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Endemic and endangered ethno-herbal medicinal climber of Darjeeling hills (*Edgaria darjeelingensis* C.B. Clarke) is a treasure of anti-cancer molecules: A study on GC-MS analysis and probable biosynthetic pathways

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Carcinogenesis, oncogenesis or tumourigenesis is a complex biomolecular mechanism that could progressively transform normal cells to malignant forms. Proper diet constituting natural fruits and vegetables enriched in bioactive components can reduce the risk factors of several forms of human cancer and other critical ailments. Endemic and endangered Cucurbitaceous climber of Darjeeling hills, *Herpetospermum darjeelingense* (C.B. Clarke) H. Schaef. & S.S. Renner [*Edgaria darjeelingensis* C.B. Clarke] is a repository of bioactive anti-cancer metabolites as depicted by the presence of fourteen anti-carcinogenic molecules explored via GC-MS analysis, which could potentially minimize the threats of various forms of cancer. The anti-cancer compounds cover about 79.22% of total GC-MS area percentage out of the total thirty four compounds detected. The explored cellular biosynthesis pathway of these anti-carcinogenic metabolites seems to follow a systemic metabologenesis pattern that may be required for the day-to-day survival of the plant and not a mere chance factor, which could have stressed the plant to produce those biochemical molecules. Isolation, pharmaceutical screening of these compounds and their medical application can revolutionise the dimension of the global cancer research in the near future.

Keywords: Anti-cancer, *Edgaria darjeelingensis,* GC-MS, Metabolites, Pathway

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Carcinogenesis is a multi-step procedure leading to genetic and epigenetic abnormalities with consequential transformation of normal cells towards malignancy[1]. The combination of healthy diet and nutritional alteration has long been thought to be an efficient regimen for cancer prevention[2]. Edible vegetables and fruits contain considerable amounts of chemopreventive molecules like vitamins, carotenoids, flavonoids, polyphenols, isoflavones, catechins including several other bioactive components which minimize the risk of several forms of human cancers[3]. Chemoprevention of cancer aims to inhibit and delay neoplastic development by blocking inception of neoplasia in addition to reversing further progression of transformed cells before manifestation of malignant lesions[4]. Most effective approach towards chemoprevention is formulating and testing new biomolecules which could proficiently act on precise cellular and molecular targets. Two main strategies include identification of natural dietary agents followed by effective bioactive anti-cancer compound screening and synthesizing pharmaceutically potent molecular target based agents as a part of chemopreventive protocols[4,5]. Plant community is the most readily available natural treasure which can be explored as a major source of chemopreventive agents for the treatment of cancer.

Numerous endemic species of plants are indigenous to Darjeeling hills. This region with its wide range of altitudinal variations is a part of eastern Himalayas and is famous for its rich biodiversity, Tea and natural beauty[6]. Darjeeling region is also inhabited by several tribes of cross border origin. Biodiversity of this region has been explored by diverse tribal communities and their traditional knowledge enriched practice of ethno-medicine in this mountainous terrain. Out of the various endemic species explored in traditional medicine, a cucurbitaceous climber *Herpetospermum darjeelingense* (C. B. Clarke) H. Schaef. & S. S. Renner [*Edgaria darjeelingensis* C. B. Clarke] is mostly unexplored. This endemic and endangered plant

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occurs at an altitude of 1450-3000m and its fruits being economically beneficial as a vegetable source to the ancient tribal ethnic group 'Tharu'[7]. *Edgaria darjeelingensis* (ED) is also used as veterinary medicine by the Tamangs and local Nepali population with pounded seeds of ED utilized in treatment of fever among the bovine animals[8]. A single report on anti-oxidant molecules in ED also emphasises the ethno-medicinal relevance of the cucurbit[9].

Though there are some reports on traditional use of ED leaves in veterinary medicine without any such delineated application in humans; comprehensive biochemical profiling of this plant is almost lacking. So, the study was conducted to overcome the knowledge gap by Gas Chromatography-Mass Spectroscopic (GC-MS) analysis for the study of metabolomics with special reference to biosynthesis pathway of the available anti-cancer compounds.

Materials and Methods

Collection of plant material

Plant specimen was identified by taxonomist of Department of Botany, Darjeeling Government College, Darjeeling (Voucher number KPGC/MB/82). Leaves of ED were collected from Darjeeling (27.050904°N and 88.260809°E). The leaves were separated from grown-up shoots, filled in zipper bags and preserved in insulated ice packed boxes for transferring to the laboratory.

Preparation of extract

The collected leaves of ED were washed properly in running tap water for removal of adherent dust particles and successively cleaned with distilled water. Healthy and clean leaves were surface dried using tissue paper and subsequently grinded mechanically to obtain a powdery mass. 3 g of powdered leaf sample was dipped in methanol and allowed to stand for 48 h at room temperature. The obtained extract was filtered through ash-less filter paper followed by drying of the complete extract at a mild temperature, with the remnants being finally dissolved in methanol for adjusting the extract concentration to 25 mg/mL.

