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Synthesis and antioxygenic activities of seabuckthorn flavone-3-ols and analogs

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ABSTRACT

A practical synthesis of polyhydroxy- and regiospecifically methylated flavone-3-ols which are components of commercial 'seabuckthorn flavone' has been achieved by modified Algar–Flynn–Oyamada method. Antioxidant activities of seabuckthorn extracts, isolated products and a number of flavone-3-ols have been determined. Structure–activity relationships have been discussed. Amongst the compounds tested, gallic acid, which is also present in seabuckthorn, was found to be the most effective antioxidant and radioprotectant.

Many of the remarkable biological activities of the plant, seabuckthorn (*Hippophae* sp., SBT) have been attributed to the presence of flavonoids. A commercial product, so called 'seabuckthorn flavone' (SBT flavone)[1] is reported to have a variety of bioactivities, although its composition was not clearly defined. Though wide-ranging bioactivities on Indian seabuckthorn (*Hippophae rhamnoides* turkestanika ssp.) have been reported,[2] not much attention has been paid to the isolation and molecular characterization of the active constituents. This attracted our attention to analyze 'SBT flavone' for flavonoids and to synthesize the flavonols (flavone-3-ols). Our analysis of commercial 'SBT flavone' samples of different origin revealed that they mainly contained a mixture of isorhamnetin **6**, quercetin **6c** and kaempferol **6b** with traces of myrcetin in highly variable proportions and yields (15–30%)[3] with more than 50% intractable materials. No evidence for presence of glycosides or tannins was found in 'SBT flavone' samples. Considerable variations in phytochemicals of SBT have been observed in related species. Thus though bioactive compounds, such as isorhamnetin **6** and ursolic acid are the major bioactive components in *H. rhamnoides* from Leh (Ladakh, India), they are only minor components in closely related *H. salicifolia* from Lachen (Sikkim, India).[4]

Ambiguous reported bioactivities and composition of 'SBT flavone' prompted us to synthesize individual flavone-3-ols, so that pure compounds are available in sufficient amounts for studies on structure–activity relationship.

Flavonoids, one of the oldest group of natural products, are attracting considerable attention for their recently discovered bioactivities, such as radio-protective,[5] kinase inhibitory[6] and apoptotic activities related to cancer.[7] They show antioxygenic and metal chelating activities[8] and have also been implicated in the conditions related to degenerative diseases (aging), wound healing and cardio-vascular problems. Flavonoids occur sporadically in nature. Procurement of desired flavonoids in sufficient quantities from the natural sources often poses logistic problems. The insights derived from the studies on bioactivities of SBT extracts made it imperative to locate the active molecule(s). Recent reports suggest that the polyhydroxy flavonoids are metabolized in the biological systems into more bioavailable partially protected forms (e.g., methyl ethers) which may be the actual active entity. For example, plasma of rats fed with quercetin **6c** were devoid of it, but contained its 3′-methyl ether namely isorhamnetin **6**.[9] Thus preparation of partially protected compounds assumes special importance in metabolic studies.

Unlike flavones, only few methods are available for the synthesis of flavonols (flavone-3-ols).[10] Allan-Robinson synthesis has been the method of choice for flavone-3-ols. But it employs very harsh experimental conditions and requires ingenious selective protection and deprotection of the free hydroxyl groups with benzyl and/or benzoyl groups.[11]

Alternate method using Algar–Flynn–Oyamada (AFO) reaction though offers flavone-3-ols directly, the yields of the reaction are variable and other side products are also formed.[12] Of the 'SBT flavones', isorhamnetin **6** seems to the most important for it shows a multitude of bioactivities.[13] Therefore we chose the synthesis of

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isorhamnetin **6** as a model candidate (Scheme 1). Isorhamnetin **6** has been synthesized earlier by Allan-Robinson method[11] or by selective methylation of quercetin **6c**.[9]

In our hands the conventional AFO reaction with protected chalcones resulted in the formation of mixture of compounds with low and variable yields. In the present synthesis, partial methyl ethers such as isorhamnetin **6**, tamarixetin **6a** and kaempferide **6d** were described. Methoxymethyl (MOM) ether was chosen because they not only offer necessary protection but are also easily deprotected.[14] The chalcone **3** was obtained by the condensation of 2,4,6-tri-MOM phloracetophenone **1** with 4-MOM vanillin **2**.[15] The chalcone **3** was obtained as a pale yellow oily product with UV λ_{max} at 324 nm. It did not respond Shinoda test (Mg–HCl); no distinct color change was observed on addition of alcoholic ferric chloride suggesting that MOM protections were intact.

