Antioxidant Activity- Guided fractionation of aqueous extracts from *Lepidium sativum* and identification of active flavonol glycosides

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ABSTRACT: *Lepidium sativum* (Fam. Brassicaceae), a popular vegetable producing plant of low reaches of Kumaun Himalaya, is an annual herb and its leaves are used as a Traditional Medicines to cure abdominal pains, asthma and lowering blood pressure. An aqueous Extract derived from the leaves of the plant, highly food supplement for human,has previously been screened for various biological activities, hypotensive, antimicrobial, bronchiodilator, hypoglycemic and allelopathic. n-Butanol fraction of aqueous-methanolic extract, a highly flavonoid enriched and antioxidative active against DPPH and ABTS free radicals, afforded three flavonol glycosides, quercetin-3-O-β-glucosyl (1 → 2)– glucopyranoside- 7-0- glucopyranoside, kaempferol-3–O– β-glucosyl (1 → 2)- glucopyranoside – 7 – O – β – glucopyransoide and isorhamnetin – 3 – O – sophoroside -7 – O – β – D – glucopyransoide . The antioxideant activity of quercetin – 3, 7 – di-O-glycoside was found higher compared to the corresponding 3,7–di-o-glycosides of kaempferol and isorhamnetin.

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KEY WORDS: - *Lepidium sativum*, antioxidative activity, Flavonol glycosides.

INTRODUCTION

Lepidium sativum (Fam. Brassicaceae) is a small annual herb with 30 to 50 cm in height and bears laciniate pinnate entire leaves. It is a food plant and leave are used as a traditional vegetable by some ethnic groups of Kumaun Himalayas. The aqueous extracts, of the plant has previously been screened for various biological activities, cardiotonic hypotensive, antimicrobial, bronchiodilator, hypoglycemic and allelopathic (Kartar and Akulyan, 1971; Singh et al, 1984; Carbajal et al, 1991; Hasegawa et al, 1992; Alcalade et al, 2005, Osana et al, 2006). Glucosinolates, a class of naturally occurring thioglycosides, have been identified as a principle bioactive constituents of *Lepidium sativum* (Fahey et al, 2001). An essential oil composition and imidazole alkaloids from the plant have been investigated (Maier et al, 1998; Mirza and Navaei, 2006). The flavonoids, a diversified group of secondary metabolites and a polyphenolic hetrocylic compounds biogenetically synthesised from phenyl propanoids, have been identified as a potent antioxidants and have widely been used to cure diseases associated to oxidative stress (Jovanovic et al, 1994; Shui and Peng, 2004).

It has previously been established that the curing of various disease from the extracts of traditional medicinal plants have been attributed to the presence of polyphenolic compounds particularly flavonoids. The polyphenolic fraction from the H_2O extract of the plants, a highly recognised hydrogen and electron donating and a class of potent radical scavenging activity bearing fraction, have highly been investigated previously from various traditional medicinal food and fodder plants for active flavonoids. Presence communication reveals isolation and characterisation of flavonol glycosides from the antioxidative active fraction of n-BuOH soluble.

MATERIAL AND METHODS

Plant material

The leaves of *L. sativum* were collected from the agriculture fields of Kumaun Himalayan hills in September 2010. The plant was identified by professor Y.S. Pangati, Department of Botany, Kumaun University,Nainital. A voucher specimen by *L. sativum* (No. 43) was deposited in the chemistry department of Kumaun University at Almora (Campus).

Extraction and isolation

5 kg. air dried and powdered aerial parts of *Lepidium sativum* was extracted with 80% aq. MeOH for six days by cold percolation method. The extract was filtered and evaporated to dryness under reduced pressure until only H_2O layer (approx 50 ml) remained. It was partitioned with dichloromethane and H_2O (1:1). After removing CH_2Cl_2 fraction, H_2O layer was further partitioned with EtOAc and n-BuOH. The n-BuOH soluble was concentrated in vacuo at 60^0C and the residue was chromatographed on sephadex LH-20 cc using H_2O as an initial eluting solvent then successively eluted with 10% MeOH to 100% MeOH. A total of 90 fraction (each with 200

ml) were collected and each fraction was chromatographically examined on cellulose TLC Using 15% HoAc as an eluent. Fractions containing similar compounds were combined into three major fractions, with fraction–I (30–50% MeOH) containing 30 – 45, fraction – II (60% MeOH), containing (60 – 65) and Fraction–III (90 – 100% MeOH) containing (70 – 75). Eluates derived from Fraction I, II and III were separately concentrated under reduced pressure and examined on 2DPC using BAW and 30% HOAc as a developing solvent and ammonical $AgNO_3$ as a spraying reagent. The fraction I, II and III produced 3, 3 and 2 flavonoid positive spots respectively on 2DPC.