Gas chromatography-mass spectrometry analysis

Methanolic extract of ED (concentration adjusted to 25 mg/mL) was used for GC-MS investigation[9,10] employing the instrument GC-MS-QP2010 Plus. 1 µL extract was injected in split mode with injection and interface temperature set at 260°C and 270°C respectively. Ion source temperature was calibrated to 230°C with Helium as the carrier gas. Total flow rate was 16.3 mL min^{-1} in addition to column flow rate being adjusted to 1.21 mL min^{-1}. Mass spectra was recorded at 5 scan sec^{-1} with corresponding scanning range of 40-650 m/z. Compound quantification was made based on the individual peak areas and respective compounds were identified by comparing the obtained spectral configurations with mass spectral databases of WILEY8.LIB (Hoboken, NJ, USA) and NIST08s.LIB (Gaithersburg, MD, USA). The generated Total Ion Chromatogram (TIC) is based upon the intensity of fragments produced due to ionization. Quantitative estimation of individual compounds (area %) was determined in compliance with comparative peak areas. The data retrieved via GC-MS probe was further examined through available resources.

Study on biosynthesis pathway of anti-cancer molecules in ED

Framing metabologenesis pathway of diversified GC-MS detected anti-cancer compounds in ED were accomplished through comprehensive literature review and bioinformatic database resources; such as KEGG pathway (https://www.genome.jp/kegg/pathway.html); PubChem (https://pubchem.ncbi.nlm.nih.gov); UniProt (https://www.uniprot.org/); MetaCyc (https://biocyc.org/META); ChEBI (https://www.ebi.ac.uk/chebi/); Good Scents Company Information System (http://www.thegoodscents company.com/) and Chem Spider (http://www.chemspider.com/).

Results

GC-MS analysis

GC-MS study of ED methanolic extract revealed presence of 34 biochemical compounds. The biological activities of the detected compounds were studied from available literature and it was observed that 27 of them had preventive and curing properties for several ailments including anti-oxidant, anti-inflammatory and anti-carcinogenic attributes while bioactivity was yet to be reported for rest of the compounds.

Anti-cancer compounds in ED

Fourteen bioactive compounds- 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- [1.52%]; Bicyclo [8.1.0]undeca-2,6-diene,3,7,11,11-tetramethyl-, (1R*,2 Z,6E,10R*)-(.+-.)- [0.42%]; 1,3,4,5-Tetrahydroxy cyclohexanecarboxylic acid [10.80%]; 3-Buten-2-ol,4-

(2,6,6-trimethyl-1-cyclohexen-1-yl) [0.76%]; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol [19.24%]; Hexadecanoic acid, methyl ester [9.47%]; 9,12-Octadecadienoic acid(Z,Z)-, methyl ester [3.54%]; 9,12,15-Octadecatrienoic acid, methyl ester,(Z,Z,Z)- [15.67%]; Squalene [1.24%]; Neryl linalool isomer [0.41%]; 1-Naphthalenepropanol,.alpha.-ethenyldecahydro-2-hydroxy-.alpha.,2,5,5,8a-pentamethyl-[1R[1.alpha. (R*),2.beta.,4a.beta.,8a.alpha.]- [1.1%]; Ergost-5-en-3-ol, (3.beta.,24R)- [0.82%]; Stigmast-5-en-3-ol,(3.beta.)- [12.96%] and Stigmasta-5,24 (28)-dien-3-ol, (3.beta.)- [1.27%] are reported to have anti-cancer, anti-tumour, anti-proliferative, anti-mutagenic, and anti-melanogenic attributes which can be correlated to effective cancer healing or remedial regimes (Table 1). These 14 compounds occupy 79.22% area to justify ED as a focal point of anti-cancer molecules.

4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl

It is a potent anti-proliferative natural compound and a typical flavonoid reported in fresh plums (*Prunus domestica* L.) including commercial and homemade prunes of the angiospermic family Rosaceae[11]. The IUPAC name of this chemical is 3,5-Dihydroxy-6-methyl-2,3-dihydropyran-4-one. It is commonly known as Pyranone with molecular formula $C_6H_8O_4$ in addition to framing the central core of several natural phenolic compounds *viz*. Kojic acid, Maltol and some important class of flavones possessing molecular weight of 144.12 g/mol.

Bicyclo[8.1.0]undeca-2,6-diene,3,7,11,11-tetramethyl-,(1R*,2Z,6E,10R*)-(.+-.)

