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Antioxidant isoflavonoids from rhizomes of *Iris kumaonensis*

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ABSTRACT

The antioxidant activity of isoflavonoids namely irigenin, iristectorin-A, tectoridin and tectorigenin isolated from the methanolic extract of *Iris Kumaonensis* rhizome was assayed by ABTS mediated TEAC methods. These compounds were moderate to good in antioxidant activities. Irigenin is responsible for better anti-oxidant properties reported here for the first time. Structures of the compounds were elucidated by classical spectroscopic methods like IR, 1D, 2D NMR and comparison with literature data.

KEYWORDS

Antioxidant activity;
Iris kumaonensis;
Isoflavonoids;
Irigenin.

INTRODUCTION

Iris kumaonensis Wall ex. Don.V. Lhathum, (Iridaceace) is a perennial herb growing wild in western Himalaya from Kashmir to Uttranchal in Central Himalaya at altitutudes 2500-4000 m. Rhizomes of this plant are used for fever[1] and roots are used for kidney infection. Genus *Iris* consists of three hundred species and is distributed throughout the world. Twelve species are found in India[2]. *Iris* species are known to be rich in isoflavones, flavones[3] and quinones[4]. These classes of compounds have attracted considerable attention because of their antioxidant[5] and cytotoxic[6] properties. Two isoflavones Iridin and Iriskumonin[7] have previously been reported from the rhizomes of *I. kumaonensis*. During our ongoing research on biopro spection of novel secondary metabolites from western Himalayan flora, we have isolated irigenin, iristectorin-A, tectoridin and tectorigenin. Several studies have revealed that plants produce potent antioxidants to control the oxidative stress caused by sunbeams and oxygen and represent source of new compounds with antioxidant activity[8]. Flavonoids and other phenolic compounds of plant origin have been earlier reported as free radical scavengers and inhibitors of lipid per oxidation[9].

In this work four pure flavonoids, both glycosides and agycones, isolated from the rhizomes of *I.kumaonensis*, were tested for the antioxidant activity by TEAC assay. Oxidation is well known to be a major cause of material degradation. More recently, oxygen-reactive species, in particular free radicals have been recognized to be involved in several diseases, including cancer and atherosclerosis. Ageing may also be the result of the deleterious free-radical reactions which occur throughout cells and tissues[10]. In this context, nowadays natural antioxidants are receiving increasing attention; particularly, flavonoids have been reported to be efficient antioxidants by scavenging oxygen radicals[11] having interesting anti-cancer, hypolidaemic, anti-ageing, and anti-inflammatory activities[12]. For all these reason the study of known and new natural derivatives could support the development of new drugs and improve the treatment of various diseases .To our knowledge, no previous investigation has been done on the radical scavenging effects of pure compounds obtained from this plant.

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EXPERIMENTAL

The rhizomes of *I.kumaonensis* were collected from Jalori Pass, Kullu district at 3500m attitudes in Himachal Pradesh, India during October 2004.

Fresh rhizomes (1 kg) were cut in small pieces and extracted with n-Hexane, followed by chloroform. The marc left after chloroform extraction was further extracted with methanol, and 80 gms of the extract was obtained.

Fresh rhizomes (1.0kg) were cut in small pieces and extracted with n-Hexane, followed by chloroform. The marc left after chloroform extraction was further extracted with methanol, and 80.0 g of the extract was obtained. 40.0g of this crude methanolic extract was chromatographed over silica gel (60-120 mesh) using pure chloroform. Fractions 1-4 did not show presence of any compound but subsequent fractions 5-17 eluted with chloroform/methanol (99:1) were pooled together and on removal of the solvent afforded compound **(1)** irigenin (114.5mg), which showed a single spot on TLC plate run in chloroform/methanol (98:2). Repeated chromatographed on silica gel using chloroform / methanol (96:4) afforded compound **(2)**, iristectorin-A, (48.0mg) was purified after crystallization in methanol. Again the fraction eluted in chloroform/ methanol (85:15) gave compound **(3)** (tectoridin), (35.4mg). Further rechromatography on silica gel of fractions 7-14 with chloroform/ethyl acetate (97:3) afforded compound **(4)** tectoregenin (43.0mg) which showed a single spot on TLC plate chloroform/ethyl acetate/acetone (96:3.5 :0.5).

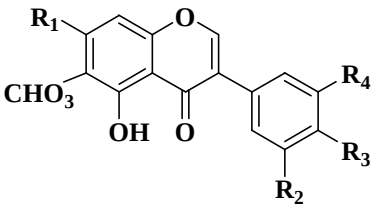
Antioxidant activity of the compounds was tested as the ability of the compounds to reduce the preformed radical mono-cation of $ABTS^+$ [2,2'–azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid], as described [13]. In brief, $ABTS^+$ was produced by the oxidation of 7.0mM ABTS by 2.45mM potassium sulfate in aqueous media under dark condition. For experimental purpose, the ABTS solution was diluted with ethanol to obtain an absorbance of 0.70 at 734nm at 30.00C. The reaction was initiated by the addition of 1.0 ml of diluted ABTS to 10.0 uL of each solution. The percentage inhibition of absorbance at 734nm was calculated and plotted as a function of concentration of antioxidants. The TEAC value is defined as the concentration of standard Trolox with the same antioxidant capacity as a 1.0 mM concentration of the antioxidant. All the four compounds identified as irigenin, iristectorin-A, tectoridin and tectorigenin give TEAC values as 4.42, 3.32, 2.83 and 3.22 mM respectively (TABLE 1).

