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Original Article

Isolation of Fisetin from *Elaeagnus indica* Serv. Bull. (Elaeagnaceae) with antioxidant and antiproliferative activity

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ABSTRACT

Background: *Eleaegnus indica* is a medicinal straggling shrub, belongs to Elaeagnaceae family and reported as potent antimicrobial, anticancer and larvicidal properties. The prime aim of this study was focused on the isolation and characterization of the most active anti-oxidant principle from the acetone leaves extracts of ***E. indica***. **Methods**: The chromatographic and spectral studies were performed in isolation of most active compound 'Fisetin' (a flavonol group) from ***E. indica***. The isolated pure compound was tested for its antioxidant and antiproliferative property (on U-937 and HT-60 cell lines) by adopting standard protocols. **Results:** The active compound was isolated as yellowish amorphous powder. The structure of the compound was identified by various spectral analysis like LC-MS, CHNS anlysis, UV, FT-IR, 1D (^{1}H and ^{13}C) and 2D NMR (HMBC and HSQC) analysis. The remarkable antioxidant activity was recorded in various assays like NO$^\bullet$ (IC_{50} 39.43 ± 0.28 µg/mL), $^\bullet$OH (IC_{50} 43.91 ± 0.35 µg/mL), $O_2^{\bullet-}$ (IC_{50} 48.30 ± 0.67 µg/mL), DPPH$^\bullet$ (IC_{50} 70.32 ± 0.89 µg/mL) and FRAP (EC_{50} 48.69 ± 1.05 µg/mL). The significant antiproliferative effect of the fisetin was noted on both U-937 (IC_{50}46. 75 ± 3.53 µg/mg) and HT-60 (IC_{50} 59.46 ± 1.81 µg/mg) cell lines. **Conclusion**: The present investigation shows that isolated fisetin harbour high antioxidant and antiproliferative potential and provide strong scientific evidence for their medicinal uses, particularly antioxidant and anticancer properties.

Key words: *Elaeagnus indica*, Antioxidant, Antiproliferative, Cancer cell lines, Spectral analysis.

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INTRODUCTION

Free radicals lead to an oxidative stress and causes damage to several cellular macromolecules like proteins, lipids and DNA.[1] The majority of the degenerative diseases, cancer, cataracts, malfunction of the immune system, atherosclerosis, myocardial infarction, arthritis, anemia, asthma, liver diseases, diabetes mellitus, inflammation, renal failure, brain dysfunction and stress are mainly linked to oxidative stress due to free radicals.[2] Cancer is the second leading cause of death, after heart disease, where one in four deaths occurs[3] and it is estimated that by the year 2020, the number of cancer patients will reach to 16 million per year.[4] Antioxidant compounds are involved in the prevention of cellular damage, aging and a variety of diseases.[5] Many plants were reported to harbor significant free radicals scavenging efficacy and produce various antioxidative compounds (phenols, alkaloids and terpenoids) which have many therapeutic potential. Bioactive plant based antioxidants were able to inhibit cancer cytogenesis by suppressing the tumor initiation, promotion and progression, which are being considered as potential biocompatible anticancer agents.[6] Recently, there has been growing interest in research about the role of phytocompounds in antioxidant and antiproliferative activity.[7,41]

Elaeagnus indica Serv., Bull. belongs to the Elaeagnaceae family, found in the hills at 750-1550 m, forest border, cleared slopes and exposed to full sun. The extracts of *E. indica* possesses good antimicrobial, anticancer[8] and larvicidal potential.[9,10] The present study deals with the isolation and characterization of potent antioxidant compound from acetone extract of *E. indica*. In addition, the isolated compound (fisetin) was tested for its proficiency in antiproliferative effect.

MATERIALS AND METHODS

Plant material

Fresh, healthy leaves of *E. indica* was collected from higher elevations (1300 to 1400 m MSL) of Shervarayan Hills (latitude 11° 47'–12° 33' N, longitude 77° 02'–78° 40' E), Salem District, Tamil Nadu, India. The collected plant sample was identified by the Botanical Survey of India (reference number: BSI/SRC/5/23/2014-15/Tech/1942), Coimbatore, Tamil Nadu, India. The plant herbarium (specimen number: PU/BT/NDRL/2010/03) was deposited in Natural Drug Research Laboratory (NDRL), Department of Biotechnology, Periyar University, Salem, Tamil Nadu, India. The collected plant material was washed with running tap water, then shade dried at room temperature for three weeks and pulverized.

