-3), 179.6(C-4), 122(C-5), 163(C-6),165.8(C-7), 94.0(C-8), 148.9(C-9), 105(C-10), 121(C-1'), 116(C-2'), 158(C-3'), 159.6(C-4'), 163.3(C-5'), 116.9(C-6'), 53.7(OCH_3)

Rhamnose

103(C-1'), 72.0(C-2'), 71.9(C-3'), 73.2(C-4'), 17.6(C-6')

It gave positive test with Molish reagent there by indicating the glycosidic nature of compound [3]. The IR spectrum displayed a peak at 1655 and 1612 cm^{-1}, which shows the presence or carbonyl function in the compound. The peaks at 3410, 1525 and 1430 were the characteristics of flavonoids. FABMS of compound provide a peak at 489[M+K] $^+$, 450[M+H] $^+$, 307[M+3H-146] $^+$, 289[M+3H-146+H_2O] $^+$, 273[M+3H-146+2H_2O] $^+$, 242[M-(146+2OH+OCH_3)]. Shows the different fragmentation pattern of compound and also loss of one deoxyhexose, two hydroxyl groups and one methoxyl group respectively. The ^{1}H-NMR spectrum displayed doublets of 1.2 H_z coupling constant at δ 6.06 and 7.1 were characteristic for H-3 and H-5', where as singlet at δ7.3, 7.7 and 6.38 were assigned for H-2', H-8 and H-5.

Further the two singlets at δ1.27 and 2.16 were assigned for rhamnose and aromatic methoxyl. The position of singlet at δ4.2 indicates a configuration of rhamnose. In ^{13}C-NMR spectrum the downfield peak at δ 179.6 assigned for C-4 (Keto group), δ 163.4 (C-6), 159.6 (C-4'), 158.0 (C-3') and 165.8 (C-7) support substitution at these positions [4]. Methoxy carbon resonated at δ53.7 which was assigned at C-6 (δ163.4) in ring A. On acidic HOH (7%MeOH + HCl) it afford an aglycone identified as 3', 4' dihydroxy 6-methyl flavone (Comparision with reported data and authentic sample) and rhamnose (Rf. Values, PC). Thus on this basis compound was identified as *Flavone 3', 4' dihydroxy 6-methyl 7-O-α-L rhamnopyranoside* (Figure-1).

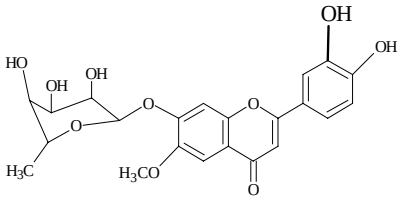

Fig. 1: Flavone 3', 4' dihydroxy 6-methyl 7-O-α-L rhamnopyranoside

Characterization of compound-3

It was crystallized from methanol as white powder.

M.P.	200-202^{0}C
Molecular formula-	$C_{22}H_{26}O_{11}$
Molecular weight-	466 amu

IR (λ_{max} ^{KBr}) cm^{-1}

3416(OH), 2927, 1607, 1518(Benzene skeleton)

^{1}H-NMR (C_5D_5N, δppm)

3.27 (^{1}H, dd, J=16.3, 8.4Hz, H-4β), 3.55(^{1}H, dd, J=16.4, 5.4Hz, H-4α), 3.69(3H, S, OCH3), 3.96(2H,M, H-6'), 4.33(^{1}H, M, H-3), 4.48(^{1}H, J=7.6Hz anomeric H), 5.08(^{1}H, d, J=2.2Hz, H-6), 5.48(^{1}H, d, J=6.6Hz, H-2), 6.72(^{1}H, d, J=1.5Hz, H-8), 6.90(2H, d, J=8.2Hz, H-5' and H-6'), 7.00(^{1}H, S,H-2')

^{13}C-NMR (C_5D_5N, δppm)

82.96(C-2), 67.66(C-3), 28.56(C-4), 155,9(C-5), 98.02(C-6), 157.6(C-7), 97.5(C-8), 157.9(C-9), 102.5(C-10), 132.8(C-1'), 115.4(C-2'), 148.7(C-3'), 148.4(C-4'), 120.0(C-5'), 112.7(C-6'), 102.0(C-1"), 75.2(C-2"), 77.9(C-3"), 70.9(C-4"), 78.7(C-5"), 62.3(C-6"), 57.2(OCH_3).

