

Research Article

Spectroscopy characterization and Antioxidant activity of 3-(1-((1Z,3Z)-5-amino-1,7-dihydroxy-3-mercapto-7-methylocta-1,3-dienyl)-2-hydroxy-5-methyl-4-((Z)-prop-1-enyl)bicyclo[3.3.1]non-6-en-3-yl)-1,7-dihydroxytetracene-5,11(4aH,11aH)-dione obtained from Nepalese pepper (*Zanthoxylum piperitum* DC) fruit

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Abstract

During present investigation attempts were made to find out the active phytochemical components. It is felt that, the investigation will enlighten the importance of much neglected species of *Zanthoxylum piperitum* DC and its taxonomic significance along with its exploitation as a medicinal plant. The quantitative and qualitative analysis is very essential for identifying the compounds present in the medicinal plants. The experiments were carried out in the laboratory using fruit parts of Nepali pepper and characterized by spectral techniques.

Introduction

Over the centuries, human beings gathered information by trial and error. In ancient times humans use various plants and herbs that grew in their environment to treat various illnesses. Medicinal plants have emerged as some of the most widely studied plants and significant interest has been shown in their chemistry because of their potential application in medicine. Many of these medicine plants contain chemical constituents that could cause harmful effects to human if taken in large quantities. Alkaloids occurring in a large amount make these plants poisonous [Tsutomu Hatano et al., 2004, & Tsutomu Hatano, 2008]. Demand on medicinal plant products such as pharmaceuticals, phytochemicals, nutraceuticals, cosmetics and other products worldwide are increasing day by day. Herbal medicinal products may vary in composition and properties unlike

conventional pharmaceutical products. Correct identification and quality assurance of the starting materials is therefore an essential prerequisite to ensure reproducible quality of herbal medicine which contributes to its safety and efficiencies. Some metals ions were present in herbs play significant role in medicine [Francesco Epifano, 2007 & K. Kamsuk, 2006]. In the same way the Nepali Pepper (*Zanthoxylum piperitum* DC) commonly named as timur vigorously uses in Himali regions and have been used as ethnomedicine as well as spices since a long time. Lipid peroxidation and free radicals are well known to be among the main causes for compromising the quality of food during processing and storage. Therefore, it is obvious that the prevention of lipid peroxidation in the food is effective in not only the stability of the nutritional content but also the extension at the best-before date. In living systems, biomembranes are composed

of lipids including unsaturated fatty acids that react easily to form lipid peroxide and free radicals. Accumulation of lipid peroxides in living systems induces functional abnormalities and pathological changes. Therefore, much attention has been paid to the antioxidants, which are expected to work effectively to prevent food and living systems from peroxidative damage. Though butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) [Lian Xia et.al.,2011 & Kyoung Hwa Jang,2008] , which are artificial strong antioxidants, have been much used in the food industry, they are suspected to be toxic in the lung and carcinogenic . Hence, the importance of research for finding natural antioxidants has been greatly increased in recent years Nepalese pepper (*Zanthoxylum piperitum* DC.) that belongs to the Rutaceae family is a deciduous and shrubby tree and is distributed in the Indian sub-continent.[Daniel F. et.al. 2010 & Xiaogen Yang,2008] The aims in this work were to isolate, identify, and characterize and antioxidant activity of isolated compound.

Materials and methods

All the chemicals used were analytical grade and Nepali pepper obtained from local market. The pericarp of *Z. piperitum* fruits was homogenized in MeOH, and the EtOAc-soluble portion of the homogenate was subjected to Toy pearl HW-40C, MCI-gel CHP-20P, and YMC-gel ODS chromatographic separations. The fractions that contained the residue were purified by preparative HPLC to obtain one compounds, which were designated as ZP-one. The ZP-one was obtained as unstable colorless syrup. The isolated residue was characterized by different spectral techniques. Their IR spectra were recorded on Perkin – Elmer FT- IR spectrophotometer in KBr pellets. The electronic spectra were recorded in water on Beckman DU-64 spectrophotometer with

quartz cells of 1 cm path length and mass spectra (TOF - MS) were recorded on Waters (USA) KC- 455 model with ES⁺ mode in DMSO-d₆. ¹H NMR spectra were recorded in DMSO-d₆ solvent (solvent peak 4.8ppm) on a Bruker Advance 400 instrument