Universally known as Bicyclogermacrene, is a class of sesquiterpenoid compound derived from a hydride of Germacrane by dehydrogenation reactions across the C(1)-C(10) and C(4)-C(5) bonds with further cyclisation across the C(8)-C(9) bond. It has been described as a formidable anti-cancer metabolite, having molecular formula $C_{15}H_{24}$ and molecular weight of 204.35 g/mol with significant cytotoxicity towards hepatic HepG2/ADM cancerous cell lines[12]. The sources include seeds of *Abelmoschus moschatus* Medik. (Malvaceae)[13]; aerial parts of *Ageratum conyzoides* L. (Asteraceae)[14] and leaves of *Cucurbita maxima* Duchesne (Cucurbitaceae)[15].

1,3,4,5-Tetrahydroxy-cyclohexanecarboxylic acid

More commonly known as Quinic acid, is known for its anti-proliferative activity[16] including ability to inhibit the master regulatory transcription factor NF-κB[17] that mediates crosstalk between inflammation and cancer at multiple levels. It is a conjugate acid of quinate and by chemical nature is a cyclitol, cyclic polyol, and cyclohexanecarboxylic acid with corresponding molecular formula and molecular weight of $C_7H_{12}O_6$ and 192.17 g/mol. Crystalline quinic acid is obtained from *Cinchona* bark, coffee beans (Rubiaceae), apples, peaches, pears, plums, vegetables, tobacco and carrot leaves. Quinic and shikimic acid are key metabolic intermediates in the biosynthesis of numerous aromatic compounds in living systems.

3-Buten-2-ol,4-(2,6,6-trimethyl-1-cyclohexen-1-yl)

A potent anti-cancer metabolite with molecular formula $C_{13}H_{22}O$ and molecular weight 194.31 g/mol is a sesquiterpene class organic compound synthesized via the terpenoid metabologenesis scheme. Commonly regarded as beta-ionol; its natural sources include grapes and raspberry fruits being attributed to exhibit anti-melanogenic effects[18].

3,7,11,15-Tetramethyl-2-hexadecen-1-ol

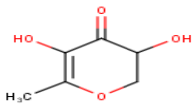
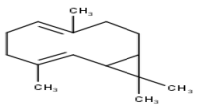
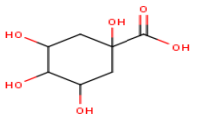
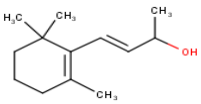
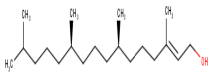
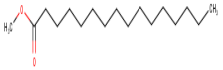
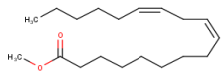
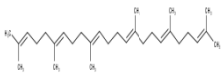
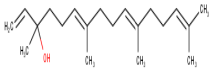
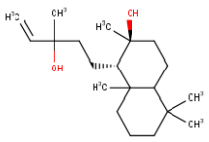
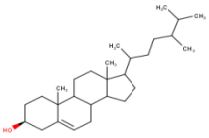
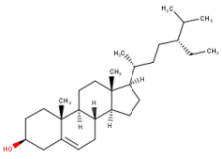
Frequently referred to as phytol is an ingredient of chlorophyll and an ubiquitous constituent in plant kingdom. It possesses anti-cancer[19,20]; anti-tumour, autophagous, apoptosis inducing and anti-teratogenic[21,22] properties. Phytol can be utilised as a chemical precursor for the manufacture of synthetic forms of vitamin E and K1. Furthermore, it has been characterised to modulate cellular transcription via transcription factor's PPAR-α and retinoid X receptor (RXR) whose altered functional expression is reported as a contributing factor in the development of numerous cancers. Phytol is an acyclic diterpene long chain primary fatty alcohol possessing molecular formula and molecular weight of $C_{20}H_{40}O$ and 296.5 g/mol respectively. Chemically it is Hexadec-2-en-1-ol with alkylation across 3,7,11 and 15 Carbon positions.

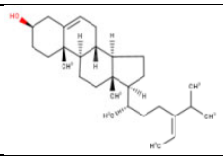
Hexadecanoic acid, methyl ester-

Widely regarded as Methyl palmitate is a class of organic compound belonging to the fatty acid methyl ester (FAME) group having molecular formula $C_{17}H_{34}O_2$ and molecular weight of 270.5 g/mol. Hexadecanoic or Palmitic acid is a saturated fatty acid (FA) and the most common FA prevalent in plants,

animals and microorganisms reported to display anti-cancer property[23]. It is also known to provide protection against Silica-induced pulmonary fibrosis and lung inflammation in rats[24]. This FAME is reported in *Eichhornia crassipes* (Mart.) Solms

