Natural anti-inflammatory terpenoids in *Camellia japonica* leaf and probable biosynthesis pathways of the metabolome

Soumya Majumder, Arindam Ghosh and Malay Bhattacharya*

**Abstract**

**Background:** Metabolomics of *Camellia japonica* leaf has been studied to identify the terpenoids present in it and their interrelations regarding biosynthesis as most of their pathways are closely situated. *Camellia japonica* is famous for its anti-inflammatory activity in the field of medicines and ethno-botany. In this research, we intended to study the metabolomics of *Camellia japonica* leaf by using gas chromatography-mass spectroscopy technique.

**Results:** A total of twenty-nine anti-inflammatory compounds, occupying 83.96% of total area, came out in the result. Most of the metabolites are terpenoids leading with triterpenoids like squalene, lupeol, and vitamin E. In this study, the candidate molecules responsible for anti-inflammatory activity were spotted out in the leaf extract and biosynthetic relation or interactions between those components were also established.

**Conclusion:** Finding novel anticancer and anti-inflammatory medicinal compounds like lupeol in a large amount in *Camellia japonica* leaf is the most remarkable outcome of this gas chromatography-mass spectroscopy analysis. Developing probable pathway for biosynthesis of methyl commate B is also noteworthy.

**Keywords:** *Camellia japonica*, Metabolomics, GC-MS, Anti-inflammatory compounds, Lupeol

**Background**

Inflammation in the body is a result of a natural response to injury which induces pain, fever, and swelling. Both corticosteroids and non-steroidal anti-inflammatory drugs are used to reduce this pain by acting on the anti-inflammatory pathways. But these drugs have undesirable side effects as gastric ulceration, infrequently myocardial infarction, and stroke (Maroon et al. 2010). For centuries, many plant and animal-derived natural compounds have been used to treat inflammation. Those compounds as dietary supplements and herbal remedies are becoming increasingly popular because of their relatively few side effects unlike steroidal and nonsteroidal anti-inflammatory drugs.

*Camellia japonica* is an ornamental flowering plant belongs to the family Theaceae. The wild plants of the genus *Camellia* originated in China (Shandong, east Zhejiang), Taiwan, southern Korea, and southern Japan (Konemann 2004). *Camellia japonica* is reported as a bioactive plant in folk medicine of South Korea, Japan, and China. Antioxidant and anti-inflammatory activities of *Camellia japonica* leaves are already reported and this plant is proved to be a source of triterpenes, flavonoids, tannin, and fatty acids having antiviral, antioxidant, and anti-inflammatory activities (Lee et al. 2017). Seed from this plant is used as a traditional medicine and in folk remedies for the treatment of bleeding and inflammation. Compounds from seed oil and stem bark extract of *Camellia japonica* are known to have anti-inflammatory activities (Lim 2014). Leaf extract of *Camellia japonica* is reported to have a high concentration of vitamin E (25.35%), n-eicosane (10.2%) with other six active components (neophytadiene; all trans-squalene; n-octacosane; 6,9-pentadecadien-1-ol, α-linolenic acid, and n-hexadecanoic acid) related to hyperuricemia (Yoon et al. 2017). However,
the seed oil of this plant is already established as a medici-
cinal ingredient in the pharmaceutical and food industry
(Akihisa et al. 1997). Camellianoside is a quercetin O-
glucoside isolated from the leaves of *Camellia japonica*
and exhibits antioxidant activity. It has a role as a metabolite
and a radical scavenger (Onodera et al. 2006). Our present
investigation aimed to make a GC-MS analysis of *Camellia
japonica* leaf extract to find out the compounds involved in
exhibiting anti-inflammatory activity and study the path-
ways involved in their synthesis. The application of pathway
study in this phytochemical analysis is an innovative strat-
gy for targeting active compounds from this plant extract.

**Methods**

**Sample collection and preparation**

Healthy leaves were collected from an organically culti-
vated *Camellia japonica* plant, which was collected from
Darjeeling Himalaya. Leaves of *Camellia japonica* were
crushed in liquid nitrogen and mixed in methanol (widely
used solvent for extraction) to make *Camellia japonica*
leaf extract (CJLE). The extract was left over-
night on a shaker at 25 °C. The extract was centrifuged
and the supernatant was collected for GC-MS analysis.