Purification and identification of the antioxidants from the methanol extract

The methanol extract of Nepalese pepper fruit was successively subjected to preparative HPLC. Two slightly yellow pigments were isolated in 45% and 50% methanol fractions; pigments 1 and 2, respectively It was confirmed that each of the purified pigment was due to a single peak as evidenced by a reversed-phase HPLC using a semi-micro column. The purified pigments were collected and used for identification and estimation of DPPH radical-scavenging activity. Each pigment was adjusted by dilution with methanol so that the absorbance of the major absorption peaks around 250 nm (band II peak region) and 355nm (band I peak region) gave reading in the region 0.6–0.8. The UV/Vis spectrum of pigment 1 showed two peaks at 255 and 368 nm and a shoulder at 270 nm, which indicated that pigment, was a flavones or flavonol. The presence of a free hydroxyl-group at the 40-position in the B-ring was indicated by a bathochromic shift of 50 nm of band I at 355 nm after addition of sodium methoxide. A new peak appeared at 338 nm suggesting the presence of a free hydroxyl-group at the 70-position. Since addition of sodium methoxide to pigment 1 did not induce immediate disappearance of band I, absence of a free hydroxyl-group at the 3-position in the C-ring was indicated. The addition of anhydrous aluminum chloride produced a bathochromic shift of 81 nm of band I at 358 nm, and a hypochromic shift of

33 nm of band I. Then two new peaks at 300 and 357 nm appeared upon the addition of hydrochloric acid (AlCl_3/HCl) indicated that pigment 1 was 5-hydroxy-3-substituted flavonol. The addition of anhydrous sodium acetate produced a bathochromic shift of 19 nm for band II at 255 nm, which indicated the presence of a free hydroxyl-group at the 7-position in the A-ring. A bathochromic shift of 22 nm of band I at 358 nm upon addition of boric acid-sodium acetate ($\text{NaOAc}/\text{H}_3\text{BO}_3$) indicated the presence of a free O-dihydroxy group in the B-ring. The UV/Vis spectrum of pigment 2 showed two peaks at 254 and 348 nm, and a shoulder at 266 nm, indicating that pigment 2 was a flavone or flavonol. The presence of a free hydroxyl-group at the 40-position in the B-ring was indicated by a bathochromic shift of 47 nm for band I at 348 nm after the addition of sodium methoxide. A new peak appeared at 324 nm suggesting the presence of a free hydroxyl-group at the 70-position. Since addition of sodium methoxide to pigment 2 did not induce immediate disappearance of band I, absence of free hydroxyl-group at the 3-position in the C-ring was indicated. The addition of anhydrous aluminum chloride produced a bathochromic shift of 83 nm of band I at 348 nm. Then a hypochromic shift of 29 nm for band I and two new weak peaks at 303, 348 nm appeared upon the addition of hydrochloric acid (AlCl_3/HCl), indicating that pigment 2 was 5-hydroxy-3-substituted flavonol. Addition of anhydrous sodium acetate produced a

bathochromic shift of 14 nm of band I at 255 nm, which indicated the presence of a free hydroxyl-group at the 7-position in the ring [Satoshi Ogawa et al. 2009]. A bathochromic shift of 20 nm of band I at 348 nm upon addition of boric acid-sodium acetate ($\text{NaOAc}/\text{H}_3\text{BO}_3$) indicated the presence of a free O-dihydroxy group in the B-ring. To identify the molecular structures, these pigments were subjected to acid hydrolysis and TLC. The hydrolysates were extracted with ether. Each of the ether and residual aqueous layer was subjected to TLC. As a result, both the ether layers of these pigments corresponded with authentic quercetin (3, 5, 7, 30, 40 pentahydroxyflavone) and the aqueous layers of pigment 1 and 2 corresponded with authentic galactose and rhamnose, respectively. Each intact pigment 1 and 2 was in good agreement with hyperoside (quercetin-3-O-galactoside) and quercitrin (quercetin-3-Orhamnoside), respectively. Pigments 1 and 2, and their ether layer of the hydrolysates were individually analyzed by ESI/MS.

Results and Discussion

The elemental analysis has found which determined the molecular formula and confirmed by ESI-MS spectra. The percentage of chemical constituents as C, 70.25%; H, 6.63%; N, 2.05%; O, 16.38%; S, 4.69%, M.P. 75°C and molecular formula is $\text{C}_{40}\text{H}_{45}\text{NO}_7\text{S}$.