Table 1 — List of anticancer compounds and their potential attributes

Name of anticancer compound	Common Name	Nature	Molecular formula	Molecular weight (g/mol.)	Structure
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Pyranone	Flavones	$C_6H_8O_4$	144.12	
Bicyclo[8.1.0]undeca-2,6-diene,3,7,11,11-tetramethyl-,(1R*,2Z,6E,10R*)-(.+-.)-	Bicyclo-germacrene	Sesquiterpenoid	$C_{15}H_{24}$	204.35	
1,3,4,5-Tetrahydroxy cyclohexane carboxylic acid	Quinic acid	Cyclitol; cyclic polyol and a cyclo-hexane carboxylic acid	$C_7H_{12}O_6$	192.17	
3-Buten-2-ol,4-(2,6, 6-trimethyl-1-cyclohexen-1-yl)-	β-Ionol	Sesquiterpenoid	$C_{13}H_{22}O$	194.31	
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Phytol	Acyclic diterpene alcohol	$C_{20}H_{40}O$	296.5	
Hexadecanoic acid, methyl ester	Methyl palmitate	Fatty acid methyl ester	$C_{17}H_{34}O_2$	270.5	
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Methyl linoleate	Fatty acid methyl ester	$C_{19}H_{34}O_2$	294.5	
9,12,15-Octa-decatrienoic acid, methyl ester, (Z,Z,Z)-	α-Methyl linolenate	Fatty acid methyl ester	$C_{19}H_{32}O_2$	292.5	
Squalene	Spinacene, Supraene	Triterpene	$C_{30}H_{50}$	410.7	
Neryl linalool isomer	(E)-Geranyl linalool	Diterpenoid tertiary alcohol	$C_{20}H_{34}O$	290.5	
1-Naphthalenepropanol,.alpha.-ethenyldecahydro-2-hydroxy-.alpha.,2,5,5,8a-pentamethyl-,[1R[1.alpha.(R*),2.beta.,4a.beta.,8a.alpha.]]	Sclareol	Labdane diterpene	$C_{20}H_{36}O_2$	308.5	
Ergost-5-en-3-ol, (3.Beta.,24R)-	Campesterol	Sterol	$C_{28}H_{48}O$	400.7	
Stigmast-5-en-3-ol, (3.Beta.)-	β-sitosterol	Sterol	$C_{29}H_{50}O$	414.7	

Stigmasta-5, 24(28)-dien-3-ol, (3.Beta.)-	Fucosterol	Sterol	$C_{29}H_{48}O$	412.7	

(Pontederiaceae)[25] and members of the family Chenopodiaceae[26].

9,12-Octadecadienoic acid (Z,Z)-, methyl ester

A FAME class biomolecule popularly known as Methyl linoleate derives from 9,12-Octadecadienoic (Linoleic) acid; an omega-6 Poly-unsaturated essential FA with two successive double bonds at C-9 and C-12 positions having Z (Cis) stereochemistry including established role as an anti-cancer metabolite[27]. Linoleic acid is widely prevalent in plant-based oils being reportedly used in the biogenesis of prostaglandins and cell membrane entities. Methyl linoleate possesses molecular formula $C_{19}H_{34}O_2$ with 294.5 g/mol molecular weight and has been isolated from *Neolitsea daibuensis* Kamik. (Lauraceae).

9,12,15-Octadecatrienoic acid, methyl ester,(Z,Z,Z)

Another FAME group compound having cancer preventive property[19,28]. Commonly referred to as α-Methyl linolenate is the corresponding methyl ester of α-Linolenic acid (ALA); one of the two essential FA's that cannot be synthesized by the human body with cardio-protective attributes. It is an omega-3 Poly-unsaturated FA (PUFA) ester possessing molecular formula and molecular weight of $C_{19}H_{32}O_2$ and 292.5 g/mol respectively. Sources include *Syzygium antisepticum* (Blume) Merr. & L.M. Perry and *Syzygium jambos* (L.) Alston [Myrtaceae].

Squalene

An organic hydrocarbon and a triterpenoid moiety of 2,6,10,15,19,23-Hexamethyltetracosane consisting of six double bonds at 2,6,10,14,18 and 22 Carbon positions with all E configuration. It is a precursor or an intermediate metabolite in the synthesis of all plant and animal sterols including cholesterol and has effective role as a human, plant, *Saccharomyces cerevisiae* (Yeast) and mice metabolite. Squalene was originally obtained from shark liver oil, a natural 30-carbon isoprenoid compound resistant to lipid peroxidation in addition to exhibiting anti-cancer, anti-neoplastic[20,29]; cyto-protective[30] and anti-tumour properties[31] labelling this bioactive molecule as an effective weapon in adjunctive cancer therapy. It also shows chemopreventive effects in colon cancer[32] with molecular formula $C_{30}H_{50}$ and molecular weight 410.7 g/mol being reported in olive oil (564 mg/100 g), soybean oil (9.9 mg/100 g), rice, wheat germ, grape seed oil (14.1 mg/100 g), peanut (27.4 mg/100 g), corn, and amaranth (5942 mg/100 g)[33].

Neryl linalool isomer

Widely recognized as (E)-Geranyl linalool is a diterpenoid tertiary alcohol and chemically modified linalool (monoterpene) with one of the terminal methyl hydrogen's being replaced by a geranyl group possessing molecular formula $C_{20}H_{34}O$ and molecular weight 290.5 g/mol. It has been characterised as an anti-cancer metabolite[34], fragrance molecule and Serine C-palmitoyltransferase (EC 2.3.1.50) inhibitor with reported occurrence in the leaves of *Ixora brachiata* Roxb. (Rubiaceae)[34].