It gave positive Molish reagent test,brown colour test with $FeCl_3$, and cherry red colour with Mg-HCl (Shinoda test) which indicates its flavonoidal glycosidic nature[3]. The IR spectrum displayed a band at 2927cm^{-1} and 1518cm^{-1} which are characteristics of benzene rings and band at 1640 cm^{-1} (>C=O of γ pyrone). On the basis of elemental analysis and molecular weight determination its molecular formula was deduced as $C_{22}H_{26}O_{11}$ which correspond 446 amu molecular weight of compound. ^{1}H-NMR spectrum of compound displayed a peaks at δ3.27 (1H, dd, J=16.3, 8.4Hz, H-4β), δ 3.55(1H, dd, J=16.4, 5.4Hz, H-4α), and peak at δ 3.69(3H, S, OCH3), 3.96(2H,m, H-6'), 4.33(^{1}H, m, H-3).The anomeric proton was observed at δ 4.48(1H, J=7.6Hz anomeric H)[5]. In ^{13}C-NMR spectrum the different carbon atoms are resonated at δ 82.96(C-2), 67.66(C-3), 28.56(C-4), 155,9(C-5), 98.02(C-6), 157.6(C-7), 97.5(C-8), 157.9(C-9), 102.5(C-10), 132.8(C-1'), 115.4(C-2'), 148.7(C-3'), 148.4(C-4'), 120.0(C-5'), 112.7(C-6'),whereas the hexose signals resonates at δ 102.0(C-1"), 75.2(C-2"), 77.9(C-3"), 70.9(C-4"), 78.7(C-5"), 62.3(C-6").The OCH_3 carbon atom was assigned at δ57.2 in ^{13}C-NMR spectrum.The compound gave tri acetate (M.P.128^{0}C) on acetylation which confirm the presence of three hydroxyl group at 3β, 7, 3' positions in the compound. These positions are further supported by data of ^{1}H-NMR spectrum.The ^{1}H and ^{13}C-NMR,especially the H-2 signal in ^{1}H-NMR and the C-2,C-3 chemical shift in ^{13}C-NMR were very close to that of (+)catechin[6].Which also support the flavonoidal skeleton or flavan-3-ol moiety in the compound.The ^{13}C-NMR signals existing at δ 62.3 - 78.7 and 102.0 ppm showed that there probably a glucose moiety is present.The m/z 304 (M^+ - 162) in FABMS further supports the presence of glucose moiety. The ^{1}H and ^{13}C-NMRspectra also indicated that the compound has a methoxyl group (δ_H 3.69 ppm, 3H, s, δ_C 57.2ppm).Thus on the basis of above spectral studies compound was identified as *3β, 7, 3' trihydroxy 4' methyl flavan 5-O-β-D glucopyranoside* (Figure-2).

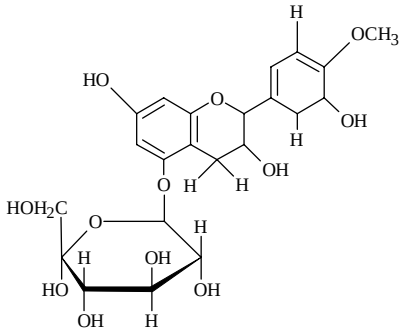

Fig. 2: 3β, 7, 3' trihydroxy 4' methyl flavan 5-O-β-D glucopyranoside

Compounds 2 and 4 were identified as epicatechin and apigenin 7-O-β-D-glucoside respectively on the basis of comparing their spectral date. Co TLC, Co IR, mixed melting point with that of the authentic sample.

Acid hydrolysis of compound

Compound 3(6.0) mg was treat with 2N methanolic HCl(2ml) under reflux at 90^o C for 1h.The mixture was extracted with CH_2Cl_2 and the aqueous layer was neutralized with Na_2CO_3 and filtered.The dried filtrate was acetylated with pyridine –Ac_2O.The GC-MS analysis showed presence of per acetyl glucose ,which was further confirmed by comparison with reference compound(TLC run parallel)

Antimicrobial activity of alcoholic extract of root of the plant

The zone of inhibition with different concentrations of extract and of the standard drugs were shown in table 1. The alcoholic extract of the root of plant was found to be having antimicrobial activity in a dose dependent manner (1µg/ml, 10µg/ml, 50µg/ml and100µg/ml)against all the test organism. Significant activity was found with the extract at a concentration of 100µg/ml as compound with standard.

Table 1: Antimicobial activity of plant materials

Type	Strains	Zone of inhibition (µg/ml)				*Standard	Control (Propylene glycol)
		1	10	50	100		
Gram positive Bacteria	*B.subtilis*	11	13	18	17	20	0
	B.pumilus	06	10	12	16	18	0
	S.aureus	08	08	03	16	13	0
	M.glutamicus	09	-	15	18	19	0
Gram Negative Bacteria	*P.aerogenosa*	07	10	15	18	18	0
	P.valgaris	11	13	15	10	18	0
	E.coli	08	11	18	12	20	0

Standard for antimicrobial activity- Ampicillin

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