Isolation and Characterization of Phenolic Glycoside from the Bark of *Symplocos Racemosa* Roxb

M. VIJAYABASKARAN*, K.R. YUVARAJA,
G. BABU, P. PERUMAL and B. JAYAKAR[§]

Department of Pharmaceutical Chemistry, J.K.K. Nataraja College of Pharmacy
Komarapalayam, Tamilnadu, India

[§]Department of Pharmaceutical Chemistry
Vinayaka Mission's College of Pharmacy, Salem, Tamilnadu, India
vijayabass@gmail.com

Received 16 April 2010; Accepted 2 June 2010

Abstract: A new phenolic glycoside, 3, 5 - dihydroxy - 2- (hydroxyl methyl) - 6-(3,4,5-trimethoxy phenoxy)tetrahydro-2H-pyran-4-yl, 4-hydroxy-3-methoxy benzoate have been isolated from the dried bark of *Symplocos racemosa*. The structure was identified by extensive spectral analysis, especially FT-IR, GC-MS, ¹H NMR and ¹³C NMR techniques. The method of isolation was simple, cost effective and efficient. The preliminary bioactivity of the compound was also evaluated. The ethanolic extract of *Symplocos racemosa* (EESR) was investigated for its anti-pyretic activity against brewer's yeast induced pyrexia. The antipyretic effect of EESR (measured as % reduction in body temperature) was compared with paracetamol (100 mg/kg, orally). EESR in dose of 200 mg/kg caused significant decrease in body temperature of rats. This study has established the antipyretic activity of *Symplocos racemosa* and thus, justifies the ethnic uses of the plant.

Keywords: *Symplocos racemosa*, FT-IR, GC-MS, NMR, Brewer's yeast, Antipyretic.

Introduction

Symplocos racemosa roxb. (Fam. symplocaceae) is a widely used ayurvedic remedy for various ailments. It is also known as lodhra and is used in Indian System of Medicine (ISM) as single drug or in multicomponent preparations (*viz.* lodhrasava). It's bark is acrid, digestible, astringent to bowels. It is useful in treatment of fever, eye diseases, for spongy gums and bleeding. It cures 'Kapha', diseases of the blood, leprosy, dropsy and liver complaints^{1,2}. It is also useful in abortions and miscarriages and for ulcers of vagina. Traditionally bark is given in menorrhagia and other uterine disorders. Unani medicine uses it as emmenagogue, aphrodisiac³. It is a potent remedy for inflammation and cleaning uterus.

This is used to treat leucorrhea and menorrhagia⁴. It contains salireposide and benzoylsalireposide which are inhibitors⁵ of phosphodiesterase I and have showed its depressant action on blood pressure and intestinal movements⁶.

Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states. It is the body's natural defense to create an environment where infectious agent or damaged tissue cannot survive⁷. Normally the infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediator's (cytokines like interleukin 1β , α , β and TNF- α), which increase the synthesis of prostaglandin E2 (PGE2) near preoptic hypothalamus area and thereby triggering the hypothalamus to elevate the body temperature⁸. As the temperature regulatory system is governed by a nervous feedback mechanism, so when body temperature becomes very high, it dilate the blood vessels and increase sweating to reduce the temperature; but when the body temperature becomes very low hypothalamus protect the internal temperature by vasoconstriction. High fever often increases faster disease progression by increasing tissue catabolism, dehydration and existing complaints, as found in HIV⁹. Most of the antipyretic drugs inhibit COX-2 expression to reduce the elevated body temperature by inhibiting PGE-2 biosynthesis¹⁰. Moreover, these synthetic agents irreversibly inhibit COX-2 with high selectivity but are toxic to the hepatic cells, golmeruli, cortex of brain and heart muscles, whereas natural COX-2 inhibitors have lower selectivity with fewer side effects. A natural antipyretic agent with reduced or no toxicity is therefore, essential. As bark of *symplocos racemosa* is used in ailments that caused fever¹¹, so it will be a cost effective alternative approach to study this plant for the development of an effective antipyretic agent.

Experimental

Column chromatography (CC): silica gel, 60–120 mesh. TLC: pre-coated Silica gel G-25-UV₂₅₄ plates: detection at 254 nm and by ceric sulphate reagent. UV and IR Spectra: Elico SL 164 and Bruker optic GMBH spectrophotometer respectively. ¹H and ¹³C NMR, Spectra: Bruker spectrometers operating at 500 and 400 MHz; chemical shifts δ in ppm and coupling constants in Hz. GC-MS: JEOL GC mate.

Plant material

The plant *Symplocos racemosa* (Family: symplocaceae) was collected from Kolli Hills at Namakkal District, Tamilnadu, India. It was authenticated by Dr. V. Sathyanathan, Taxonomist, Epoch Pharma and Research Labs Pvt. Ltd. Chennai and its voucher specimens were deposited in the Herbarium for further reference.