1 IR spectra and mode of bonding

The infrared spectrum of isolated compound appeared at (OH) (3363cm^{-1}), (CH₂)stretching, CH₃, Ch-aromatic ring (2924cm^{-1}), (CH) (2854cm^{-1}), (C=O) (1709cm^{-1}), (aliphatic) (1365cm^{-1}), (CSH) (1258cm^{-1}), (aliphatic and aromatic) (828cm^{-1}).

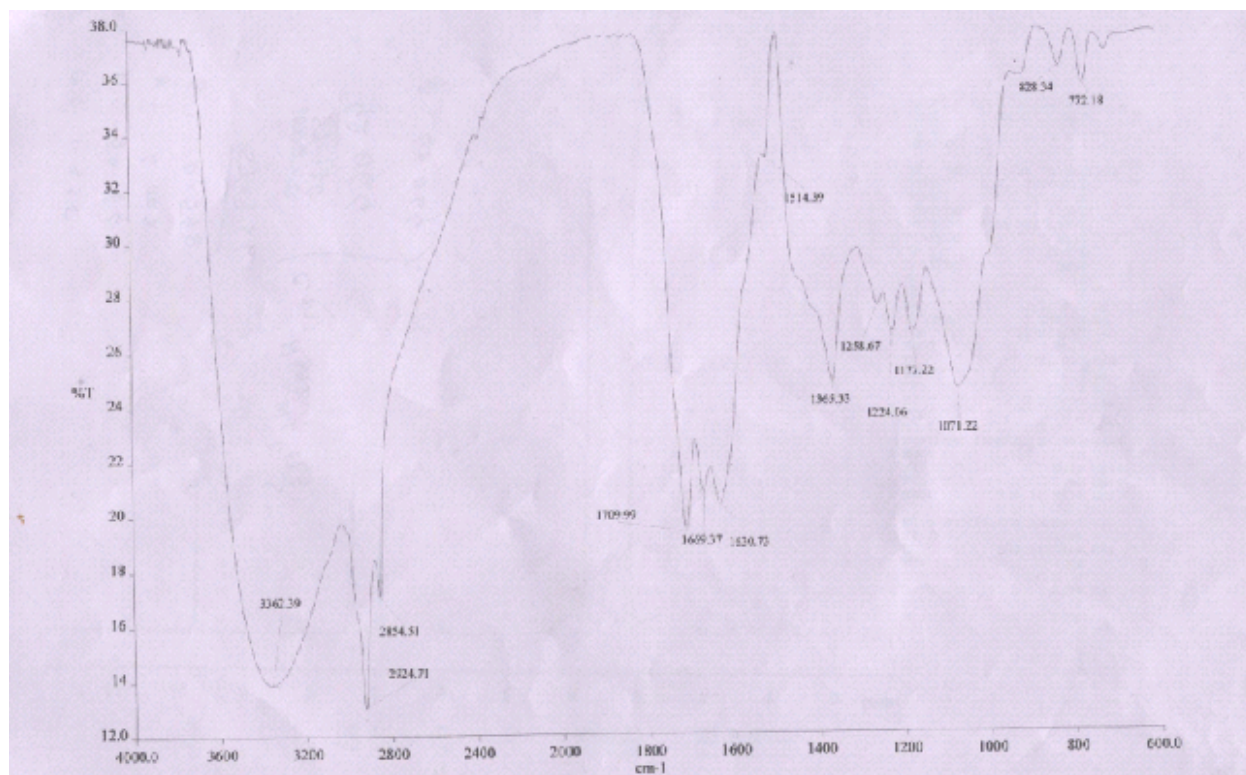


Figure 1. FT-IR spectra of isolated compound

3.3 ¹H NMR spectra

The ¹H nmr spectra in a DMSO-d₆ solvent of the various assignments of ¹H nmr of the isolated compound is summarized as chemical shift are in ppm from TMS & multiplicity in parentheses (bd: broad; d: doublet; m: multiplet) The ¹H NMR spectrum [in (showed signals of three pairs of protons

on trans-olefinic carbons [d 6.04 (1H, dt, J = 15.5, 1 Hz, H-2), 6.78 (1H, dt, J = 15.5, 7 Hz, H- 3); 6.28 (1H, dd, J = 6, 15 Hz, H-7), 6.43 (1H, br dd, J = 11, 15 Hz, H-8); 7.22 (1H, dd, J = 11, 15.5 Hz, H- 9), 6.10 (1H, d, J = 15.5 Hz, H-10)], protons of one methine [d 4.23 (1H, br q, J = 6 Hz, H-6)], three methylene [d 2.27 (2H, m, H-4), 1.65 (2H, m, H-5), and