**Gas chromatography-mass spectrometry analysis**

Methanolic extract (CJLE) with a concentration of 25
mg/ml was used for GC-MS. This method was adopted
from Das et al. (2014) and Labar et al. (2019) with a
slight modification. One microliter of CJLE was injected
in split mode in the instrument (GCMS-QP2010 Plus).
Injection temperature was 260 °C and the interface
temperature was set to 270 °C. Ion source temperature
was adjusted to 230 °C. Helium was used as carrier gas.
Total flow rate was 16.3 ml min⁻¹ and the column flow
rate was 1.21 ml min⁻¹. Mass spectra were recorded at 5
scan s⁻¹ with a scanning range of 40-650 m/z. Quantifi-
cation of compounds was done on the bases of their
peak areas. The data obtained from GCMS analysis were
further analyzed from available literature.

**Studies on biosynthesis pathways of different terpenoids
found in CJLE**

Studies on biosynthesis pathways of different com-
 pounds detected in GC-MS were done by reviewing several litera-
tures and databases (The Kyoto Encyclopedia of Genes
and Genomes database 2020 and The PubChem 2020).

**Results**

**Gas chromatography-mass spectrometry analysis**

Total of fifty peaks were found in the chromatogram
(Table 1; Fig. 1) showing forty-seven different compounds
where twenty-nine compounds are reported natural
anti-inflammatory agents with a summative value of
83.96% in total area. The major anti-inflammatory
molecules detected in the extract were squalene
(27.25%), lupeol (17.26%), diethyl phthalate (5.11%),
vitamin E (5.01%), and patchouli alcohol (3.49%) along
with other twenty-four anti-inflammatory compounds
contributing a total share over eighty-three percentage
of peak area (shown in Table 2; Fig. 2) where some of
them are reported as pain relievers and as anti-arthritis.

Compounds are arranged on the bases of their chemical
groups regarding their biosynthesis pathways, which
are mainly sesquiterpenes, triterpenes, diterpene, mono-
terpenes, tocopherols, phthalate esters, cannabinoid, and
others (Table 2; Fig. 3). Interestingly, more than seventy-
four percentage of the extract belongs to terpenoids and
their derivatives. Total of twenty compounds (patchou-
lool, alpha-, and gamma-patchouline, alpha-gurjunene,
caryophyllene, isolede, etc.) with 19.58% percentage of
total peak area belong to the class sesquiterpene while
triterpenoids like squalene, oxidosqualene, methyl com-
mate B, and lupeol have occupied 50.02% of peak area
(shown in Fig. 3). Furthermore, five compounds are
present from the group monoterpines (including their
derivatives as linalyl acetate) while sclareol (diterpene),
CB-86 (cannabinoid), and vitamin E (tocopherol) are
lone representatives of their chemical groups having rich
bioactivities. A total of 7.52% area belongs to phthalate
esters where two of them are reported bioactive but
their biosynthesis or biodegradation pathway in this par-
ticular plant is not clear due to absence of previous re-
ports and precursors or intermediates in our result.

**Studies on biosynthesis pathways of different terpenoids
found in CJLE**

Triterpenes, sesquiterpenes, monoterpenes, and tocoph-
erol are major groups of bioactive components found in
plant bodies where their pathways are closely related
with each other as isopentyl diphosphate (IPP) and
dimethylallyl diphosphate (DMAPP) play the role of pre-
cursors in their biosynthesis (Fig. 4). The biosynthesis
pathway of terpenoids in plants involves and regulates a
number of pathways, where bioactive compounds like
squalene, lupeol, vitamin E, patchouli alcohol, eucalyptol,
and linalyl acetate are synthesized which have been
found in CJLE.