Preparation of extracts

Powdered plant material (2 kg) was successively extracted with various solvents (hexane, chloroform, ethyl acetate, acetone and methanol) in an increasing polarity method in a Soxhlet apparatus until the efflux solvent became colorless. All extractives were filtered through Whatman filter paper (No: 1) and concentrated *in vacuo* at 40°C which yield 1.67%, 3.48%, 4.68%, 5.33% and 6.21% (hexane, chloroform, ethyl acetate, acetone and methanol respectively) of dark green to brown color viscous extracts.

Isolation of antioxidant compound

Based on the preliminary results of phytochemicals and antioxidant properties,[11] the acetone extract was selected for isolation of antioxidant compound. *E. indica* acetone extract (50 g) was fractioned through

a silica gel (60-120 mesh) column chromatography (column length 60 cm, diameter 4 cm) using hexane:ethyl acetate (100:0 to 0:100) solvent system (increasing 10% polarity) which yielded 58 fractions (F). All the fractions were tested for their DPPH radical scavenging potential. Among 58 fractions, only six fractions (F15, F28 F33, F35, F42 and F52) were showed considerable antiradical activity (Table 1). However, remarkable DPPH radical scavenging potential was observed in fraction F52 (hexane:ethyl acetate 40:60) with significant low IC_{50} value (37.33 µg/mL). Hence, fraction F52 (2.88 g, brownish yellow color, solid in nature) was permeated through a silica gel column with hexane:ethyl acetate (100:0 to 70:30) in order of increasing 5% polarity which yielded 10 subfractions (EASF=*Elaeagnus* Antioxidant Sub-Fractions) and their DPPH radical scavenging potential was tested. Lowest IC_{50} value (12.22 µg/ml) (Table 1) was noticed in EASF9 (hexane: ethyl acetate 45:55) which was renamed as EIA (*E. indica* antioxidant compound). The results of analytical TLC (pre coated silica gel $F_{254,}$ Merck, Germany) of EIA fraction showed single spot with R_f value of 0.75 (under UV light, iodine vapor and after spraying CAM stain) using *n*-Butanol-Acetic acid-Water (BAW) (4:1:5) solvent system as mobile phase.

The purity of isolated EIA was confirmed by analytical LC (Thermo/Finnigan Surveyor System) which eluted with methanol/water and LC column outlet was coupled to a Thermofleet (LCQ-Fleet) Ion Trap mass spectrometer equipped with an ESI ion source. Data acquisition and mass spectrometric evaluation were carried out in Qual Browser; Thermo Electron, San Jose, CA. Melting point (m.p.) of EIA was determined using a hot stage melting point apparatus (Leica GALEN III) equipped with microscope and are uncorrected. UV λ_{max} of EIA was recorded on Perkin Elmer, Lambda-650 UV-Visible Spectrophotometer. The IR spectrum of the EIA was recorded in PerkinElmer, Spectrum RX-I spectrophotometer in the range of 400 to 4000 cm^{-1} wavelength with a resolution of 1 cm^{-1}. The elemental analysis, of EIA was carried out in PerkinElmer 2400 Series II. 1D (^1H and ^{13}C) and 2D NMR (HMBC and HSQC) spectra were recorded (DMSO-d_6) using a Bruker AV-500 MHz NMR spectrometer with TMS as internal standard.

In Vitro Antioxidant Studies

Different concentrations of isolated bioactive compound (0–100 µg/mL) from *E. indica* was tested for various types of radical scavenging potential. Ascorbic acid and BHA were served as standard reference compounds for all *in vitro* antioxidant assays like DPPH Radical (DPPH•) Scavenging,[12] Hydroxyl Radical (•OH) Scavenging,[13] Nitric Oxide Radical (NO•) Scavenging,[14] Superoxide Anion Radical ($O_2^{\bullet-}$) Scavenging,[15] Ferric Reducing Power Ability (FRAP) Assays.[16]

Antiproliferative Activity

The antiproliferative activity of isolated compound was determined by methylthiazolyl diphenyl-tetrazolium bromide (MTT) assay as described by Mosmann[17] on U-937 (human leukemic monocyte lymphoma) and HT-60 (human acute promyelocytic leukemia) cell lines. The U-937 and HT-60 cell lines were obtained from National Institute of Cell Sciences, Pune, India. The cells were treated with various concentrations of the isolated compound (3.13-1000 µM/ml). The effect of the isolated compound on the proliferation of U-937 and HT-60 cells was expressed as percentage of cell viability. IC_{50} values are calculated graphically from the curve plotted for the percentage of cell viability against different concentrations of the compound.

Statistical analysis

All the experiments were carried out at least in triplicates. Data were represented as mean ± standard deviation (SD) of three determinations. The Inhibitory Concentrations (IC_{50}) were determined graphically from the curve fitted (nonlinear regression) to the mean values of quotients.