3.22 (2H, d, $J = 6$ Hz, H-10)] and three methyl [d 1.11 (6H, s, H-30/H-40) and 2.21 (3H, s, H-12)] carbons, and an NH proton [d 7.42 (1H, br t, $J = 6$ Hz)]. The correlations H-2–H-3–H-4–H-5–H-6–H-7–H-8–H-9–H-10, and H-10–SH among these signals were revealed by

vicinal correlations that were observed in the 1H–1H COSY spectrum. The spectrum also showed the presence of the 1H–1H long-range correlations H-10–H-12 and H-10–H-30/H-40.

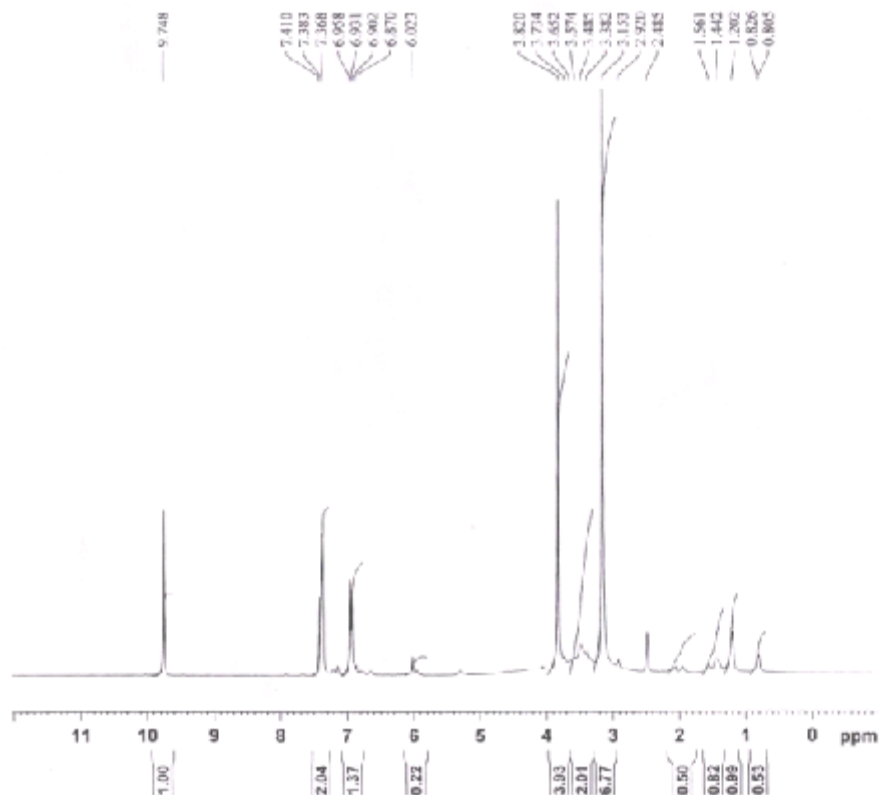


Figure 2. ^1H NMR Spectra of isolated compound

ESI- MS

The electrospray-ionization mass spectrometry (ESI-MS) showed ion peaks corresponding to the molecular formula $\text{C}_{40}\text{H}_{45}\text{NO}_7\text{S}$, and this was confirmed by high-resolution (HR) ESI-MS. The fragmentation of the molecule is m/z : 683.29 (100.0%), 684.30 (44.0%), 685.30 (10.9%), 685.29 (5.0%), 686.30 (2.1%), 686.29 (2.0%), 684.29 (1.2%)