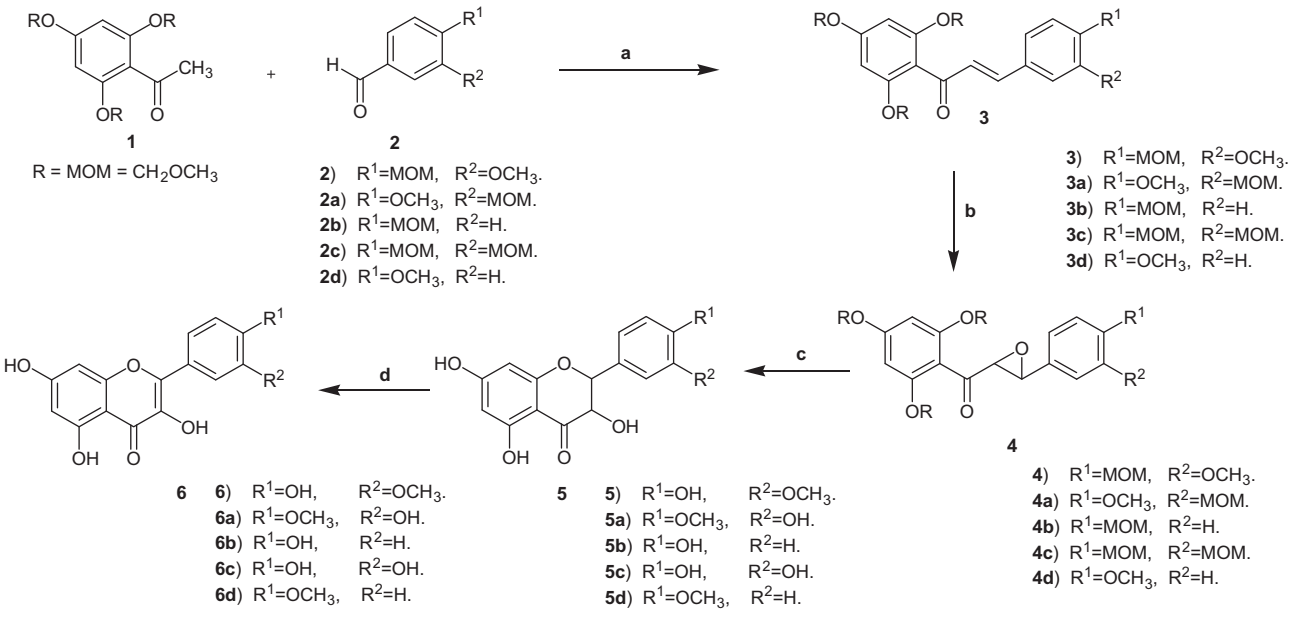
The chalcone **3** was converted to epoxide **4** using alkaline H_2O_2 in 70–85% yield.[16] The formation of the epoxide **4** was confirmed with UV (λ_{max} 285 nm) and IR (ν 883 cm^{-1}) spectral data.[12]

Treatment of epoxide **4** with methanolic HCl resulted into regio-specific opening of the epoxide ring with concurrent removal of MOM protections to give dihydroflavonol **5** in 55–60% yield. The dihydroflavonol **5** showed expected spectroscopic data (UV λ_{max} 285 nm, IR ν 1644 cm^{-1}). It responded to Shinoda's (Mg–HCl) and Pew's (Zn–HCl) color reactions (pink color).