Table 1: DPPH radical scavenging activity of column fractions of acetone extract of *E. indica*

Fraction no	IC_{50} (µg/mL)*
F15	61.51 ± 1.22^{d}
F28	45.56 ± 0.78^{b}
F33	54.46 ± 1.04^{c}
F35	$48.97 \pm 0.95^{b,c}$
F42	41.42 ± 0.52^{b}
F52	37.33 ± 1.15^{a}
EASF2	23.58 ± 0.76^{b}
EASF5	27.16 ± 0.96^{c}
EASF9	12.22 ± 0.57^{a}
Ascorbic acid	18.77 ± 0.52
BHA	15.48 ± 0.61

*-The values are mean of three replicates with (±) standard deviations (mean ± S.D; n=3). Different superscript letters (a-d) in a column within treatments indicate significant differences (at $p<0.05$) when subject to Tukey's multiple comparison test.

Table 2: Antiproliferative activity of isolated fisetin from *E. indica* on U-937 and HL-60 cell lines

Concentration (µM/mL)	% Cell viability*	
	U-937 CL#	HL-60 CL#
03.13	92.01 ± 1.64^{i}	85.47 ± 2.28^{i}
06.25	87.08 ± 2.34^{h}	77.07 ± 1.74^{h}
12.50	78.44 ± 1.72^{g}	71.45 ± 1.07^{g}
25.00	64.32 ± 1.11^{f}	65.43 ± 1.11^{f}
50.00	56.33 ± 0.78^{e}	57.40 ± 0.84^{e}
100.0	49.64 ± 0.68^{d}	52.22 ± 1.00^{d}
250.0	44.28 ± 1.06^{c}	37.99 ± 0.86^{c}
500.0	34.48 ± 1.23^{b}	31.97 ± 0.65^{b}
1000	28.82 ± 0.95^{a}	24.89 ± 2.85^{a}
IC_{50}	98.22 ± 3.22	121.61 ± 7.20

*-The values are mean of triplicates with standard deviation (mean ± S.D; n=3). Different superscript letters (a-i) in a column within treatments indicate significant differences (at $p<0.05$) when subject to Tukey's multiple comparison test, CL#–Cell line.

The analyses were performed by logarithmically transforming the data to comply with analysis of variance (ANOVA) in a completely randomized design and Tukey's multiple range test (at $p<0.05$) by employing SPSS (16.0) software.

RESULTS AND DISCUSSION

Characterization of isolated compound

Fisetin; $C_{15}H_{10}O_{6,}$ Yellowish amorphous powder and m.p. 299-300°C; UV (Methanol) λ_{max} nm (log ε) 248 (3.10) and 350 (3.91); IR υ_{max} cm^{-1} (KBr): 3350 (OH), 1683 (C=O), 1512, 1444 (-C=C-), 1327, 1276 (-C-O-bend), 1113, 1014 (-C-O-strech), 932, 874, 844, 809, 671 (-C-H out of plane bending); LC-ESIMS *m/z* (% intensity): 287 (100) (M^++1); CHNS analysis: C=62.99%, H=3.47%, N=0%, S=0%, O=33.54% (Calcd for $C_{15}H_{10}O_6$, 286, degree of unsaturation is 11); ^1H-NMR (500 MHz, DMSO-d_6): δ 6.91 (1H, m, H-5), 7.93 (1H, dd, *J*=9.5, 2.5 Hz, H-6), 6.91 (1H, m, H-8), 6.91 (1H, m, H-2'), 7.70 (1H, d, *J*=1.5 Hz, H-5'), 7.56 (1H, dd, *J*=8.5, 2 Hz, H-6'), 9.04 (1H, br s, 3-OH), 10.74 (1H, br s, 7-OH), 9.29 (1H, br s, 3'-OH), 9.51 (1H, br s, 4-OH); ^{13}C-NMR (500 MHz, DMSO-d_6): δ 147.7

