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FREE RADICAL SCAVENGING PROPERTY OF β-AESCIN AND TRANS-CHALCONE: IN VITRO STUDY

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ABSTRACT

The aim of the present study was to investigate the antioxidant activity of β-aescin and trans-Chalcone by using in-vitro DPPH assay. Ascorbic acid was used as standard antioxidant compound. β-aescin is active phytoconstituent found in the extract of horse chestnut (Aesculus hippocastanum L.) seed. trans-Chalcone is open chain flavonoid biosynthesized in plant, Piper methysticum and synthesized in laboratory by Claisen-Schmidt condensation. The concentration range of 10, 20, 40, 60, 80 and 100 µg/mL of both the drug solutions was used. Our data showed that β-aescin and trans-Chalcone exhibited antioxidant activity in a concentration dependent manner, indicated by % inhibition and IC_{50} value. The IC_{50} value of β-aescin, trans-Chalcone and ascorbic acid (standard drug) were found to be 1.54, 1.45 and 1.47 respectively. Moreover, on the basis of IC_{50} values, trans-Chalcone exhibited more potent antioxidant activity when compared with standard compound and β-aescin. The range of antioxidant behavior is trans-Chalcone > ascorbic acid > β-aescin.

KEYWORDS: trans-Chalcone; β-aescin; Oxidative stress.

INTRODUCTION

Oxidative stress is the main culprit in the development of various pathological conditions. It is imbalance between the reactive oxygen species and antioxidant defenses. The increased production of oxidizing species such as O_2^- (superoxide radical), OH (hydroxyl radical) and H_2O_2 (hydrogen peroxide) and subsequent decrease in the level of antioxidant defenses such as glutathione (GSH) are majorly involved in the development of oxidative stress.[1] Further, it is mainly implicated in the progression of neurodegenerative disorders, cardiovascular disorders, diabetes mellitus, cancer, etc.[2] Therefore, promising antioxidant activities can be used as hopeful agent in treating multifarious disorders.

β-aescin is the major component isolated from the seeds of horse chestnut tree Aesculus hippocastanum (Hippocastanum). Aescin exist mainly in two forms α and β-aescin. Out of these two forms, β-aescin come out with active part of the muddle. The pleiotropic properties of β-aescin are antiedematous, anti-inflammatory, venotonic, etc. Clinically, β-aescin has been known to possess venotonic property and is efficacious in treating venous insufficiency, varicose vein and venous stasis ulcers.[3] It has been reported that Aescin is used in the treatment of hemorrhoids.[3] The anti-inflammatory effect of β-aescin has been revealed in various studies via decreasing the levels of IL-6, IL-8 and VEGF, inhibiting the expression of Nuclear Factor kappa B, Tumor Necrosis Factor α.[4,5] In addition, β-aescin has been demonstrated to exhibit hepatoprotective effect via its anti inflammatory, up-regulating GR expression, down-regulation 11 β-HSD2 expressions and antioxidant action.[6] Numerous studies have reported that β-aescin exhibit anticancer property via inhibiting STAT-3 activation by down-regulating cyclin D1, Bcl-2, Bcl-XL, etc.[7, 8] Aescin has been reported to be anti allergic in murine and porcine model.[9] Aescin possesses anti histaminic, antiserotinergic, anti hyluronidase activity, anti ulcer, antisecretory acitivity and releases PGF 2α from veins.[8]

Chalcones are open chain flavonoids which are obtained naturally as well as can be synthesized in laboratory. Chalcones play an important role in pigmentation of flowers and thus helps in pollination. Chalcones are used in cosmetic preparation and food additive. Further, chalcones help to protect the plant against pathogens and insects.[10] Chalcone and its derivative have been documented as antioxidant, anti inflammatory, analgesic, antiulcer and antihistaminic.[10,11] The pharmacological properties of chalcone and its derivative covers antimicrobial activity such as antibacterial,

antituberculotic, antifungal, antiviral, anti HIV, anti protozoal, etc.[12,13] Moreover, Chalcones and their derivatives have been reported to possess antileishmanial and antimalarial activity by inducing ultra structural changes in mitochondria of parasite.[14] It is interesting to note that chalcone and its derivative are effective in treating different types of cancer.[11,15] Chalcone and its derivative have shown hepatoprotective potential in galactosamine and carbon tetrachloride-induced hepatotoxicity in mice and rat by decreasing the oxidative stress, decreasing the level of TNF alpha, attenuating hepatocyte apoptosis, and inducing reduction in histological changes-induced by toxicants. [16, 17]
Thus, the present study was undertaken to determine whether β-aescin and trans-Chalcone possess antioxidant behavior by using in vitro technique.