Extraction and isolation of plant materials

After proper identification, the bark of *Symplocos racemosa* was dried under shade and then coarse powdered with a mechanical grinder. The coarse powder was stored in an airtight container for further use. The dried coarse powder material of bark (250 g) was extracted with ethanol (95%) for 72 h in soxhlet apparatus. The extract was made solvent free distillation process under reduced pressure and the resulting semisolid residue was vacuum dried using rotary flash evaporator. The yield of the ethanolic extract was 18.28% w/w and it was used for the isolation and study of antipyretic activity.

The column was packed with (EESR 4 g) silica gel and eluted with isoamyl alcohol: acetic acid: water (1:1:2) to afford 1.84 g of compound as a brown solid. It was recrystallized from hexane to afford compound 1 (0.98 mg). Thin layer chromatography of

EESR in isoamyl alcohol: acetic acid: water (1:1:2) showed the presence of brown coloured single spot. (R_f 0.89). IR absorption at 3473 cm^{-1} (OH) 2924 cm^{-1} (CH), 1615 cm^{-1} (C=C aromatic), 1247 cm^{-1} (C–O C), 1744 cm^{-1} ester function, and broad (C–O) stretching bands in the region $1077\text{--}1050\text{ cm}^{-1}$. HREIMS $[M+H]^+$ ion peak m/z at 498, confirmed the possibility of molecular formula of $C_{23}H_{29}O_{12}$. The fragments at m/z 92 $[\text{HOC}_6\text{H}_3]^+$, 108 $[\text{CH}_3\text{OC}_6\text{H}_4\text{O}]^+$, 138 $[\text{HOC}_6\text{H}_4\text{COOH}]^+$, 167 $[(\text{CH}_3\text{O})_3\text{C}_6\text{H}_2]^+$, 328 $[M - \text{glucose}]^+$. ^1H NMR and ^{13}C NMR of compound **1** were presented in Table 1.

Table 1. NMR data (in CDCl_3) for compound **1**

Position	δ_{H}	δ_{C}
1	-	155.0
2	3.02	93.7
3	3.72	151.8(56.2 –OCH ₃)
4	3.72	131.5(56.2 –OCH ₃)
5	3.72	151.8(56.2 –OCH ₃)
6	3.04	93.7
1'	4.62(d, J = 2.0 Hz)	71.3
2'	4.44	55.9
3'	4.76	79.0
4'	-	85.8
5'	4.58	69.1
6'	4.27(d, J = 1.8 Hz, H-6 _a)	58.7
	4.10 (d, J = 8.0 Hz, H-6 _b)	170.3(RCOOR)
1''	-	122.1
2''	3.06	115.3
3''	3.72	149.8(56.2 –OCH ₃)
4''	-	149.8
5''	3.09	115.3
6''	3.11	122.1

All spectra were recorded at 400 MHz (^1H) and 100 MHz (^{13}C). ^{13}C NMR multiplicities were determined by DEPT 135°

Animals

Male albino Wistar rats of body weight 150–250 g were obtained from the Sri Venkateshwara Enterprises, Bangalore and were maintained in J.K.K. Nataraja College of Pharmacy animal house. The animals were housed in well ventilated large spacious polypropylene cages and had 12 ± 1 h light and dark cycles throughout the experimental period. The animals received a balanced diet of commercially available pellet rat feed and water *ad libitum*. The Guidelines for Breeding and Experiments on Animals, 1998 defined by the Ministry of Social Justice and Empowerment of India were followed and the protocol was approved by the Institutional Animal Ethics Committee (887/ac/05//CPCSEA).

Acute toxicity studies

Ethanol extract of *Symplocos racemosa* was studied for acute oral toxicity as per revised OECD (Organization for Economic Cooperation and Development) guidelines No. 423. The extract was devoid of any toxicity in rats when given in doses up to 2000 mg/kg by oral route. Hence, in our study 100 and 200 mg/kg doses of extract were dissolved in 0.1% Carboxy Methyl Cellulose (CMC) and used for the study¹².

Screening for antipyretic activity

The antipyretic activity of EESR was evaluated using Brewer's yeast-induced pyrexia in rats. Fever was induced by administering 20 mL/kg of 20% aqueous suspension of Brewer's yeast in normal saline subcutaneously. The EESR (100 and 200 mg/kg, orally) was administered orally, paracetamol (100 mg/kg, orally) was used as reference drug and control group received distilled water. Rectal temperature was determined by thermal probe Eliab thermistor thermometer at 1, 2 and 3 hrs after test extract/reference drug administration¹³.

Statistical analysis

Data were recorded as mean $\pm$ SEM. The statistical significance of differences between groups was determined by analysis of variance (ANOVA), followed by Dunnett's test for multiple comparisons among groups. Differences of $p < 0.05$ were considered statistically significant.

Results and Discussion

Scheme 1. (3R, 5S, 6S)-3, 5-dihydroxy-2-(hydroxymethyl)-6-(3, 4, 5-trimethoxyphenoxy)-tetrahydro-2H-pyran-4-yl 4-hydroxy-3-methoxybenzoate.