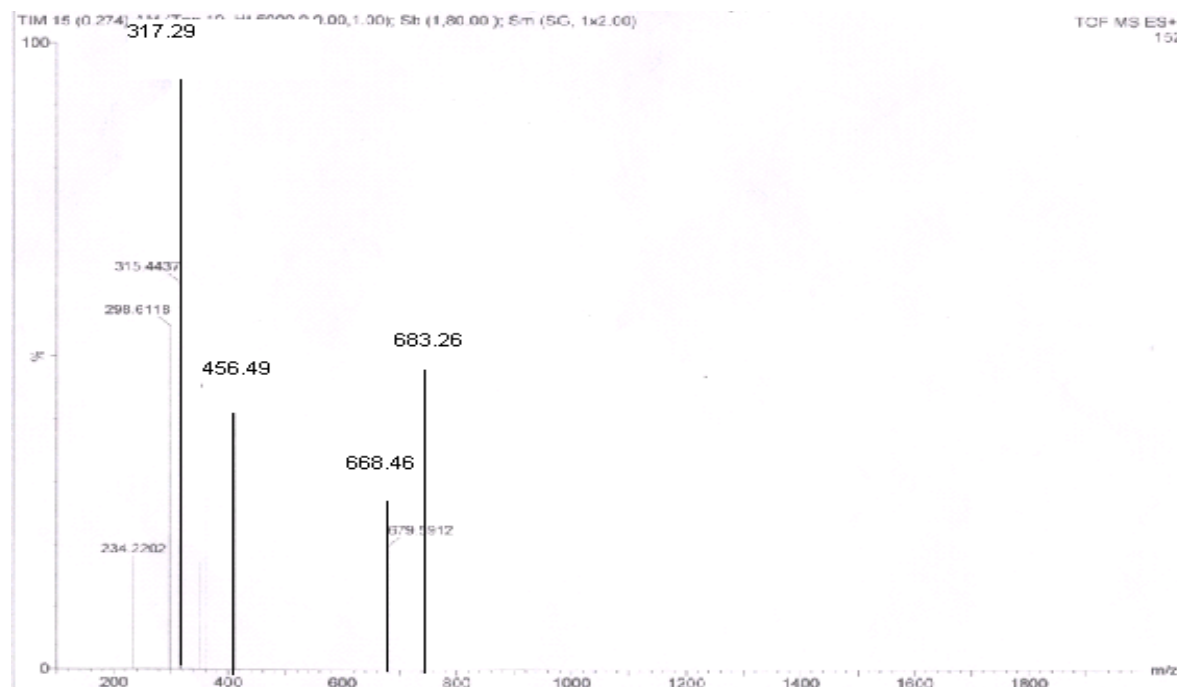


Figure 3. ESI mass spectra of isolated compound.

3D- Molecular modeling

3D molecular modeling of the proposed structure of the complexes was performed using CsChem3D Ultra 12 program package. The correct stereochemistry was assured through the manipulation and modification of the molecular coordinates to obtain reasonable low energy molecular geometries. The potential energy of the molecule was the sum of the following terms: $E = E_{\text{str}} + E_{\text{ang}} + E_{\text{tor}} + E_{\text{vdw}} + E_{\text{oop}} + E_{\text{ele}}$. Where all E's represent the

energy values corresponding to the given types of interaction (kcal/mol). The subscripts str, ang, tor, vdw, oop, and ele denote bond stretching, angle bonding, torsion, deformation, Van der Waals interactions, out of plane bending and electronic interaction, respectively. The molecular mechanics describe the application of classical mechanics to determination of molecular equilibrium structures. The optimized structure to be proposed.