MATERIALS AND METHODS

The gift sample of DPPH in pure form was obtained from Herb heal industry, Amritsar. Beta-Aescin (E1378, >95%, powder) was procured from Sigma Aldrich, invoice number A/5012, Batch No. SLBC6907V. trans-Chalcone was purchased from Sigma Aldrich, Invoice No. A/5012, (Batch No. STBC8746V). All other chemicals were used as fresh solutions.

Antioxidant activity (DPPH Method)

Principle of DPPH assay

It is based on the reduction of DPPH (1, 1-diphenyl-2-picrylhydrazyl), a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm with purple color. When Antioxidants compounds react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor (e.g., a free radical-scavenging antioxidant) and is reduced to the DPPHH and absorbance decreases and loss of its violet color. More de-colorization more is the reducing ability. The antioxidant potential of an agent is determined through its free radical scavenging in terms of change in optical density of DPPH.[18]

Procedure for Antioxidant Activity

The working solutions (10, 20, 40, 60, 80, 100 µg/mL) of extracts were prepared in methanol. Ascorbic acid was used as standard. 1 ml of DPPH solution (0.1 mM in methanol) was mixed with 3 ml of sample extracts and standard solutions separately. The mixture was shaken and kept for 30 minutes at room temperature. The decrease of solution absorbance due to proton donating activity of components of extracts was determined at 517 nm using UV spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.
The DPPH radical scavenging activity was calculated using the following formula:

DPPH Radical Scavenging Activity (% inhibition) = $[(A_0–A1/A_0) \times 100]$

Where A_0 is the absorbance of the blank, and A1 is the absorbance of extract mixed with DPPH. IC_{50} value (inhibitory concentration at which DPPH radicals where scavenged by 50%) was obtained by interpolation from linear regression analysis.

RESULTS

Effect of β-aescin on DPPH free radical scavenging

The solution of different concentration ranging from 10-100 µg/mL of β-aescin was used. The DPPH free radical scavenging activity of β-aescin was evaluated by using ascorbic acid as standard antioxidant drug and the entire test was performed in triplicate series. The % inhibition and IC_{50} were estimated. The % inhibition was estimated by using formula and IC_{50} value was calculated by linear regression analysis of concentration response curve plotting between % inhibition and log concentration. The DPPH radical scavenging effect increased with increase in concentration of β-aescin solution. The IC_{50} value for β-aescin was found to be 1.54, which was slightly higher than IC_{50} value of ascorbic acid (Figure 1, 3).

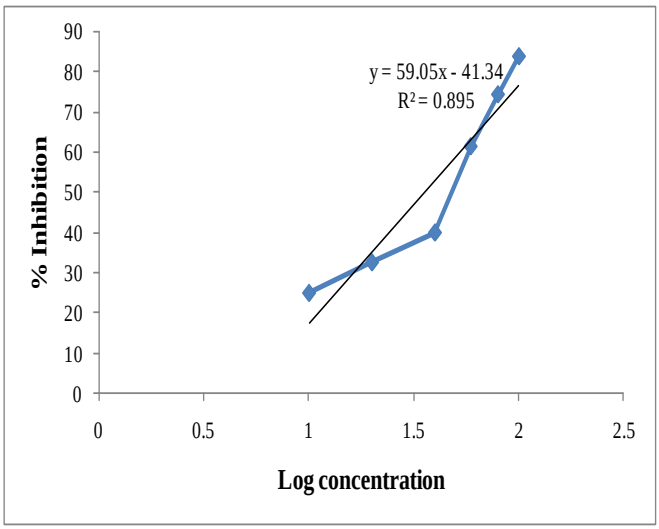

Figure 1. Effect of β-aescin on DPPH free radical scavenging **(IC_{50} value 1.54)**

Effect of trans-Chalcone on DPPH free radical scavenging

The trans-Chalcone of 10-100 µg/mL solution was used. The % inhibition was found to be increased with increase in concentration of the drug solution. It was found that trans-Chalcone exhibited more potent antioxidant activity when compared with ascorbic acid in terms of % inhibition and IC_{50} value. The IC_{50} value for trans-Chalcone was found to be 1.45, which was slightly lower when compared with IC_{50} value of ascorbic acid, indicating marked antioxidant activity (Figure 2, 3).

