SHORT COMMUNICATION

Anti-tumour-promoting and thermal-induced protein denaturation inhibitory activities of β-sitosterol and lupeol isolated from Diospyros lotus L.

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In this study, the anti-tumour-promoting and thermal-induced protein denaturation inhibitory activities of β-sitosterol (1) and lupeol (2), isolated from Diospyros lotus L., were explored. Compound 1 showed a marked concentration-dependent inhibition against 12-O-tetradecanoylphorbol-13-acetate (20 ng/32 pmol)-induced Epstein–Barr virus early antigen activation in Raji cells with IC 50 of 270 μg/ml, without significant toxicity (70% viability). Compound 2 showed significant anti-tumour-promoting effect with IC 50 of 412 μg/ml, without significant toxicity (60% viability). In heat-induced protein denaturation assay, compound 1 exhibited a concentration-dependent attenuation with a maximum effect of 73.5% at 500 μg/ml with EC 50 of 117 μg/ml, while compound 2 exhibited a maximum effect of 59.2% at 500 μg/ml with EC 50 of 355 μg/ml. Moreover, in silico docking studies against the phosphoinositide 3-kinase enzyme also show the inhibitory potency of these compounds. In short, both the compounds exhibited a marked anti-tumour-promoting and potent inhibitory effect on thermal-induced protein denaturation.

Keywords: Diospyros lotus; β-sitosterol; lupeol; anti-tumour-promoting and inhibitory activities of thermal-induced protein denaturation; docking studies against the phosphoinositide 3-kinase enzyme
1. Introduction

*Diospyros lotus* belongs to the family of Ebenaceae, which consists of about 500 species. This genus is widely distributed in tropical and subtropical regions throughout the world, and is a native to the Himalayan region (Uddin et al. 2011). *D. lotus* grows up to 9 m in height in semi-shaded area (Chittendon 1956). The extracts and chemical constituents obtained from *D. lotus* have already been reported for their significant anti-nociceptive, anti-inflammatory and sedative activities (Uddin et al. 2014). Different *in vitro* protocols have shown that *D. lotus* fruit contained significant antioxidant activities (Gao et al. 2014).

The enzyme phosphoinositide 3-kinases (PI3Ks) belong to the family of lipid kinases. It has key regulatory roles in many cellular processes including cell survival, proliferation and differentiation (Vivanco & Sawyers 2002; Bader et al. 2005; Engelman et al. 2006). This study was designed to assess the anti-tumour-promoting and thermal-induced protein denaturation inhibitory activities of β-sitosterol and lupeol (Figure S1), which were isolated from *D. lotus*, based on various established protocols. Furthermore, *in silico* docking studies of PI3K enzyme was also carried out to correlate between *in vitro* and *in silico* studies.

2. Results and discussion

The effect induced by compounds 1 and 2 on Epstein–Barr virus early antigen (EBV-EA) activation is given in Table S1. Compound 1 showed a profound concentration-dependent inhibitory action against 12-O-tetradecanoylphorbol-13-acetate (TPA; 20 ng/32 pmol)-induced EBV-EA activation in Raji cells with IC$_{50}$ of 270 μg/ml without significant toxicity and with 70% viability. Similarly, compound 2 showed significant anti-tumour-promoting effect with IC$_{50}$ of 412 μg/ml without significant toxicity and with 60% viability.

The EBV-EA induced by TPA in Raji cells is frequently used for the assessment of the anti-tumour-promoting activity (Tatsuzaki et al. 2010; Maoka et al. 2012). The results of our study reflect significant anti-tumour-promoting effect of the compounds by showing potent inhibitory effect against TPA (20 ng/32 pmol)-induced EBV-EA activation in Raji cells (8.2% and 6.9% induction of EBV-EA at 100 mg/ml concentration, respectively). Moreover, 70% and 60% viability was observed for compounds 1 and 2, respectively.

Compound 1 exhibited a concentration-dependent protein denaturation with a maximum effect of 73.5% at 500 μg/ml with EC$_{50}$ 117 μg/ml (Figure S2). Compound 2 exhibited a significant concentration-dependent protein denaturation with a maximum effect of 59.2% at 500 μg/ml with EC$_{50}$ 355 μg/ml. It is established that conventional non-steroidal antiinflammatory drugs such as phenylbutazone and indomethazine also inhibit production of endogenous prostaglandins by preventing denaturation of proteins (Chandra et al. 2012). Denatured proteins are considered as one of the inflammatory mediators; therefore, agents that cause prevention of denatured protein aggregation and precipitation as well as protein condensation are useful in treatment of such as rheumatic disorders, cataract and Alzheimer’s diseases (Saso et al. 2001). Our result showed a marked attenuation of heat-induced protein denaturation by compound 1, followed by compound 2.

*In silico* docking studies were carried out to observe the inhibitory potency of β-sitosterol and lupeol against the enzyme PI3K obtained from *Mus musculus*, based upon the hydrogen bonding and hydrophobic interactions. Computationally, the results of both compounds have showed a best docking effect against the PI3K enzyme, as compared with the standard antioxidant curcumin. The compounds (β-sitosterol and lupeol) were docked into the active site of PI3K enzyme (Figure S3). The docking energies (Table S2) of the compounds, including binding energy, ranges from −7.6 to −7.2 kcal/mol (Autodock vina score), while the total energy ranges from −103 to −98 kcal/mol (a Generic Evolutionary Method for molecular DOCKing score). Docking analysis of β-sitosterol (Figure S4) shows that it forms only one
hydrogen bond on interaction with Ser831 at a distance of 2.80 \( \text{Å} \). Four hydrophobic contacts were observed from the residues of Lys708, Met752, Asp753 and Asn836 within the active site of PI3K. Moreover, lupeol exhibited a slightly strong interaction than that of \( \beta \)-sitosterol (Figure S5), because it forms two hydrogen bond on interaction with Ser831 at the distances of 2.78 \( \text{Å} \) and 3.06 \( \text{Å} \) while similar hydrophobic contacts were observed from the residues Lys708, His830, Asn836 and Lys841.

3. Conclusions

In conclusion, the compounds \( \beta \)-sitosterol (1) and lupeol (2) isolated from \textit{D. lotus} L. demonstrated marked anti-tumour-promoting effect and inhibited thermal-induced protein denaturation. Moreover, \textit{in silico} docking studies against the PI3K enzyme also show the inhibitory potency of these compounds. Thus, we conclude that these compounds could be used as natural healing agents in effective management of tumour associated with inflammatory conditions.

Supplementary material

Experimental details related to this article are available online, alongside Figures S1–S5 and Tables S1 and S2. http://dx.doi.org/10.1080/14786419.2015.1046381.

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