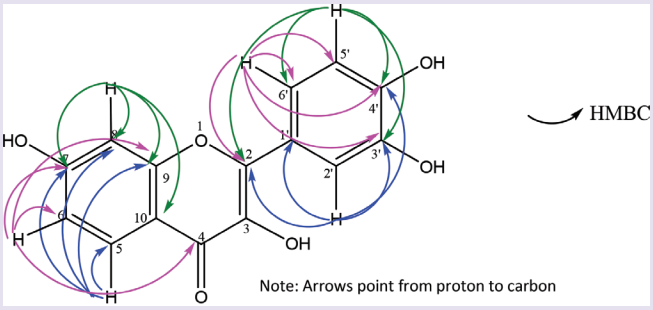

Figure 1: The key HMBC correlations of Fisetin

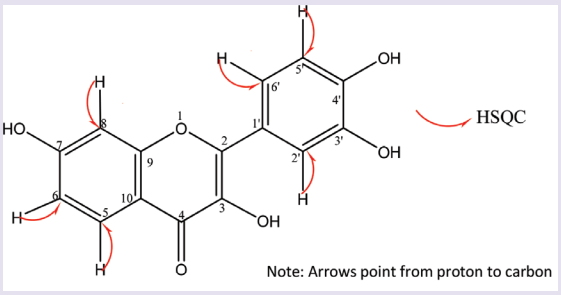

Figure 2: The key HSQC correlations of Fisetin

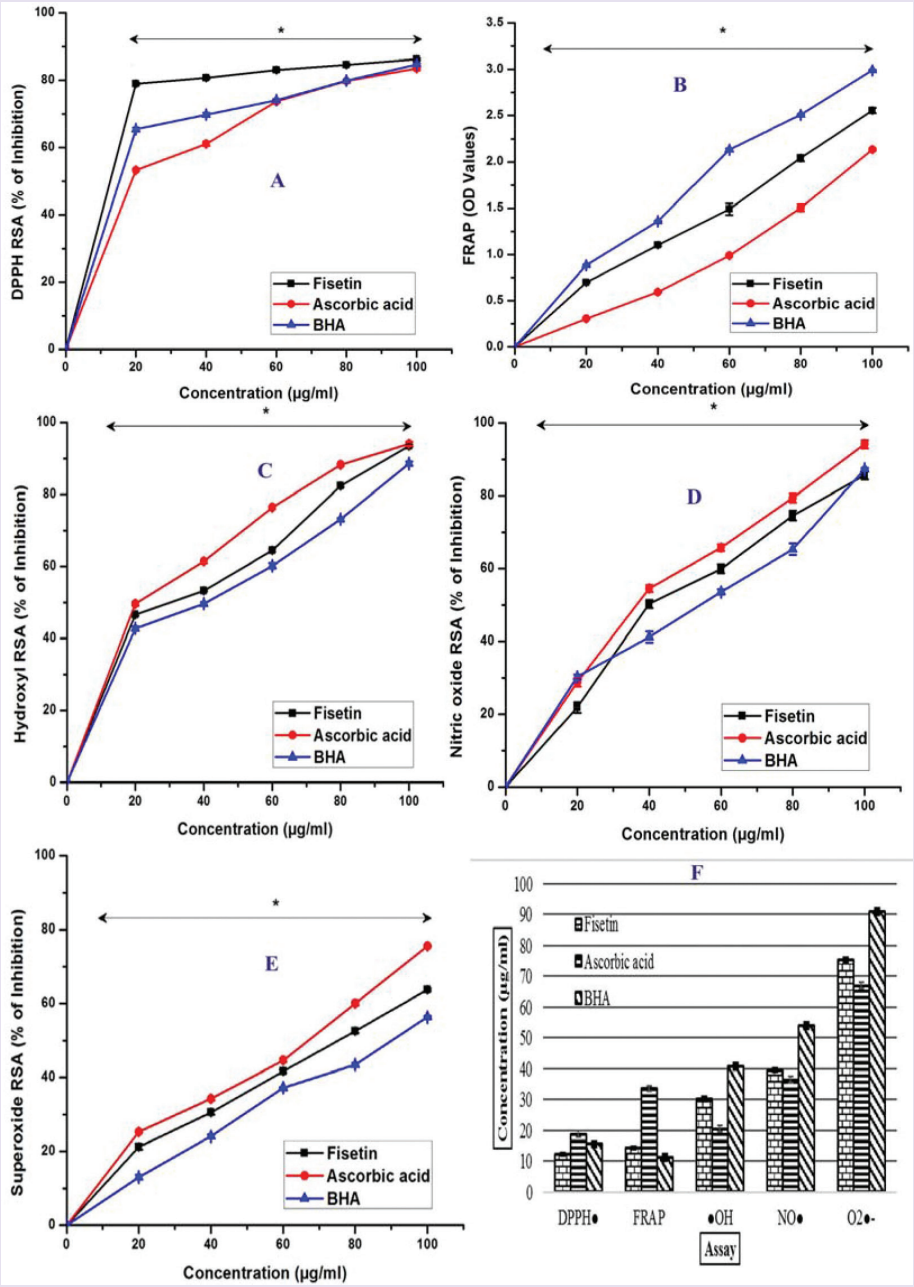

Figure 3: Radical scavenging activity of isolated fisetin compound from *E. indica*
A=DPPH, B=FRAP, C=Hydroxyl, D=Nitric oxide, E=Superoxide radical scavenging activity, and F=IC_{50} values. *-Significant differences at $p<0.05$.