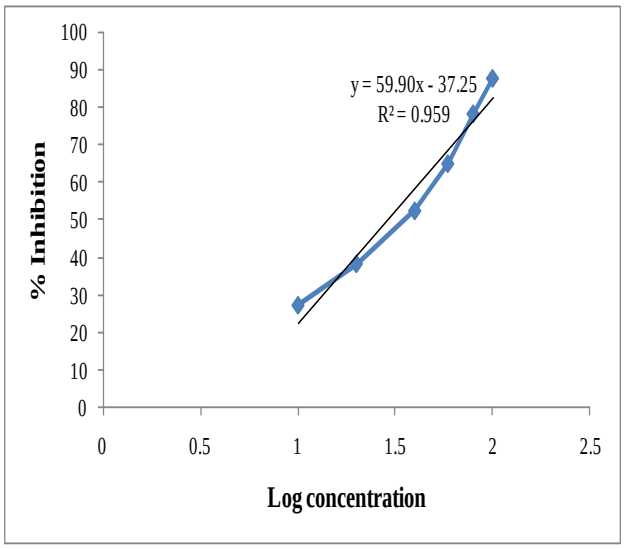

Figure 2. Effect of *trans*-Chalcone on DPPH free radical scavenging **(IC_{50} value 1.45)**

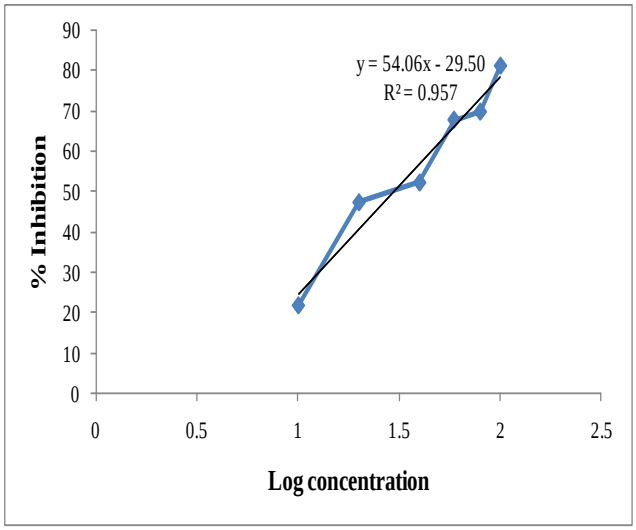

Figure 3. Effect of Ascorbic acid on DPPH free radical scavenging **(IC_{50} value 1.47)**

DISCUSSION

The results of present study confirmed the antioxidant effect of β-aescin and trans-Chalcone by DPPH free radical scavenging method. The findings also suggest that trans-Chalcone was more efficacious antioxidant compound than ascorbic acid and β-aescin.

Recently many researchers have found that large number of plant products and its major phytoconstituents have been shown to exhibit antioxidant behavior with free-radical scavenging property and have huge importance as therapeutic agents in number of pathological conditions caused due to oxidative stress.[19] At present, the trend is trickling towards identification and isolation of compounds having antioxidant behavior from plant origin with lesser side effect. In modern era, increased exposure to triggers such as smoke, alcohol, chemicals, pollutant, pesticides and even number of synthetic drugs are the major factors for the development of disorders due to increased production of free radicals.[20]

% inhibition of β-aescin was found to be ranging from 25-83.75% at 10-100 µg/mL concentration of drug solution. On the other hand, trans-Chalcone exhibited % inhibition from 27.3-87.95% at 10-100 µg/mL. Moreover, standard drug (Ascorbic acid) showed % inhibition from 21.8-81.25 % at the same range of concentration. On the basis of IC_{50} values, the trans-Chalcone showed lesser values when compared with standard drug and β-aescin. The lesser IC_{50} value indicates more marked oxidative activity.

β-aescin, a major phytoconstituent from the seeds of horse chestnut tree Aesculus hippocastanum (Hippocastanum) demonstrated antioxidant activity which may be attributed due to its flavonoidal nature by increasing antioxidative defence system. The chemical nature of the trans-Chalcone (1,3-diphenyl-2-propenone) is open chain flavonoid, exhibited potent antioxidant activity. The marked scavenging property of trans-Chalcone may be due to its flavonoidal nature of phytoconstituent that can provide the necessary component as a radical scavenger.

Therefore, by exploring antioxidant activity from natural resources and isolation of their phytoconstituents are concurrently presenting colossal scope for their better therapeutic application for treatment of various disorders. Hence to explore our traditional therapeutic knowledge and plant sources and interpret it according to the recent advancements to fight against oxidative stress, in order to give it a commendable place.