1-Naphthalenepropanol,.alpha.-ethenyldecahydro-2-hydroxy-.alpha.,2,5,5,8a-pentamethyl-[1R[1.alpha.(R*),2.beta.,4a.beta.,8a.alpha.]]

Commonly known as Sclareol is a representative member of the labdane diterpene family with varied biological attributes viz. anti-cancer, anti-inflammatory and anti-oxidant properties[35] having molecular formula $C_{20}H_{36}O_2$ and molecular weight 308.5 g/mol. Sources include essential oil of *Salvia sclarea* L. (Lamiaceae)[36].

Ergost-5-en-3-ol, (3.beta.,24R)

Regularly referred to as Campesterol is an anti-cancerous phytosterol[37] that has the ability to prevent proliferation of various cancer cell lines, including pulmonary cancer[38]. The bioactive compound is a steroid derivative possessing molecular formula $C_{28}H_{48}O$ and molecular weight 400.7 g/mol. It is the simplest sterol, featured by the presence of hydroxyl group in position C-3 of the steroid skeleton in addition to saturated bonds throughout the sterol structural framework, with exception in the existence of 5-6 double bonds in B ring. It is common in banana, pomegranates, pepper, coffee, grapefruit, cucumber, oat, potato and lemon grass.

***Stigmast-5-en-3-ol,* (*3.beta.*)**

Synonymous to β-Sitosterol; a triterpene sterolic entity common in plants with chemical structure similar to that of cholesterol. It is an alcohol-soluble and hydrophobic-natured metabolite with molecular formula $C_{29}H_{50}O$ and molecular weight of 414.7 g/mol. Predominantly known for its anti-cancer activities[39]; β-Sitosterol induces apoptosis in MCF-7 cells by activating key caspases in MDA-MB-231 epithelial breast cancer cell lines and thus serves as a preventive biomolecule in the treatment of breast cancer[40]. It also inhibits the growth of several specific types of tumour cell under *in vitro* conditions including decrease in the size and extent of tumour metastasis *in vivo* situation[41]. β-Sitosterol has been attributed to possess chemopreventive potential by virtue of its *in vivo* radical quenching ability causing minimal toxicity to normal cells as a part of collateral damage[42]. It is commonly found in rice bran, wheat germ, corn oils, soybeans, and peanuts.

***Stigmasta-5,24* (*28*)-*dien*-3-*ol,* (*3.beta.*)**

Usually designated as Fucosterol, is a phyto-sterol possessing the molecular formula $C_{29}H_{48}O$ and molecular weight of 412.7 g/mol. It is a potential anti-cancer compound[43] isolated from marine macro brown algae (Phaeophyceae) *viz*. *Ecklonia cava* and *Ecklonia stolonifera* of the order Laminariales including cyanobacteria and diatoms with anti-oxidant and hepatoprotective activity.

Discussion

Critical analysis of GC-MS results exemplify five out of the fourteen bioactive compounds exhibiting anti-tumour characteristics share a comparatively higher proportion among the available anti-cancer metabolomes in ED. The five metabolites namely- 1,3,4,5-Tetrahydroxy-cyclohexanecarboxylic acid; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; Hexadecanoic acid, methyl ester; 9,12,15-Octadecatrienoic acid, methyl ester,(Z,Z,Z)-; and Stigmast-5-en-3-ol, (3.beta.)-, cover about 68.14% of total GC-MS area percentage and 86.01% among the total detected anti-neoplastic molecules (Fig. 1).

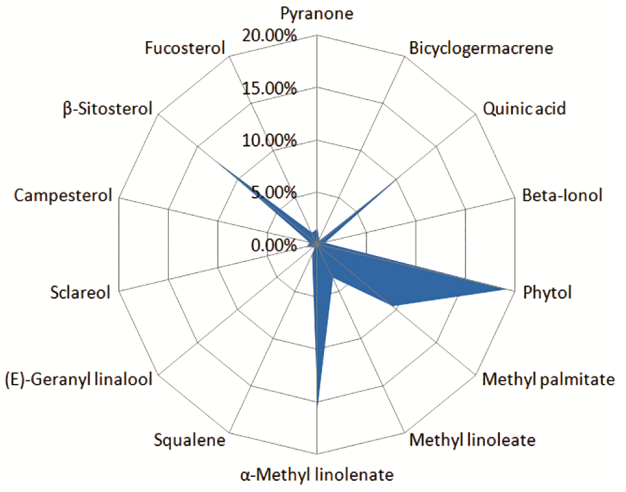


Fig. 1 — Graphical representation of the percentage of anticancer compounds in ED

All of the 14 anti-cancer compounds are of immense medicinal values and are very important in ascertaining bioactivity to the highly endemic and endangered underexplored Cucurbit climber. However, without the metabolic pathway analysis of these anti-tumour metabolites, the chemical nature and origin of these biomolecules remains untraceable and incomplete. Therefore, the metabologenesis pattern of these anti-angiogenic molecules was undertaken using KEGG Pathway Database for exploration of their biochemical precursors, intermediates, site of origin and biosynthetic pathways involved.