RESULTS AND DISCUSSION

Fraction, I, II and III were separately evaluated for antioxidant activity by DPPH assay and characterized by the concentration which produced 50% quenching of the free radical.

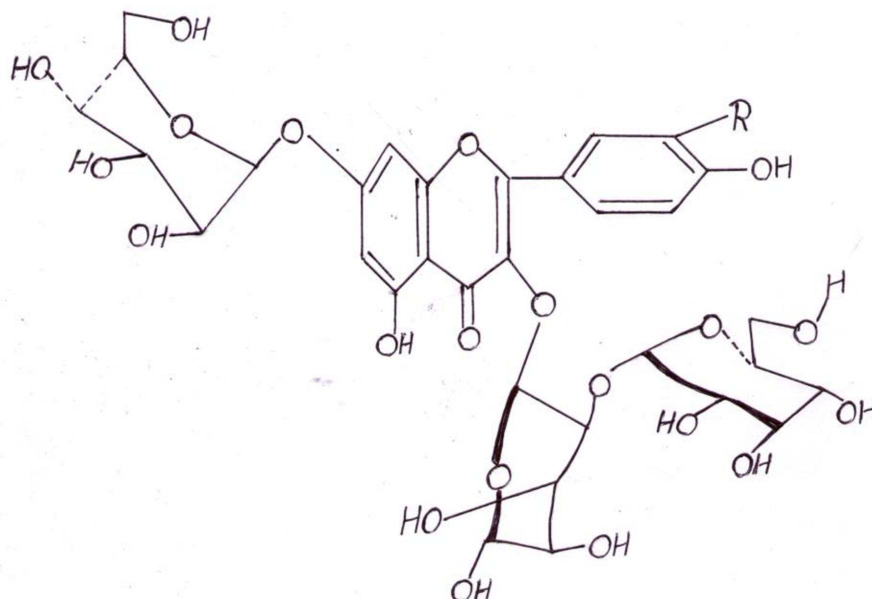
Fractions	DPPH Assay IC 50 ±SD (µg/ml)
Fraction–I	5.04 ±0.40
Fraction–II	12.05 ±0.17
Fraction–III	13.06 ±0.05

Fraction–I, which has been identified as a prominent antioxidant compared to Fraction–II and III, was analysed for positive flavonoid compounds. Fraction-I was evaporated to dryness and it was dissolved into MeOH. The MeOH soluble was band on whatman N.3 PC and repeatedly developed three times in BAW (4:1:5). Three Fluorescent (dark purple violet) band were observed on PC and each was cut and eluted separately in 70% aq. EtOH. An eluate derived from faster fluorescent bands representing compounds 1, 2 and 3 respectively.

Compound 1, representing a violet fluorescent spot on PC at Rf 43 in BAW, was identified as a flavonol glycoside on the basis colour reactions with $FeCl_3$, α–napthol, Mg+HCl, ammonical $AgNO_3$ and NA reagent (Mabry et al, 1970; Markham, 1982).Complete acid hydrolysis of 1 with 2NHCl afforded isorhamnetin and glucose and were characterized by comparing with their standards on PC. LC-MS-FABMS (deprotonated) exhibited molecular ion at m/e 801 $(M\text{-}H)^-$ and other prominent ions observed at m/e 639 (m/e 801– glucose)– and m/e 315 $[(m/e\ 639–(glucose + glucose)]^-$ suggesting abstraction of three molecules of glucose from isorhamnetin. H_2O_2 oxidation of 1 gave a disaccharide sugar molecule, sophrose(glucose 1 → 2 glucose) and an aglycone representing compound 1(a). The UV, MS, ^{1}HNMR and ^{13}CNMR Data of 1(a) were found similar to those reported in the literature to isorhamnetin-7–O–β–D-glucopy ranoside. Thus, compound 1 could be identified are isorhamnetin–3–0-sophoroside–7–0–glucoside.