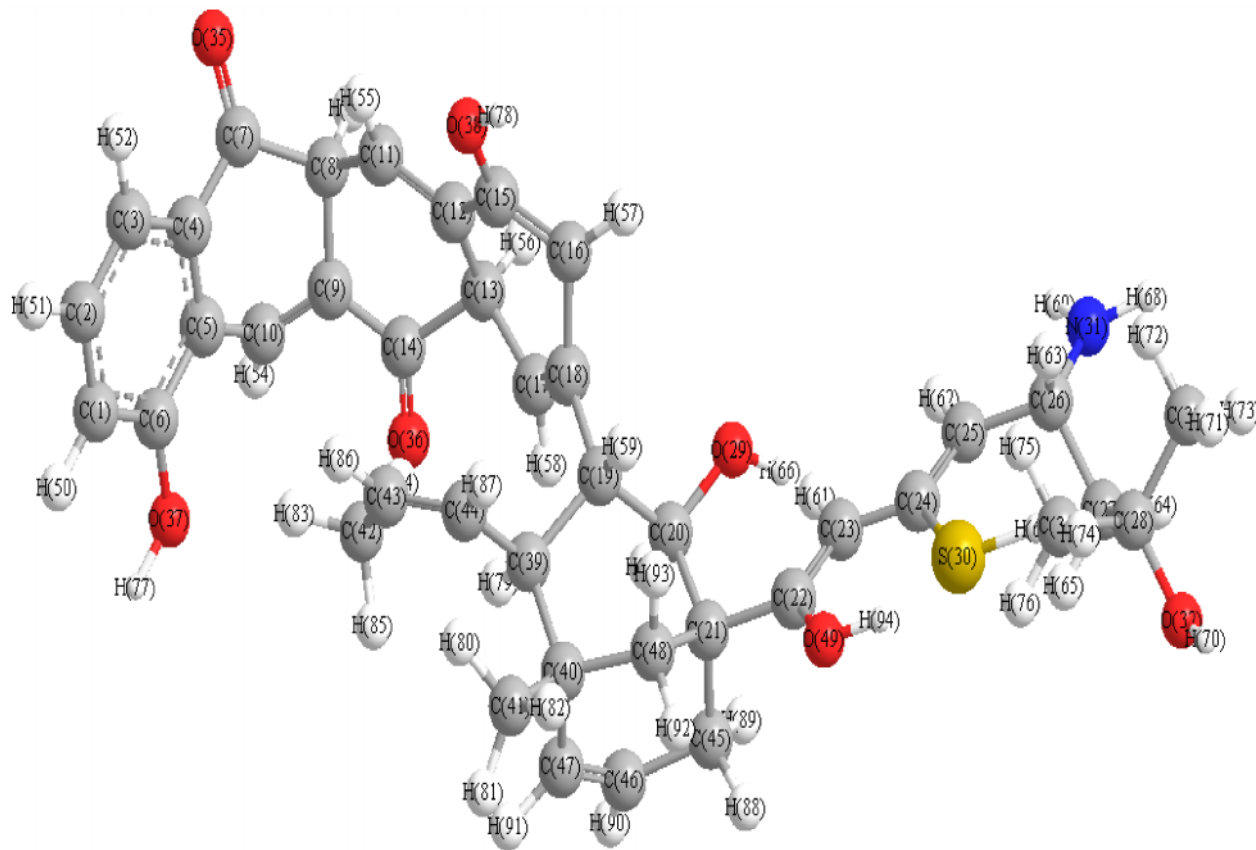


Figure 4. Optimised structure

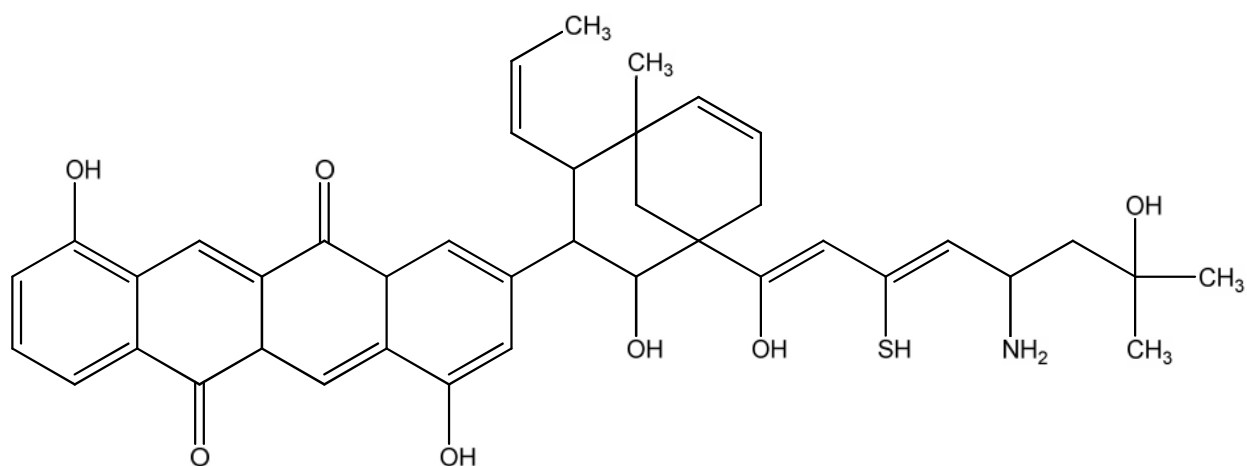


Figure 5.3-1-((1Z,3Z)-5-amino-1,7-dihydroxy-3-mercapto-7-methylocta-1,3-dienyl)-2-hydroxy-5-methyl-4-((Z)-prop-1-enyl)bicyclo[3.3.1]non-6-en-3-yl)-1,7-dihydroxytetracene-5,11(4aH,11aH)-dione[C₄₀H₄₅NO₇S]