Conversions of **5** to isorhamnetin **6** was achieved by treating **5** with potassium metabisulfite solutions for 5 h at 100 °C.[17] After the completion of reaction, monitored by TLC (methanol 0.8: formic acid 0.2: toluene 3: ethyl acetate 3), the reaction mixture was poured into crushed ice when **6** precipitated as a pale solid and collected by centrifugation (55% yield). Isorhamnetin **6** was purified by column chromatography (SiO_2). It was characterized by UV, IR, NMR and HPLC and direct comparison with an authentic sample isolated from *Pluchea lanceolata*.[18]

Two other partial methyl ethers, tamarixetin **6a** and kaempferide **6d** were synthesized by the same sequence of reactions using **1** and MOM isovanillin **2a** and *p*-anisaldehyde **2d**, respectively. This protocol was also useful for the synthesis of polyhydroxy flavone-3-ols such as quercetin **6c** and kaempferol **6b**, which are the constituents of 'SBT flavone'. Identity of the compounds (Table 1) were established by comparison of the properties with authentic samples and literature data.[19]

Antioxygenic activities of flavonoids of seabuckthorn extracts have been associated with many bioactivities, such as radioprotection, cancer prevention, anti-aging and prevention of cardiovascular diseases.[20] Therefore it was of interest to carry out a comparative study of antioxygenic activities of SBT extracts, 'SBT flavone' and other common flavonoids (Table 2, a–y). For this the free radical scavenging activities were determined using quenching of DPPH. EC_{50} were calculated as μg required for inhibition of 50% of activity.[21] Trolox (g, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a reference antioxidant. Amongst the SBT extracts, 70% aqueous acetone extract of SBT leaves was found to be the most active; the seed and pulp oils showed lower activities. A sample enriched in phenolic PC (h) was isolated from 70% aqueous acetone extract was found to have high antioxygenic activity (EC_{50}, 12.8). Analysis of PC (h) showed the presence of quercetin **6c** (i), isorhamnetin **6** (p) and gallic acid (a). Amongst isolated pure compounds, gallic acid (a) showed the maximum activity followed by ellagic acid (b). Both these may be artifact of hydrolysable tannins present in SBT leaves. Quercetin **6c** (i) and kaempferol **6b** (j) showed much better activity compared to the major flavonoid, isorhamnetin **6** (p) of 'SBT flavone'. Isorhamnetin **6** (p, 3′-methyl ether), tamarixetin **6a** (u, 4′-methyl ether), kaempferide **6d** (r, 4′-methyl ether) are less active than the parent compounds viz. quercetin **6c** (i) and kaempferol **6b** (j). Gossypetin (c, 8-hydroxyquercetin) and quercetagetin (e, 6-hydroxyquercetin) showed high activities. Patuletin (f, 6-methyl quercetagetin) also showed similar trend in activities. Blocking of hydroxyl by glycosylation decreases the bioactivities considerably as can be seen for rutin (k, quercetin-3-rhamnoglucoside) and gossypin (o, gossypetin-8-glucoside). 8-Glucronide of gossypetin (l, hibifolin) showed good activity. Dihydroflavonols (v–y) did not show significant activity except dihydroquercetin **5c** (n). Thus it may be inferred that radical scavenging activities are dependent on number and location of hydroxyl groups. Presence of *o*-dihydroxy group acts as metal chelating systems also.



Scheme 1. Synthesis of flavone-3-ols: reagents and conditions: (a) KOH, DMF and 0 °C; (b) 30% H_2O_2, NaOH and methanol; (c) HCl, methanol and reflux; (d) $K_2S_2O_5$ soln, 100 °C.

Table 1
Yields, UV and IR spectral data of compounds

Chalcones	Yield (%)	UV nm	IR (CO) cm^{-1}	Epoxides	Yield (%)	UVnm	IR (CO) cm^{-1}	Dihydroflavonols	Yield (%)	UV nm	IR (CO) cm^{-1}	Flavonols	Yield (%)	UV nm
3	80–85	327	1677	**4**	85–90	285	1700,883.3*	**5**	56–59	288	1644	**6**	50–55	254,370
3a	78–82	305	1676	**4a**	73–79	283	1696,882.5*	**5a**	52–56	286	1635	**6a**	40–50	256,372
3b	85–88	322	1647	**4b**	79–85	276	1700,884.8*	**5b**	56–60	292	1636	**6b**	55–60	266,366
3c	80–83	330	1676	**4c**	70–75	282	1699,884.3*	**5c**	55–60	293	1640	**6c**	45–50	255,371
3d	80–88	328	1676	**4d**	75–80	275	1700,884.5*	**5d**	53–58	283	1634	**6d**	50–55	268,364

* Epoxide ring.