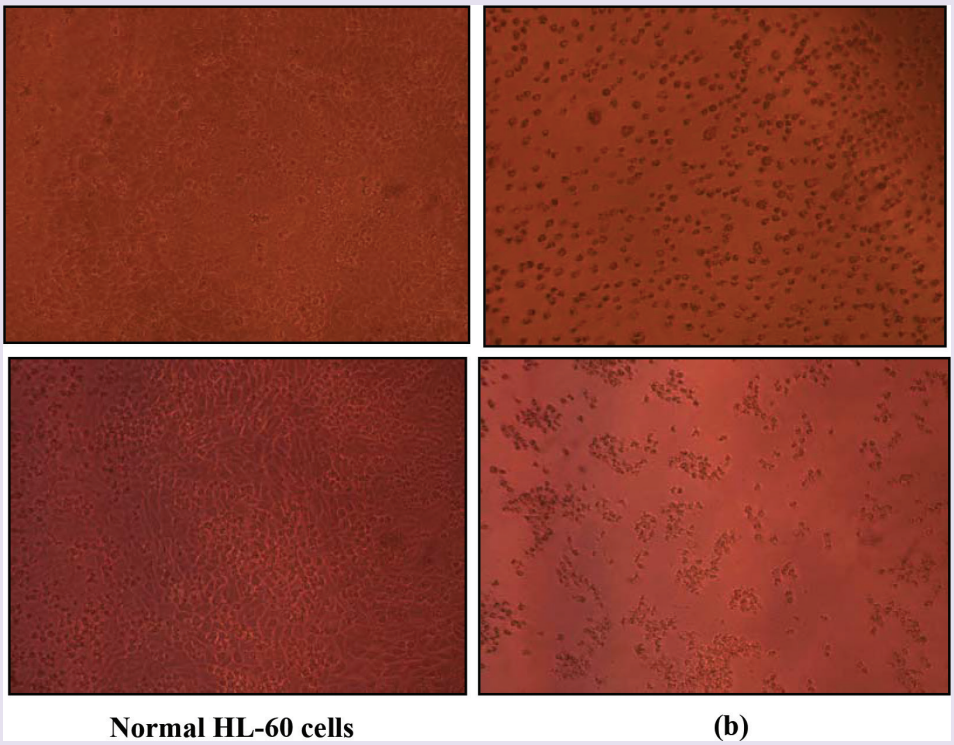

Figure 4: Fisetin induced morphological changes in U-937 (a) and HL-60 cells (b) at 1000 μM/mL concentration (magnification 100X).

(C-2), 137.6 (C-3), 172.4 (C-4), 115.4 (C-5), 126.9 (C-6), 162.7 (C-7), 102.3 (C-8), 156.7 (C-9), 114.7 (C-10), 123.0 (C-1'), 116.0 (C-2'), 145.5 (C-3'), 145.5 (C-4'), 115.1 (C-5'), 120.1 (C-6').

To the best of our knowledge, this is the first report on the Heteronuclear Multiple Bond Correlation (HMBC) and Heteronuclear Single Quantum Correlation (HSQC) spectroscopy correlations of fisetin. The HMBC spectroscopy show the coupling correlation of H-5 (δ_H 6.889-6.921) with C-5 (115.40), C-7 (162.70), C-8 (102.28) and C-9 (156.74); H-6 (δ_H 7.924-7.943) with C-4 (172.42), C-6 (126.92), C-7 and C-9; H-8 (δ_H 6.889-6.921) with C-7, C-8, C-9 and C-10 (114.67); H-2' (δ_H 6.889-6.921) with C-1' (122.96), C-3' (145.48), C-4' (145.50) and C-2 (116.03), H-5' (δ_H 7.702-7.705) with C-2, C-3', C-4' and C-6' (120.10); H-6' (δ_H 7.545-7.566) with C-2, C-3', C-4' and C-6' confirmed long range couplings of fisetin (Figure 1). The HSQC spectroscopy results report that aromatic protons are coupled with corresponding carbons. The H-5 proton (δ_H 6.889-6.921) was coupled with C-5 (δ_C 115.40); H-6 (δ_H 7.924-7.943) with C-6 (δ_C 126.92); H-8 (δ_H 6.889-6.921) with C-8 (δ_C 102.28); H-2' (δ_H 6.889-6.921) with C-2' (δ_C 116.03); H-5' (δ_H 7.702-7.705) with C-5' (δ_C 115.12); H-6' (δ_H 7.545-7.566) with C-6' (δ_C 120.10) (Figure 2).