Finally, the structure of 1 was identified by ^{1}HNMR in DMSO-d_6 (400 MHz); ^{1}HNMR of 1 showed five signals in aromatic region at δ 6.42 (1 H,d, J = 2.0 Hz), δ 6.77 (1 H,d, J = 2.0 Hz), δ 6.96 (1 H, d, J= 8.5 Hz), δ 7.66 (1H,d,2.0Hz) and δ 7.82 (1H,dd, 2.0 and 8.5 Hz) representing H-6,H-8,H-5',H-2' and H-6',respectively of isorhamnetin. The anomeric protons at δ 5.72 (d, 7.6Hz) and δ 5.10(d, J=7.5Hz) were attributed to two glucose moieties (β –configuration) directly linked to the aromatic rings at 3- and 7– positions, respectively. A third anomeric proton was located upfield at δ 4.60 (1H, d, 7.8Hz) was assigned to a glucose moiety linked to the 3-0-glucosyl moiety with interglycosidic linkage, 1 → 2 (Altona and Haasnoot, 1980). Thus, compound 1 was identified as isorhamnetin – 3 – 0 – glucosyl (1 → 2) – glucophyranoside – 7 – 0 – β – D – glucopyranoside.

Compound 2, representing a dark purple fluorescent on PC under UV light at Rf 40 (in BAW) gave positive colour reactions to Mg+HCl, α-naphthol and $FeCl_3$ – K_4 Fe $(CN)_6$, complete acid hydrolysis of 2 with 2 NHCl afforded Kaempferol (CoPC), and glucose (CoPC). H_2O_2 oxidation of 2 gave sophrose (CoPC) and a fluorescent yellow aglycone 2(a). The 2(a) was identified as kaempferol – 7-0 – β – D – glucopyranoside by UV, ^{1}HNMR and FABMS studies. LC–FABMS (deprotonated) gave a molecular ion at m/e 771 $(M–H)^-$ and other prominent ions observed at m/e 609 (m/e 771 – glucose) and m/e 285 (m/e 609 – (glucose + glucose) – representing three molecules of glucose from kaempferol.



COMPOUNDS:

1. R=OCH_3
2. R=H
3. R=OH

^{1}HNMR of 2 in DMSO-d_6 (400 MH_z) gave four signals in aromatic region at δ 6.47 (1H, d, 2.0 H_z), δ 6.81 (1H, d, 2.0 Hz), δ 6.90(2H, d, 8.5 Hz) and δ 8.05(2H,d,8.5 Hz) were assigned to H-6, H–8, H–3'/5' and H–2'/6' of kaempferol. The anomeric proton signals appeared at δ 5.72 (d, J = 7.6 H_z), δ 5.10 (d, J=7.5 z_2) and δ 4.60 (d, J = 7.8 Hz) were found similar to the corresponding anomeric proton signals of compound 1. Thus, 2 was identified as kaempferol – 3 – 0 – β –glucosyl – (1 → 2) – β – glucopyranoside – 7 – O – β – glucoyranoside.

Compound 3, representing a dark purple fluorescent spot on PC at Rf 36 (in BAW), gave a positive colour reactions with $FeCl_3$, Mg + HCl, $AgNO_3$ (Ammonical), α-naphthol and NA (in MeOH), suggesting a flavonol glycoside. Complete acid hydrolysis of 3 with 2 NHCl afforded quercetin (CoPC) and glucose (CoPC). LC – FABMS (–) of 3 exhibited a molecular ion at m/e 787 $(M–H)^-$ and other prominent ions observed at m/e 625 (m/e 787 – glucose) $^-$ and m/e 301 (m/e 787 – (glucose + glucose)$^-$, representing abstraction of three molecules of glucose from quercetin. ^{1}HNMR showed five signals in aromatic region at δ 6.41 (1H, d, 2.0), δ 6.74 (1H, d, 2.0 Hz), δ 6.80 (1H, d, 8.5), δ 7.50 (1H, d, J = 2.0 Hz) and δ 7.50 – 7.60 (1H, dd, 2.0 and 8.5 Hz), representing H – 6', H – 8, H – 3', H-2' and H-6', respectively of quercetin. The anomeric proton signals of 3 were found similar to the corresponding anomeric protons of 2 and 1. Thus compound 3 was identified as quercetin – 3 – O – β – glucosyl (1 → 2) – glucopyranoside – 7 – O – β – D – glucopyranosides.

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