Metabolic activities in a plant cell are far more complex compared to an animal cell owing to the maximum amount of abiotic and biotic stresses encountered by it as a characteristic feature of its immobility. ED is no exception to it as evidenced by the major chemical compounds revealed through GC-MS analysis of which fourteen metabolites are attributed to be of anti-cancer values. The source of these anti-neoplastic biomolecules can be well understood with reference to the major metabolic pathways operating in plants. Figure 2 provides a bird's eye view of the nature and origin of these anti-angiogenic molecules within the cytosol in addition to the semi autonomous organelle chloroplast and mitochondria being the other two major core site of biosynthesis. The anti-carcinogenic metabolites based on their chemical nature can be depicted to originate via three unique metabologenesis scheme namely the terpenoid biogenesis system, the FA fabrication mechanism and the Shikimate pathway (SP) organization. Two long chain FA derivatives (Methyl linoleate and Methyl linolenate) and four triterpenoids (Squalene, Campesterol, β-Sitosterol and Fucosterol) are discretely produced in the cytoplasm while two sesquiterpenes (Bicyclogermacrene and β-Ionol) are

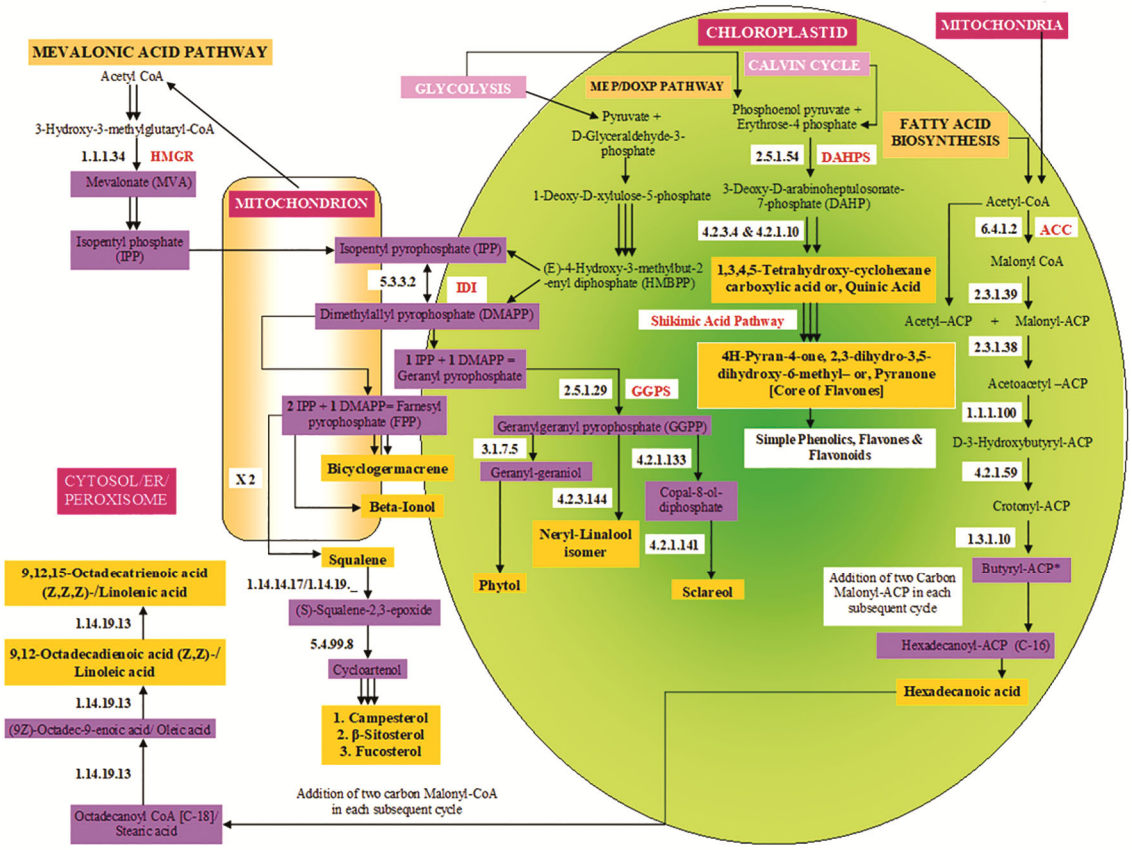


Fig. 2 — Pathway diagram depicting the major metabolic pathways leading to the synthesis of the anticancer compounds in ED

