Biomedicine & Pharmacotherapy 85 (2017) 444–456

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Characterization of bioactive constituents from the gum resin of *Gardenia lucida* and its pharmacological potential

Priyanka Maurya[a,b,1], Shilpi Singh[b,1], Madan Mohan Gupta[a], Suaib Luqman[b,c,*]

[a] *Analytical Chemistry Department, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow-226015, Uttar Pradesh, India*
[b] *Academy of Scientific and Innovative Research (AcSIR), CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow-226015, Uttar Pradesh, India*
[c] *Molecular Bioprospection Department of Biotechnology Division, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow-226015, Uttar Pradesh, India*

ARTICLE INFO

Article history:
Received 22 September 2016
Received in revised form 8 November 2016
Accepted 11 November 2016

Keywords:
Gardenia lucida
Antiproliferative
ODC
CATD
Polymethoxyflavones

ABSTRACT

In the present study we mined the information on *Gardenia lucida* (Dikamali) and identified seven polymethoxyflavones from its gum resin. We also investigated its antiproliferative and antioxidant potential. Xanthomicrol (8) found as potent DPPH scavenger (85.86 ± 1.3%) along with strong ferric plummeting ability (53.60 ± 2.0 FSE) and reducing potential (1.07 ± 0.01) as compared to ascorbic acid. Gardenin B (5) strongly inhibit biochemical production of nitric oxide (IC_{50} 10.59 ± 0.4 μg/mL) followed by 5-Desmethylnobiletin (7) and Gardenin E (10, IC_{50}11.01 ± 0.7–34.53 ± 2.7 μg/mL). Methanol extract, chloroform fraction and Acerosin (11), Gardenin D (9) and Gardenin B (5) exhibited superior antiproliferative activity against lung, breast, colon, hepatic and leukaemia cell lines as well as in keratinocytes (IC_{50} 12.82 ± 0.67–94.63 ± 1.27 μg/mL) whereas other fractions and isolated compounds moderately affect the cell proliferation (21.40 ± 0.12–48.12 ± 0.47%) with least and non-specific interaction against succinate dehydrogenase. Except compound 2, 3, 6, 8 and 11, others were found as a significant inhibitor of ODC (IC_{50} 2.36 ± 0.7–8.53 ± 0.32 μg/mL) with respect to DFMO (IC_{50} 10.85 ± 0.28 μg/mL). *In silico* analysis also revealed enervated binding energy (−4.30 to −5.02 kcal/mol) and inhibition constant (704.18–210.26 μM) wherein 5, 7, 8, 9 and 10 showed specific interaction with the receptor while rest were non-specific. Except butanol fraction and Gardenin E, others were potently inhibited the cathepsin D activity with non-specific interaction and better binding energy (−5.78 to −7.24 kcal/mol) and inhibition constant (57.87–4.90 μM). In conclusion, it can be interpreted that isolated polymethoxyflavones (Gardenin B, 5-Desmethylnobiletin, Gardenin E) could be taken up as a lead for target specific studies. Methanol extract and chloroform fraction prevails in all the tested activity therefore cumulative and composite intervention of polymethoxyflavones present in it reveals its pharmacological attributes and traditional value.

1. Introduction

The exploration of drugs for cancer pain management has been a long-standing goal in basic and clinical research. Phytochemicals including flavonoids are known to inhibit cancer cell proliferation, regulate inflammatory and immune response and protect against lipid oxidation [1,2]. Flavonoids, polyphenolic compounds are ubiquitous in nature and have aroused considerable interest because of their beneficial effects on human health. There are about 80 species of Gardenia found in different tropical and subtropical regions of the world. Dikamali is obtained from *Gardenia lucida* which belongs to the family Rubiaceae. It is a gum resin which exudes from the leaf buds of *G. lucida.* The gum resin secretes from the leaf buds appears as yellow tears and marketed in the herbal shops in form of greenish-yellow lumps. It has an

Abbreviations: DPPH, 2, 2-diphenyl-1-picryl hydrazyl; GAE, gallic acid equivalent; FRAP, ferric reducing antioxidant power; FSE, ferrous sulphate equivalent; QCE, quercetin equivalent; AAE, ascorbic acid equivalent; MTT dye, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; DMEM, Dulbecco's modified essential eagle medium; FBS, fetal bovine serum; ODC, ornithine decarboxylase; CATD, cathepsin D; SDH, succinate dehydrogenase; PDB, protein data bank; PEP, pepstatin A; DFMO, difluoromethylornithine.

* Corresponding author at: Academy of Scientific and Innovative Research (AcSIR), CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow-226015, Uttar Pradesh, India.
E-mail address: s.luqman@cimap.res.in (S. Luqman).

[1] These authors equally contributed to this work.

http://dx.doi.org/10.1016/j.biopha.2016.11.049
0753-3322/

offensive odor and a sharp pungent taste [3]. According to a review [4], a number of flavonoids have been isolated from Gardenia genus. In the Indian Traditional System of Medicine, Dikamali gum have been reported to possess various properties such as antispasmodic, expectorant, diaphoretic, carminative, antimicrobial, antihelminthic and many more [5]. It is also claimed to be useful in dyspepsia, flatulence for cleaning foul ulcers and wounds, and to keep off flies from wounds in veterinary practice [6,7]. Owing to its high medicinal value, the gum resin of the plant is of exclusive commercial importance along with its traditional significance.