TABLE 1: Antioxidant activity of isoflavonoid compounds as measured by TEAC assay

Compounds	TEAC (mM)
Irigenin	4.42± 0.17
Iristectorin-A	3.32±0.17
Tectoridin	2.83±0.31
Tectorigenin	3.22±0.12

RESULTS AND DISCUSSION

The structures of the compounds were elucidated by classical spectroscopic methods like IR, 1D, 2D NMR and comparison with literature data. Compound **(1)** irigenin (114.5mg) was isolated with chloroform/ methanol, which showed a single spot on TLC plate run in $CHCl_3$/MeOH (98:2) and analysed for $C_{16}H_{12}O_6$. ^{13}C NMR (300MHz, $CDCl_3$-d_1): δ 153.9(C-2), 122.2.0 (C-3), 181.5 (C-4), 153.5 (C-5), 130.3 (C-6), 153.5 (C-7), 93.9 (C-8), 157.7 (C-9), 105.6 (C-10), 122.2(C-1′), 105.6 (C-2′), 153.9 (C-3′), 131.7 (C-4′),153.9 (C-5′), 115.2 (C-6′), 59.8 (6-OMe), 58.4 (4′-OMe), 56.3 (5′-OMe). On repeated chromatography on silica gel using $CHCl_3$ / MeOH (96:4) afforded compound **(2)**, iristectorin-A, (48.0 mg) analysed for $C_{17}H_{14}O_7$. ^{13}C NMR (300MHz, $CDCl_3$-d_1): δ 154.5 (C-2), 120.9 (C-3), 181.0 (C-4), 153.1 (C-5), 130.9 (C-6), 153.6 (C-7), 93.3 (C-8), 156.5 (C-9), 104.8 (C-10), 122.1(C-1′), 115.2 (C-2′), 112.4 (C-3), 159.2 (C-4′),113.0 (C-5′), 123.4 (C-6′), 61.4 (6-OMe), 57.4 (4′-OMe). Again the fraction eluted in $CHCl_3$ / MeOH (85:15) gave compound **(3)** (tectoridin), (35.4 mg) analysed for $C_{22}H_{22}O_{11.}$ ^{13}C NMR (300MHz, DMSO-d_6): δ 157. (C-2), 121.8 (C-3), 181.4 (C-4), 153.3 (C-5), 131.0 (C-6), 151.2 (C-7), 94.7 (C-8), 153.6 (C-9), 107.3 (C-10), 121.8 (C-1′), 131.0 (C-2′), 116.0 (C-3′), 157.2 (C-4′), 116.0 (C-5′), 133.1 (C-6), 61.2 (6-OMe) 58.4 (4′-OMe), 56.3 (5′-OMe), 100.8 (Glu-C-1″), 73.8(C-2″), 77.2 (C-3″), 69.0 (C-4″), 77.8(C-5″/), 60.7(C-6″). Further rechromato graphy on silica gel of fractions 7-14 with chloroform/ ethyl acetate (97:3) afforded compound **(4)** tectoregenin (43.0mg) which showed a single spot on TLC plate chloroform/ethyl acetate/acetone(96:3.5:0.5) analysed for $C_{18}H_{16}O_7$. ^{13}C NMR (300MHz, $CDCl_3$-d_1): δ



	R_1	R_2	R_3	R_4
1	OH	OH	OMe	OMe
2	OH	H	OMe	OH
3	OGlu	OH	OMe	OMe
4	OH	H	OH	H

Figure 1: Isoflavonoids from *Iris kumaonensis*

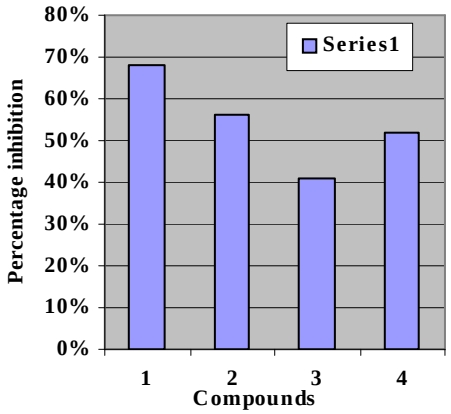


Figure 2: Antioxidant actvity of isoflavonoids from methanolic extract of *I.kumaonensis*

153.3 (C-2), 120.0 (C-3), 181.6 (C-4), 153.4 (C-5), 130.9 (C-6), 153.4 (C-7), 93.6 (C-8), 155.5 (C-9), 106.8 (C-10), 124.1(C-1′), 115.2 (C-2′), 111.7 (C-3), 130.2 (C-4′),111.0 (C-5′), 115.4 (C-6′), 61.2 (6-OMe).

The identification of these compounds was carried out by spectral analysis (IR, MS, 1D and 2D NMR). Compound **(1-4)** (figure 1) were identified as irigenin, iristectorin-A, tectoridin and tectorigenin with 68%, 56%, 41% and 52% antioxidant activity respectively (figure 2). The result of our experiment showed that irigenin posses a potent oxidation inhibition, a value greater than the remaining compounds (TABLE 1). Antioxidant activity of natural isoflavonoids is governed by number and location of total hydroxyl and methoxy group[14]. The antioxidant activity may increase with the number of total hydroxyl groups probably due to the vulnerable loss of proton and stability of the radical intermediate because of the resonance delocalization[15]. The TEAC radical-scavenging activities of compounds **(1-4)** were detected (TABLE 1). It is noticed that most of the isolated compounds showed obvious scavenging activity on ABTS radicals. All of the isoflavonoids derivatives displayed stronger activities than that of Trolox. The orders of the activities of flavonoids derivatives were to be as 1,2,4,3. High antioxidant activity of irigenin could be attributed to the highest number of hydroxyl and methoxy group present in the compounds. In our knowledge no previous investigation has been done on the antioxidant activity of isoflavonoids from *I.kumaonensis*.

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