anticipated to be biosynthesized collaboratively in the plant cytosol, mitochondrion and chloroplastid respectively. The diterpene entities (Phytol, (E)-Geranyl linalool and Sclareol) together with Quinic acid, Pyranone and Methyl palmitate are exclusively anabolized in the chloroplast. The terpenoid backbone metabolism involves joint association of the MVA (Mevalonic acid/Mevalonate) reaction machinery operative in cytosol in addition to the MEP/DOXP (2-C-Methyl-D-erythritol-4-phosphate/1-deoxy-D-xylulose-5-phosphate) pathway functional in chloroplast. The SP is responsible for generation of flavonoids, flavones and pyranones; while the FA biogenesis route is attributed to aid in the production of FA derived biomolecules exclusively in the chloroplastid compartments of the plant cell.

The MVA pathway is initiated utilizing the end products of glycolysis as raw materials namely Pyruvate being converted to Acetyl-CoA during its entry to the Tricarboxylic acid cycle and its subsequent metamorphosis to 3-Hydroxy-3-methylglutaryl-CoA (HMG). The prime enzyme controlling the MVA biogenesis scheme is HMG reductase [HMGR and EC 1.1.1.34] that aid in the conversion of HMG to MVA. HMGR helps to maintain a metabolic pool of MVA, with the gene HMG1 and HMGR1 (592 amino acids in length); reported and annotated in *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) playing a pro-active role in its synthesis. MVA is further transformed to Isopentyl pyrophosphate (IPP); which may also be generated via the MEP/DOXP metabolegenesis scheme commencing from Glycolytic produce Pyruvate and D-Glyceraldehyde-3-phosphate as initial reactants. It consequently produces 1-Deoxy-D-xylulose-5-phosphate in presence of the biocatalyst 1-Deoxy-D-xylulose-5-phosphate synthase [EC 2.2.1.7]; being further anabolised to (E)-4-Hydroxy-3-methylbut-2-enyl diphosphate (HMBPP) and subsequently converting to IPP or Dimethylallyl pyrophosphate (DMAPP) respectively. IPP may also be isomerised to DMAPP catalyzed by Isopentyl-diphosphate δ-isomerase [IDI and EC 5.3.3.2], a critical enzyme regulating the metabolic homeostasis between IPP

and DMAPP. IDI has been attributed to be a product of the gene IPP1 and IPP2 (291 and 284 amino acidsin length) as explored in the model plant *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae).1 molecule each of IPP and DMAPP combine to form Geranyl pyrophosphate (GPP) while 2 molecules of IPP and 1 molecule of DMAPP condenses to produce Farnesyl pyrophosphate (FPP). FPP serves as the common precursor for all sesquiterpene metabolites whereas GPP is converted to Geranylgeranyl pyrophosphate (GGPP) in aid of the biocatalyst Geranylgeranyl pyrophosphate synthase [GGPS and EC 2.5.1.29]; that acts as the mother compound for all diterpenoid biomolecules. The biological catalyst GGPS too have been regarded as an important factor regulating the metabologenesis of diterpene metabolites. GGPS is encoded by the gene named GGPP6 and GGPPS2 (336 and 376 amino acids in length), as characterized in *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae). FPP is either transformed to Germacrene-D via the enzyme (-)-Germacrene-D synthase [EC 4.2.3.75]; in addition to being further metamorphosed to its dehydrogenated derivative Bicyclogermacrene or divergently gives rise to β-Ionol through separate set of reactions. GGPP may be directly anabolised to Neryl linalool isomer catalyzed by Geranyl-linalool synthase [EC 4.2.3.144]; else is either converted to Geranyl-geraniol by geranylgeranyl diphosphate diphosphatase [EC 3.1.7.5] or is transformed to Copal-8-ol-diphosphate aided by Copal-8-ol diphosphate hydratase [EC 4.2.1.133]. Geranyl-geraniol yields Phytol while Copal-8-ol-diphosphate synthesizes Sclareol in assistance of Sclareol synthase [EC 4.2.3.141]. Two molecules of FPP aggregate to produce Squalene; which is subsequently converted to (S)Squalene-2,3-epoxide in presence of the biocatalyst Squalene monooxygenase [EC 1.14.14.17]/Alternative squalene epoxidase [EC 1.14.19.] being successively transformed to Cycloartenol by the enzyme Cycloartenol synthase [EC 5.4.99.8] through a sequence of reactions yields phytosterol representatives Campesterol, β-sitosterol and Fucosterol.