From the ethanol extract of *Symplocos racemosa* (EESR) a new compound **1** was isolated as an amorphous powder. Its FAB-MS showed a $[M]^+$ ion at m/z 498, which established that the molecular formula was $C_{23}H_{29}O_{12}$. Compound **1** exhibited a UV band at $\lambda_{\max}$ 285 nm typical of phenolic compounds. There was an intense IR absorption at 1744 cm^{-1} in indicating the presence of an ester function. Other strong bands were observed at 3373 cm^{-1} (OH), 1615 cm^{-1} (C=C, Aromatic), 1247 cm^{-1} (C-O-C) and $1077\text{--}1050\text{ cm}^{-1}$ (C-O). The EIMS spectrum of compound **1** exhibited an anion at m/z 328 $[M\text{ Benzoyl glucose}]^+$. This ion disintegrated further to the following characteristic fragments: $[\text{HOC}_6\text{H}_3]^+$ (m/z 92), $[\text{CH}_3\text{OC}_6\text{H}_4\text{O}]^+$ (m/z 108), $[\text{HOC}_6\text{H}_4\text{COOH}]^+$ (m/z 138) and $[(\text{CH}_3\text{O})_3\text{C}_6\text{H}_2]^+$ (m/z 167).

^1H NMR ($\text{DMSO-}d_6$) spectrum displayed an anomeric hydrogen due to sugar unit at 4.62 (d, $J = 2.0\text{ Hz}$, H-1') and the geminal carbon C-6' showed two set of peaks for hydrogen (H_a and H_b) due to long range two bond coupling at 4.27 (d, $J = 1.8\text{ Hz}$, H-6_a') and 4.10 (d, $J = 8.0\text{ Hz}$, H-6_b'), respectively. The other glycosidic hydrogen displayed as broad unresolved multiplets in the region of 4.44 - 4.58 (m, H-2', 5') and 4.76 - 4.92 (m, H-3', 6'), respectively. The methoxy groups appeared in the region of 3.72 along with the water present in the NMR solvent $\text{DMSO-}d_6$. The aromatic hydrogen (Ar H-2,6,2'', 6'', 5'') appeared as broad unresolved multiplets in the region between 6.22 - 6.92. The hydroxyl groups appeared as broad peaks at 7.24 and 9.28 respectively. ^{13}C NMR spectra showed resonance that was readily attributed to β -glucose and two phenolic esters.

The results of effect of EESR on yeast-induced pyrexia in rats are depicted in Table 2. EESR produced significant ($P < 0.05$) antipyretic effect in a dose dependent manner. Normal rats did not show any decrease in the body temperature on intraperitoneal administration EESR. The initial and final rectal temperature ($^{\circ}\text{C}$) for two groups of extract administered

rats was 38.85 ± 2.16 and 37.64 ± 2.15 (100 mg/kg); and 38.28 ± 1.14 and 37.15 ± 1.59 (200 mg/kg). Based on the result, it can be concluded that EESR produced antipyretic effect.

Table 2. Effect of EESR on Brewer's yeast induced pyrexia in rats

Treatment	Dose mg/kg	Rectal temperature °C		Rectal temperature after administration of drug °C		
		Normal (A)	18 h after yeast admn (B)	1 h (C ₁)	2 h (C ₂)	3 h (C ₃)
Control (saline)	0.5 mL	36.97±1.17	39.01±1.13	38.79±1.12	38.63±1.23	38.25±1.25
Paracetamol	100	36.99±2.19*	39.05±1.16	38.05±2.15	37.22±1.13	37.02±1.88*
EESR	100	37.16±1.16	39.25±1.12	38.85±2.16*	38.25±1.14	37.64±2.15*
EESR	200	36.84±1.17	39.31±1.13	38.28±1.14	37.75±1.11	37.15±1.59

All values are expressed as mean (n=6), EESR = Ethanolic Extract of *Symplocos racemosa* bark,

*P < 0.05, Experimental animals were compared with control

Figure 1. Effect of EESR on body temperature of control and experimental group of rats

Conclusion

Search for safe herbal remedies with potent antipyretic activity received momentum recently as the available antipyretic, such as paracetamol, aspirin, nimusulide *etc.* have toxic effect to the various organs of the body¹⁴. The acute toxicity result reveals that this plant might be considered as a broad non-toxic one. The antipyretic activity exhibited that the both doses of ethanol extract of barks possess a significant antipyretic effect in maintaining normal body temperature and reducing yeast induced fever in rats and their effect are comparable to that of standard antipyretic drug paracetamol. Such reduction of rectal temperature of tested animals by the both doses of EESR appears to be due to the presence of a single bioactive principles or mixture of compounds in them. The phytochemical analysis of the EESR showed the presence of carbohydrates, sterols, glycosides, alkaloids, phenols and saponins. The antipyretic activity observed can be attributed to the presence of steroids. Moreover, in many studies, glycosides, alkaloids have been reported to exhibit antipyretic effect¹⁵⁻¹⁷.

It was also evident from the study that the antipyretic activity of EESR at 100 mg/kg body weight is almost similar to the standard paracetamol group and more than the 200 mg/kg body weight dose. The present study, therefore, supports the claims of traditional medicine practitioners as an antipyretic remedy. However, to know the exact mechanism of action of EESR further study with purified fractions/ bioactive compounds are warranted.

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