Antioxidant activity of fisetin

The findings of antioxidant properties of fisetin show significant antiradical capacity and is directly related to the concentration of fisetin (Figure 3). Fisetin showed remarkable strong inhibition activity on DPPH radicals than others having IC_{50} value (12.23 μg/mL) which was lower than the reference compounds (ascorbic acid and BHA). Fisetin showed good Fe^{3+} reduction property in FRAP assay with minimal EC_{50} value (14.20 μg/mL) which was lower than positive controls. Fisetin harbor excellent hydroxyl radical neutralization potential with least IC_{50} value (30.17 μg/mL) which are quite comparable with standards. Fisetin was found to be powerful quenchers of nitric oxide radicals with significant IC_{50} value (39.57 μg/mL). Considerable superoxide radicals scavenging activity were detected in fisetin with sustainable IC_{50} value (75.26 μg/mL). Recently, several researchers worked on the antioxidant potential of fisetin by performing various tests.[18-20] Wherein Phenolic hydroxyl groups are prone to donate a hydrogen atom to free radicals and extend conjugated aromatic system to delocalize an unpaired electron.[21]

DPPH radical scavenging activity guided isolation of acetone extract of *E. indica* yielded a flavonol compound, *i.e*. fisetin. Similarly several researchers have reported the isolation and structural elucidation (through ^1H and ^{13}C-NMR studies) of fisetin from various parts of many plants such as, xylem sap of *Hymenaea courbaril,*[22] aerial parts of *Tanacetum parthenium,*[23] fruit and leaf of *Vitex rotundifolia,*[24] leaves of *Mayodendron igneum,*[25] roots of *Boesenbergia rotunda*[26] and *Sanguisorba officinalis.*[27]

The scavenging activity of flavonoids depends on the number of free hydroxyl groups in the molecule. The *ortho*-dihydroxy structure of the B ring, of a flavonoid has the best electron-donating properties and confers higher stability in the radical form and participates in electron delocalization. Similarly, the 2, 3-double bond with a 4-*oxo* function in the C ring, are responsible for electron delocalization from the B ring. The 3-and 5-hydroxyl groups with the 4-*oxo* function in A and C rings, are essential for maximum radical scavenging potential of flavonoids. The 3-glycosylation reduces flavonoids activity when compared with corresponding aglycones which support the present results of antiradical activity of fisetin and the possible mode of inhibition of radicals.[28]

Antiproliferative activity of fisetin

The results of antiproliferative property of fisetin isolated from *E. indica* showed good inhibitory effect on the growth of both U-937 and HL-60 cells in a dose dependent manner (Table 2, Figure 4). Moreover, the present results revealed that U-937 cells (IC_{50}=98.22 μM/mL) were more susceptible to fisetin than HL-60 cells (IC_{50}=121.61 μM/mL). Fisetin expressed superior antiproliferative activity due to the high antioxidant

potential. Agullo *et al*.[29] stated that the antiproliferative effects of compounds on cancer cells are mainly due to their antioxidant potential which strengthens the findings of the present study.

The extensive studies have been conducted to show that fisetin could inhibit several molecular targets, including cyclin-dependent kinases,[30-32] DNA topoisomerases I and II,[33,34] urokinase,[35] actin polymerization[36] and androgen receptor signaling.[37] Moreover, fisetin has been recently reported to possess interesting anticancer activity in mice lung carcinoma[38] and prostate tumours.[37,39] Murtaza *et al*.[40] reported that fisetin inhibited the proliferation of various types of human cancer cells and induces the cell cycle arrest or apoptosis as depicted in this study.

CONCLUDING REMARKS

Elaeagnus indicia is a good source for a variety of phytoconstituents. DPPH radical scavenging activity guided isolation method resulted in isolation of fisetin. The structure of fisetin was established by spectroscopic analysis and it expresses significant antioxidant and antiproliferative activity on U-937 and HL-60 cell lines.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests in this paper.

ABBREVIATION USED

DPPH: 2,2-diphenyl-1-picrylhydrazyl Radical (DPPH•) Scavenging, Hydroxyl Radical (•OH) Scavenging, Nitric Oxide Radical (NO•) Scavenging, Superoxide Anion Radical (O_2•-) Scavenging, **FRAP:** Ferric Reducing Power Ability, **NMR:** Nuclear Magentic Resonance, **IC_{50}:** Half maximal Inhibitory concentration, **EIA:** Elaeagnus indica acetone extract, **TLC:** Thin Layer Chromatography, **BHA:** Butylated hydroxyanisole, **LC-MS:** Liquid Chromatography-Mass Spectrometry, **FTIR:** Fourier Transform Infrared Spectroscopy, **UV:** Ultra violet.