SP is accountable for the synthesis of major phenolic counterparts. Glycolytic product Phosphoenol pyruvate (PEP) and Erythrose-4-phosphate either from the Calvin cycle or Pentose phosphate scheme are fed as raw materials into the SP that consequently generates 3-Deoxy-D-arabinoheptulosonate-7-phosphate (DAHP) catalyzed by DAHP synthase [DAHPS and EC 2.5.1.54]; being further transformed to 3-Dehydroquinate by the enzyme 3-Dehydroquinate synthase [EC 4.2.3.4], followed by a dehydrogenation reaction in aid of the biocatalyst 3-Dehydroquinate dehydratase [EC 4.2.1.10] leads to the formation of Quinic acid; which via a chain of reactions is finally converted to Chorismate; the terminal biomolecule of the Shikimic acid metabologenesis design. Chorismate ultimately gives rise to Pyranone, an intermediate metabolite in the biosynthesis of simple phenolics, flavones and flavonoid molecules. DAHPS may be depicted as the key biocatalyst controlling the biogenesis of the compounds related to SP, as it serves as an entry point regulator in the production of the first metabolic entity DAHP. It is a transferase class enzyme coded by the gene SHKA and ARO1 as described in *Solanum tuberosum* L. (Solanaceae) with 1617 nucleotides encoding 538 amino acids (AA) respectively.

The FA metabologenesis commences with TCA cycle derived Acetyl-CoA being converted to Malonyl-CoA by the action of the enzyme Acetyl-CoA carboxylase [ACC and EC 6.4.1.2]. Malonyl-CoA is transformed to Malonyl-ACP with the aid of the biocatalyst Malonyl transferase [EC 2.3.1.39] while Acetyl-CoA is successively modified to Acetyl-ACP parallely. Acetyl-ACP and Malonyl-ACP condenses together to produce Acetoacetyl-ACP in presence of the enzyme ACP-acetyltransferase [EC 2.3.1.38]. The synthesized Acetoacetyl-ACP is anabolized to D-3-Hydroxybutyryl-ACP by β-Keto-acyl reductase [EC 1.1.1.100] followed by subsequent conversion to Crotonyl-ACP through biocatalytic activity of 3-Hydroxyacyl-ACP dehydratase [EC 4.2.1.59]. Crotonyl-ACP is further metabolized to Butyryl-ACP aided by the enzyme NADPH 2-enoyl CoA reductase [EC 1.3.1.10], which serves as the basic metabolite in the biosynthesis of FA molecules. Addition of repetitive two Carbon entity Malonyl-ACP through consecutive cycles gives rise to Hexadecanoyl-ACP, the precursor of Palmitic acid and its corresponding methyl ester derivative Methyl Palmitate. Hexadecanoyl-ACP is converted to Octadecanoyl-CoA by addition of another Malonyl-CoA unit including introduction of successive unsaturation at C-9(Δ9), C-12(Δ12) and C-15(Δ15) positions in existence of the

biocatalyst Fatty acyl-CoA-desaturase (EC 1.14.19.13) leading to the metabologenesis of Linoleic and Linolenic acid along with their corresponding FA methyl esters Methyl linoleate and Methyl linolenate respectively. Long chain FA's beyond Hexadecanoic acid is biosynthesized in the smooth endoplasmic reticulum (SER) and the prime enzyme controlling the FA biogenesis pathway in ED is ACC[10]. ACC, a ligase class biocatalyst has been characterised to be encoded by plastidal Acc-1 (2254 AA) and cytosolic Acc-2 (2355 AA) gene respectively as reported in *Triticum aestivum* L. (Poaceae)[44].

Conclusion

The eastern Himalaya biodiversity hotspot is a reservoir of endemic, endangered, highly medicinal but biochemically and pharmaceutically unexplored floral varieties. Focus was to identify an unexplored endemic plant and study its biochemical attributes. The GC-MS investigation of ED uncovered the existence of various bioactive compounds possessing anti-oxidant, anti-inflammatory, and anti-carcinogenic properties; among which fourteen compounds were strictly anti-cancerous in nature as per available literature. Now, it was a question whether those anti-cancerous compounds were metabolized in the plant system either due to chance factors, or biotic and abiotic stress mechanisms play a role in their biosynthesis or do they have a specific biological pathway? The answers revealed seems interesting as all of the fourteen anti-cancer compounds seem to follow a specific and ordered metabologenesis pathway that may be essentially required for the survival of the plant. The anti-cancer compounds thus prove ED to be a repository of bioactive molecules, of which five compounds constitute 68.14% of the total biochemical components. The biochemical pathway revealed can help in easy screening or isolation of those components in a large scale contemporary to other favourable factors. Those anti-cancer molecules of ED after isolation, screening and sequential pharmaceutical trial followed by medicinal application can change the dimension of the future cancer research in the global arena and reduce the ill effects of this deadly ailment. Besides that, conservation strategies could also be implemented to preserve these natural ethno-medicinally important floral varieties that could possibly cure even some other complex ailments too presently way beyond the imagination of current medical research.

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Conflict of Interest

There is no conflict of interest.

Author's Contributions

SC and MB conceived the idea and designed the research work. SC, SM and AG carried on all the experiments. SC and MB wrote the draft manuscript. All the authors read and accepted the manuscript.

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