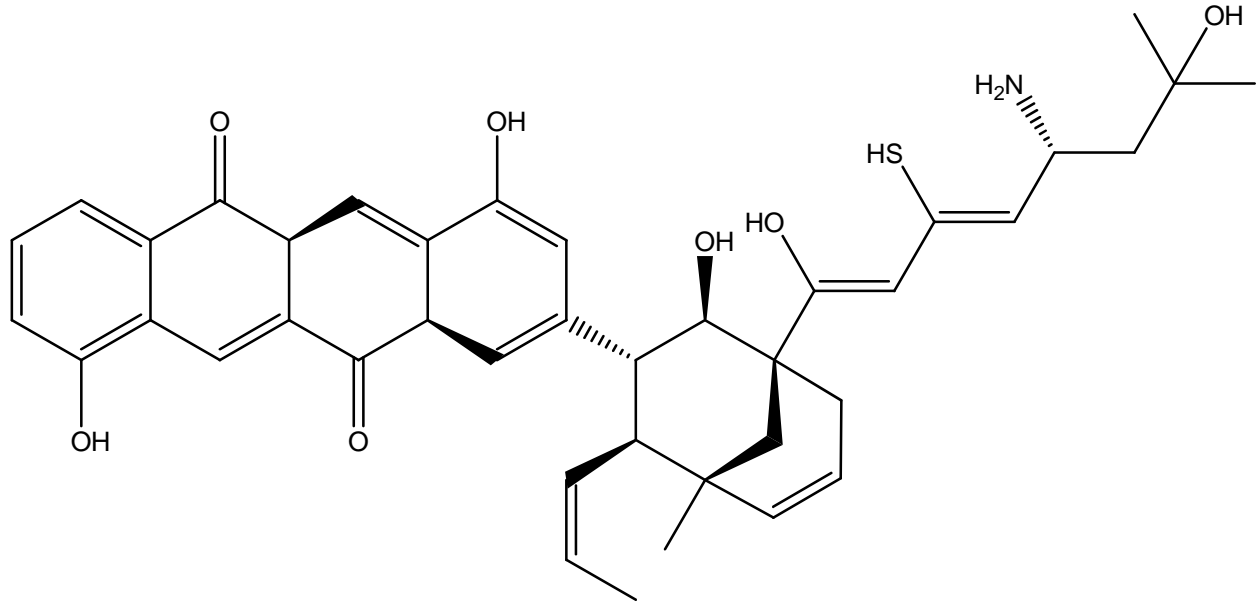


Figure 6. Asymmetry structure of isolated compound

Antioxidant Activity

Antioxidant activity and radical-scavenging activity of flavonoids depend highly on their structure; especially a free C-3-OH, a free C-4-OH, a double bond between C-2 and C-3, and an O-dihydroxy grouping in the B-ring are important [Satoshi Ogawa et.al.2008.]. Both hyperoside and quercitrin were good

match for these requirements with the exception of the absence of a free C-3-OH. However, in biological systems both quercetin glycosides might undergo enzymatic hydrolysis, resulting in the formation of quercetin, a highly antioxidative aglycone with a free C3- OH[Jun-Song Xiao et.al.2008]

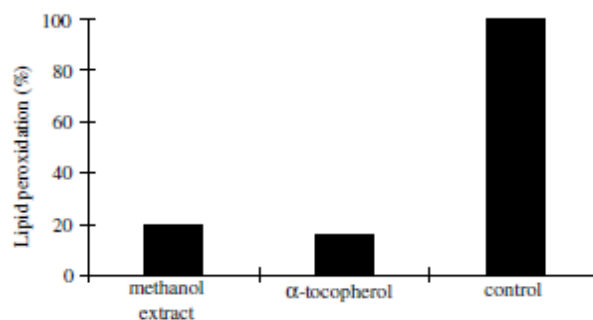


Figure7. Antioxidant activity of the methanol extract from the Nepalese pepper fruit measured by the thiocyanate method. Tocopherol (100 lg) was used for the standard sample.

Conclusions

In conclusion, hyperoside and quercitrin from the methanol extract of Nepalese pepper fruit

inhibited lipid peroxidation effectively and exhibited strong radical-scavenging activity, which would be expected to increase shelf life of foods and protect against peroxidative

damage in living systems in relation to aging and carcinogenesis. Thus, Nepalese pepper fruit presents a potentially valuable source of natural antioxidant and bioactive material of the isolated component playing vital role as ethanomedicine

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