REFERENCES

1. Pan Y, Wang K, Huang S, Wang H, Mu X, He C, ***et al***. Antioxidant activity of microwave-assisted extract of longan (*Dimocarpus longan* Lour.) peel. Food Chem. 2008;106(3):1264-70.
2. Cho YK, Yun JW, Park JH, Kim HJ, Park DI, Sohn CI, *et al*. Deleterious effects of silymarin on the expression of genes controlling endothelial nitric oxide synthase activity in carbon tetrachloride-treated rat livers. Life Sci. 2009;85(7):281-90.
3. Vijaya T, Mouli KC, Rao SD. Phytoresources as potential therapeutic agents for cancer treatment and prevention. J Global Pharm Technol. 2009;1(1):4-18.
4. Jemal R, Siegel E, Ward T, Xu J, Thun MJ. Cancer statistics. A Cancer J Clin. 2007;57(1):43-66.
5. Rang HP, Dale MM, Ritter JM, Moore PK, Dale R. Pharmacology (5th ed). Elsevier published by India private limited, New Delhi. 2005;493.
6. Trouillas P, Calliste CA, Allais DP. Antioxidant, anti-inflammatory and antiproliferative properties of sixteen water plant extracts used in the Limousin countryside as herbal teas. Food Chem. 2003;80(3):399-407.
7. Halabi F, Sheikh Y. Anti-proliferative effect and phytochemical analysis of *Cymbopogon citratus* extract. BioMed Res. Int. 2014;1:8.
8. RameshKannan N, Nayagam AAJ, Gurunagara S, Muthukumar B, Ekambaram N, Manimaran A. Photochemical screening from *Elaeagnus indica* activity against human pathogens and cancer cells. Adv Biol Res. 2013;7(3):95-103.
9. Shivakumar MS, Srinivasan R, Natarajan D. Larvicidal potential of some Indian medicinal plant extracts against *Aedes aegypti* (L.). Asian J Pharm. Clinical Res. 2013;6(3):77-80.
10. Srinivasan R, Shivakumar MS, Natarajan D. Medicinal plants for *Anopheles stephensi* Liston larvae management. J. Biologically Active Prod Nat. 2015; 4(5-6):391-9.
11. Srinivasan R. Bioactivity Guided Isolation and Structural Elucidation of Antimicrobial, Antioxidant and Larvicidal Compounds from *Elaeagnus indica* and *Memecylon edule* and their Molecular Docking Studies. Ph.D. Thesis, Periyar University, Salem, India. 2014;154.
12. Chew YL, Goh JK, Lim YY. Assessment of *in vitro* antioxidant capacity and polyphenolic composition of selected medicinal herbs from Leguminosae family in peninsular malaysia. Food Chem. 2009;116(1):13-8.
13. Halliwell B, Gutteridge JMC, Aruoma OI. The deoxyribose method: A simple 'test tube' assay for determination of rates constants for reactions of hydroxyl radical. Anal Biochem. 1987;165(1):215-24.
14. Garrat DC. The Quantitative analysis of Drugs. Chapman and Hall Ltd., Japan. 1964;3rd edition,:456-8.
15. Liu F, Ooi VEC, Chang ST. Free radical scavenging activity of Mushroom polysaccharide extracts. Life Sci. 1997;60(10):763-71.
16. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239(1):70-6.
17. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol. Methods. 1983;65(1):55-63.
18. Prasath GS, Sundaram CS, Subramanian SP. Fisetin averts oxidative stress in pancreatic tissues of streptozotocin-induced diabetic rats. Endocr Int J Basic Clin Endocrinol. 2013;44:359-68.
19. Sakai EM, Sugawara S, Yamaguchi Y, Sakamoto H, Fumimoto R, Fukuma Y, *et al*. Fisetin inhibits osteoclastogenesis through prevention of RANKL induced ROS production by Nrf2-mediated up-regulation of phase II antioxidant enzymes. J Pharmacol Sci. 2013;121(4):288-98.
20. Murugesan J, Perumal S. Antihyperammonemic effect of fisetin on hyperammonemic rats: a biochemical study. Int J Mod Res Rev. 2014;2(1):33-9.
21. Shahidi F, Wanasundara PK. Phenolic antioxidants. Crit Rev Food Sci. Nutr. 1992;32(1):67-103.
22. Costa MPD, Bozinis MC, Andrade WM, Costa CR, Silva AL, Oliveira CMA, *et al*. Antifungal and cytotoxicity activities of the fresh xylem sap of *Hymenaea courbaril* L. and its major constituent fisetin. BMC Complementary Altern Med. 2014;14(1):245.
23. Shafaghat A, Salimi F. Extraction and determining of chemical structure of flavonoids in *Tanacetum parthenium* (L.) Schultz. Bip. from Iran. J Sci. 2008;18(68):39.
24. Yoshioka T, Inokuchi T, Fujioka S, Kimura Y. Phenolic compounds and flavonoids as plant growth regulators from fruit and leaf of *Vitex rotundifolia*. Z Naturforsch. 2004;59c(7-8):509-14.
25. Shabana H, Hashem AM, Abdel-Naser S, Sally K, Abdel-Razik F. Protective and therapeutic activities of *Mayodendron igneum* Kurz against Paracetamol induced liver toxicity in rats and its bioactive constituents. J Appl Pharm Sci. 2013;3(7):147-55.
26. Taechowisan T, Srisakul C, Wanwikar R, Waya PS. Antibacterial activity of new flavonoids from *Streptomyces* sp. BT01; an endophyte in *Boesenbergia rotunda* (L.) Mansf. J Appl Pharm Sci. 2014;4(4):8-13.
27. Zhang S, Liu X, Zhang LZ, Lu H, Zhe W, Shu WG. Isolation and identification of the phenolic compounds from the roots of *Sanguisorba officinalis* L. and their antioxidant activities. Molecules. 2012;17(12):13917-22.
28. Bors W, Michel C. Chemistry of the antioxidant effect of polyphenols. Ann N. Y. Acad Sci. 2002;957(1):57-69.
29. Agullo G, Payrastre LG, Fernandez Y, Anciaux N, Demigne C, Remesy C. Comparative effects of flavonoids on the growth, viability and metabolism of a colonic adenocarcinoma cell line (HT29 cells). Cancer Lett. 1996;105(1):61-70.
30. Lu H, Chang B, Baratte L, Gahmen M. Crystal structure of a human cyclindependent kinase 6 complex with a flavonol inhibitor, fisetin. J Med Chem. 2005a; 48(3):737-43.
31. Lu X, Jung J, Cho HJ, Lim DY, Lee HS, Chun HS, *et al*. Fisetin inhibits the activities of cyclin-dependent kinases leading to cell cycle arrest in HT-29 human colon cancer cells. J Nutr. 2005b;135(12):2884-90.
32. Sung B, Pandey MK, Aggarwal BB. Fisetin, an inhibitor of cyclin dependent kinase 6, down regulates nuclear factor kappa B-regulated cell proliferation, antiapoptotic and metastatic gene products through the suppression of TAK-1 and receptor interacting protein-regulated I-kappa B-alpha kinase activation. Mol Pharmacol. 2007;71(6):1703-14.
33. Constantinou A, Mehta R, Runyan C, Rao K, Vaughan A, Moon R. Flavonoids as DNA topoisomerase antagonists and poisons: structure-activity relationships. J Nat Prod. 1995;58:217-25.
34. Olaharski AJ, Mondrala ST, Eastmond DA. Chromosomal malsegregation and micronucleus induction *in vitro* by the DNA topoisomerase II inhibitor fisetin. Mutat Res. 2005;58(2):79-86.
35. Jankun J, Selman SH, Aniola J, Jankun ES. Nutraceutical inhibitors of urokinase: potential applications in prostate cancer prevention and treatment. Oncol Rep. 2006;16(2):341-6.