In nut shell, our study demonstrated the antioxidant role of β-aescin and trans-Chalcone. This is attributed to its flavonoidal nature. However, further studies are warranted to elucidate its mechanism at molecular level.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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REFERENCES

1. Dorman HJD, Surai P, Deans SG. In vitro antioxidant activity of a number of plant essential oils and phytoconstituents. J Ess Oil Res., 2000; 12: 241-248.
2. Sen S, Chakraborty R, Sridhar C, Reddy YSR, De B. Free radicals, current status and future prospect. Int J Pharma Sci Rev Res., 2010; 3: 91-100.
3. Otajagic S, Pinjic Dz, Cavar S, Vidic D, Maksimovic M. Total phenolic content and antioxidant activity of ethanolic extracts of Aesculus

hippocastanum L. Bull Chem Technol Bosnia Herzegovina, 2012; 38: 35-39.
4. Li ST, Zhou ZY, Xu GC. Effects of β-Aescin on the expressions of IL-6, IL-8 and VEGF in the vitreous humor of high-oxygen-induced neonatal rat retinopathy. Acta Academiae Med Quingdao Univers, 2009; 3: 289-292.
5. Wang H, Zhang L, Jiang NA, Wang Z, Chong Y, Fu F. Anti-inflammatory effects of Escin are correlated with the glucocorticoid receptor/NF-κB signaling pathway, but not the COX/PGF2α signaling pathway. Exp Therap Med., 2013; 6: 419-422.
6. Jiang N, Xin W, Wang T, Zhang L, Fan H, Du Y, Li C, Fu F. Protective effect of Aescin from the seeds of Aesculus hippocastanum on liver injury induced by endotoxin in mice. Phytomed, 2011; 18: 1276-1284.
7. Tan SML, Li F, Rajendran P, Kumar AP, Hui KM, Sethi G. Identification of Escin as a novel inhibitor of signal transducer and activator of transcription 3/janus-activated kinase 2 signaling pathway that suppresses proliferation and induces apoptosis in human hepatocellular carcinoma cells. J Pharmacol Exp Ther., 2010; 334: 285-293.
8. Patlolla JMR, Raju J, Swamy MV, Rao CV. β-Escin inhibits colonic aberrant crypt foci formation in rats and regulates the cell cycle growth by inducing p21waf1/cip1 in colon cancer cells. Mol Cancer Ther., 2006; 5: 1459-1466.
9. Sipos W, Reutterer B, Frank M, Unger H, Grassauer A, Prieschl-Grassauer E, Doerfler P. Escin inhibits type 1 allergic dermatitis in a novel porcine model. Int Arch Allergy Immunol., 2013; 161: 44-52.
10. Batovska DI, Todorova IT. Trends in utilization of the pharmacological potential of chalcones. Curr Clin Pharmacol., 2010; 5: 1-29.
11. Ravichandran R, Rajendran M, Devapiriam D. Studies on chalcone derivatives antioxidant and stability constant. J Chem Bio Phy Sci Sec., 2013; 3: 2446-2458.
12. Prasad YR, Rao AS, Rambabu R. Synthesis of some 4'-amino chalcones and their antiinflammatory and antimicrobial activity. Asian J Chem. 2009; 21: 907-914.
13. Tiwari B, Pratapwar AS, Tapas AR, Butle SR, Vatkar BS. Synthesis and antimicrobial activity of some chalcone derivatives. Int J Chem Tech Res., 2010; 2: 499-503.
14. Zhai L, Chen M, Blom J, Theander TG, Christensen SB, Kharazami A. The antileishmanial acitivity of novel oxygenated chalcones and their mechanism of action. J Antimicrobial Chemother., 1999; 43: 793-803.
15. Shibata S. Anti-tumorigenic chalcones. Stem Cells., 1994; 12: 44-52.
16. Guan LP, Nan JX, Jin XJ, Jin QH, Kwak KC, Chai KY, Quan ZS. Protective effects of chalcone derivatives for acute liver injury in mice. Arch Pharm Res., 2005; 28: 81-86.
17. Yu WG, Qian J, Lu YH. Hepatoprotective effects of 2', 4'-dihydroxy-6'-methoxy-3', 5'-dimethylchalcone on CCl_4-induced acute liver injury in mice. J Agri Food Chem., 2011; 59: 12821-12829.
18. Alam MN, Bristi NJ, Rafiquzzaman M. Review on in vivo and in vitro methods evaluation of antioxidant activity. Saudi Pharma J., 2013; 21: 143-152.
19. Prasad C, Singh D, Shukla O. Antioxidant activity and trace elements profile of extracts of Cymbopogon jwarancusa (Jones.) leaves. J Pharmacogn Phytochem, 2013; 2: 33-37.
20. Khilfi S, El Hachimi Y, Khalil A, Es-Safi N, Belahyan A, Tellal R, El Abbouyi A. In vitro antioxidants properties of Salvia verbenaca L. hydromethanolic extract. Indian J Pharmacol, 2006; 38: 276-280.