Table 2
Antioxidant properties

Products	EC_{50} value (μg)
(a) Gallic acid	3.8
(b) Ellagic acid	4.8
(c) Gossypetin	5.5
(d) Tannic acid	8.8
(e) Quercetagetin	10.5
(f) Patuletin	10.6
(g) Trolox	12.5
(h) PC	12.8
(i) Quercetin (**6c**)	14.4
(j) Kaempferol (**6b**)	14.6
(k) Rutin	15.5
(l) Hibifolin	16
(m) SBT flavone	18.5
(n) Dihydroquercetin (**5c**)	24
(o) Gossypin	25.5
(p) Isorhamnetin (**6**)	26.2
(q) Seabucthorn leaf 70% acetone ext	40
(r) Kaempferide (**6d**)	97
(s) SBT seed oil	>100
(t) SBT pulp oil	>100
(u) Tamarixetin (**6a**)	>100
(v) Dihydroisorhamnetin (**5**)	>100
(w) Dihydrotamarixetin (**5a**)	>100
(x) Dihydrokaempferol (**5b**)	>100
(y) Dihydrokaempferide (**5d**)	>100

In conclusion, a synthetic route to polyhydroxy- or partial methyl ethers of flavone-3-ols of biological importance has been achieved using readily accessible starting materials and mild reaction conditions. Amongst the compounds tested, gallic and ellagic acids were found to have higher antioxygenic activities compared to flavone-3-ols. Also we have found anti-diabetic and radio-protective activity of gallic acid.[22] Therefore due importance should be given to the presence of gallic and ellagic acids for bioactivity studies of SBT extracts. Since many flavone-3-ols show better activities when compared to flavones from SBT, it should be feasible to develop improved bioactive formulations compared to SBT products.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.07.008.

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16. *General synthetic procedure:* (a) **3**, **3a**, **3b**, **3c** and **3d**; The chalcone (**3**) was obtained by the condensation of 2,4,6-tri-MOM phloracetophenone (**1**, 1 mmol) with 4-MOM vanillin (**2**, 1 mmol) in DMF (5 ml) using powdered KOH (1 mmol) under anhydrous conditions at 0–4 °C for 4 h. Water (20 ml) was added and extracted with dichloromethane (30 ml × 2), dried with anhydrous sodium sulfate and solvent removed under reduced pressure. The product was purified by column (SiO_2) chromatography.
(b) Epoxides **4**, **4a**, **4b**, **4c** and **4d**: An aqueous solution of NaOH (4.8 ml, 5%) and H_2O_2 (5.0 ml, 30%) were added to a stirred solution of chalcone (**3**, 1 mmol) in methanol (20 ml) at rt (4 h). Water (100 ml) was added and extracted with ethyl acetate (30 ml × 2), dried with anhydrous sodium sulfate and solvent removed under reduced pressure. The products were purified by column (SiO_2) chromatography.
(c) Dihydroflavonols **5**, **5a**, **5b**, **5c** and **5d**: Epoxide (**4**, 0.9 mmol) in methanol (44 ml) and concentrated HCl (2 ml) were refluxed for 20 (min) and poured into ice-water. The product was collected by centrifugation.
(d) Flavonols **6**, **6a**, **6b**, **6c** and **6d**: Dihydroflavonols (**5**, 0.3 mmol) in ethanol (2.5 ml) was added to potassium metabisulphite, (5.0 ml, 20%) and heated at 100 °C (5–8 h). The reaction mixture is poured into crushed ice. The centrifuged product was purified by column (SiO_2) chromatography. The purity of final products were analyzed by HPLC and NMR (Supplementary data).
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