Polymethoxyflavones (PMFs) exhibit a broad spectrum of biological properties including anticancer, antiatherogenic and neuroprotective effects. In quest of our ongoing interest in natural products, we have investigated the antiproliferative and antioxidant efficacy of crude methanol extract (1), hexane (2), butanol (3), chloroform fractions (4) and seven polymethoxyflavones (5–11) isolated from the gum resin of *G. lucida*. Additionally, a probable mechanism for antiproliferative activity has been worked out by targeting succinate dehydrogenase (SDH), ornithine decarboxylase (ODC) and cathepsin D (CATD). Moreover, molecular interaction studies of ODC, CATD and SDH with that of the isolated compounds (5–11) have also been explored by utilizing *in silico* approach.

2. Materials and methods

2.1. Collection of plant material

The gum resin was obtained from *G. lucida*. Dikamali were purchased from the local market of Lucknow, Uttar Pradesh, India. A voucher specimen (No. 11190) has been deposited in the crude drug repository of CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, India.

2.2. Reagents and chemicals

Compounds were isolated in the laboratory from *G. lucida* for bioactivity evaluation. Silica gel (60–120 mesh) for column chromatography was purchased from M/s Merck India (Mumbai, India). All the solvents Petroleum ether (PE), Ethyl acetate (EtOAc), Acetone (Ac), Methanol (MeOH) used were of AR grade.

2.3. Equipment used for analysis

The 300-MHz and 500-MHz NMR spectra were recorded with tetramethyl silane as an internal standard on a Bruker Advance instrument. ^{13}C NMR and DEPT spectra were recorded at 75 MHz and 125 MHz. The DEPT experiments were used to resolve multiplicities of carbon atoms. Chemical shifts are given in parts per million. COSY, HSQC, and HMBC were performed using standard Bruker pulse programs. The ESI–MS was obtained on a MS-2010EV (Shimadzu, Japan) at 70 eV by flow injection into the electrospray source. The instrument was operated in positive and negative ion modes both. One of the compounds was purified on a semi-preparative LC-8A (Shimadzu) using reverse phase column (Ascentis C18 HPLC column, 10 cm × 10 mm, 10 μm).

2.4. Extraction and isolation of polymethoxyflavones

The air-dried gum resin of *G. lucida* (1 kg) was finely powdered and percolated with methanol (8L, 2L every 24 h) at room temperature; the process was repeated four times and evaporated *under vacuo* to yield crude extract (675.2 g). This crude extract was suspended in water (600 ml) and extracted successively with hexane (3 × 500 ml), chloroform (3 × 500 ml) and butanol (3 × 500 ml). Vacuum concentration yielded hexane extract (0.81 g), chloroform extract (641.2 g) and butanol extract (6.07 g). Classical glass column chromatography of chloroform extract (180 g) over silica gel (60–120 mesh) with a gradient elution of hexane-ethyl acetate was conducted.

2.5. DPPH (2, 2′-diphenyl-1-picrylhydrazyl) assay

Free radical scavenging ability of extract, fractions and isolated compounds were performed according to the method reported earlier [8,9]. Inhibition of dark violet color produced due to DPPH was measured spectrophotometrically at 517 nm. Concentration ranges from 0.4–50 μg/mL were added to 0.1 M Tris buffer pH (7.4) and 0.5 mM of DPPH, after half an hour incubation at room temperature, scavenging capacity were noted spectrophotometrically against a buffer blank and reagent control.

2.6. Nitric oxide assay

The scavenging potential of extract, fractions and isolated compounds were estimated according to the procedure described earlier [10]. Different concentration ranges from 0.4–50 μg/mL were added to sodium nitroprusside (10 mM in phosphate buffer saline) followed by incubation at room temperature for 30 min generate nitric oxide, released nitric acid get transformed into nitrous acid upon addition of 100 μl Griess reagent and absorbance was recorded at 546 nm. Results were expressed in terms of percent inhibition/scavenging which was calculated and compared with reagent control.

2.7. Ferric ion reducing antioxidant power (FRAP) estimation

FRAP assay estimate antioxidant capacity of samples by reduction of ferric tripyridyltriazine (Fe (III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe (II)-TPTZ). In FRAP assay, extract, fractions and isolated compounds (0.4–50 μg/mL) were added to the freshly prepared FRAP reagent (TPTZ + ferric chloride + acetate buffer in 1:1:10 ratio) and absorbance was recorded as described by Benzie & Strain (1996). The ferric reducing capacity was expressed as ferrous sulfate equivalent described earlier [8,9].

2.8. Reducing power determination

The assay was performed according to Yen and Chen [11] with slight modification [12]. The method is important for determining the ferric reducing potential of samples. A concentration range from 0.4–50 μg/mL were mixed with phosphate buffer (200 mM, pH 6.6) followed by addition of 1% potassium ferricyanide and incubation in a water bath for 20 min at 50° C. The reaction mixture would precipitate upon addition of 10% TCA, followed by centrifugation for 5 min at 5000 rpm. After addition of 0.1% (w/v) ferric chloride to the supernatant absorbance was read at 700 nm.

2.9. Total phenolic determination

Total phenolic content were determined by Folin reagent as described previously [13]. For the estimation of phenolic content, extracts, fractions and isolated compounds were added to Folin's reagent and sodium carbonate, then absorbance was read at 765 nm after incubation at 37 °C for 90 min. Results of total