36. Bohl M, Tietze S, Sokoll A, Madathil S, Pfennig F, Apostolakis J, *et al*. Flavonoids affect actin functions in cytoplasm and nucleus. Biophys J. 2007;93(8):2767-80.
37. Khan N, Afaq F, Mukhtar H,. Cancer chemoprevention through dietary antioxidants: progress and promise. Antioxid Redox Signal. 2008;10(3):475-510.
38. Touil SY, Seguin J, Scherman D, Guy CG. Improved antiangiogenic and antitumour activity of the combination of the natural flavonoid fisetin and cyclophosphamide in lewis lung carcinoma-bearing mice. Cancer Chemother Pharmacol. 2010;68(2):445-55.
39. Khan N, Asim M, Afaq F, Abu ZM, Mukhtar H. A novel dietary flavonoid fisetin inhibits androgen receptor signaling and tumor growth in athymic nude mice. Cancer Res. 2008;68(20):8555-63.
40. Murtaza I, Adhami VM, Hafeez BB. Fisetin, a natural flavonoid, targets chemoresistant human pancreatic cancer AsPC-1 cells through DR3-mediated inhibition of NF-kappaB. Int J Cancer. 2009;15(10):2465-73.
41. Srinivasan R, Natarajan D, Shivakumar MS. Antioxidant compound Quercetin-3-O-α-L-rhamnoside (1→6)-β-D-glucose (Rutin) isolated from ethyl acetate leaf extracts of *Memecylon edule* Roxb (Melastamataceae). Free Rad Antiox. 2015;5(1):35-42.

SUMMARY

- Fisetin compound isolated from the *Elaeagnus indica* leaves.
- Spectral characterization of Fisetin compound by NMR, HMBC and HMQC analysis.
- Fisetin compound has strong Antixoidant and aniproliferative potential.

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