**Triterpenoids**

Triterpenoid (50.02%) is the major group of compounds
reported in this GC-MS analysis of CJLE. Squalene, one
of the triterpenes found in CJLE, is involved as a com-
mon precursor for synthesis of various hormones in ani-
mals and sterols in plants. Moreover, squalene, itself is a
major bioactive compound having anti-inflammatory
property (Table 2). Oxidosqualene, also named as epox-
yqsualene and (3S)-2,3-epoxy-2,3-dihydroxysqualene, is
another triterpene compound found in our GC-MS as
| Peak index | R. time (min) | Area (AU) | Area%  | Name                                                                 |
|------------|--------------|-----------|--------|----------------------------------------------------------------------|
| 1          | 7.368        | 493233    | 1.18   | Eucalyptol                                                            |
| 2          | 10.733       | 274998    | 0.66   | Linalyl acetate                                                       |
| 3          | 11.342       | 182020    | 0.43   | 1,7,7-TRIMETHYL-1,10BICYCLO[2.2.1]HEPT-2-YL ACETA                     |
| 4          | 12.157       | 751582    | 1.79   | 3-CYCLOHEXENE-1-METHANOL, ALPHA, ALPHA,                               |
| 5          | 12.753       | 129266    | 0.31   | EPIGLOBULOL                                                           |
| 6          | 13.026       | 794979    | 1.89   | 1H-CYCLOPROP[1]AZULENE, 1A,2,3,4,4A,5,6,7-B-OC                         |
| 7          | 13.207       | 555776    | 1.32   | Caryophyllene                                                         |
| 8          | 13.360       | 551887    | 1.32   | Isoledene                                                             |
| 9          | 13.567       | 93953     | 0.22   | 3-Chloropropane-1,2-diol, bis(tert-butyldimethylsilyl) ethene         |
| 10         | 13.639       | 93299     | 0.22   | Seychellene                                                           |
| 11         | 13.737       | 59132     | 0.14   | NEOALLOOCIMENE                                                        |
| 12         | 13.792       | 65085     | 0.16   | 1H-3a,7-Methanazulene, 2,3,6,7,8a-hexahydro-1,4,9,9-t                 |
| 13         | 13.881       | 65370     | 0.16   | 1H-3a,7-Methanazulene, octahydro-1,9,9-trimethyl-4-methyl              |
| 14         | 14.116       | 140615    | 0.34   | BICYCLO[7.2.0]UNDEC-4-ENE, 4,11,11-TRIMETHYL-                         |
| 15         | 14.439       | 118763    | 0.28   | (1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo[8.1.0]undec               |
| 16         | 15.137       | 329377    | 0.79   | (1aR,3aS,7S,7aS,7bR)-1,1,3a,7-Tetramethyldecahydro-1H-                 |
| 17         | 15.224       | 255742    | 0.61   | 1H-Cycloprop[1]azulen-7-ol, decahydro-1,1,7-trimethyl-4-               |
| 18         | 15.286       | 2142686   | 5.11   | 1,2-BENZENEDICARBOXYLIC ACID, DIETHYL ESTE                           |
| 19         | 15.447       | 757801    | 1.81   | Epicurzerenone                                                        |
| 20         | 15.531       | 308410    | 0.74   | Chenodiol                                                             |
| 21         | 15.617       | 15884     | 0.04   | Unidentified compound                                                |
| 22         | 15.673       | 412507    | 0.98   | 1,1,4,7-Tetramethyldecahydro-1H-cycloprop[1]azulene-4,                |
| 23         | 15.787       | 716851    | 1.71   | TRIDUETRIOMETHYL-10-EPOXY-7-ETHYL-3,11-DI                             |
| 24         | 15.988       | 768032    | 1.83   | METHYL (3-OXO-2-PENTYL)CYCLOPENTYLACETA                              |
| 25         | 16.159       | 1287600   | 3.07   | 1-(4-ISOPROPYLPHENYL)-2-METHYLPROPYL ACET                              |
| 26         | 16.248       | 79006     | 0.19   | 1-(4-ISOPROPYLPHENYL)-2-METHYLPROPYL ACET                              |
| 27         | 16.345       | 544685    | 1.30   | 2-PENTEN-1-0L, 5-(2,3-DIMETHYL)TETRACYCLO[2.2.1.0]                    |
| 28         | 16.394       | 1466093   | 3.49   | Patchouli alcohol                                                     |
| 29         | 16.590       | 257277    | 0.61   | 1-Naphthalenepropanol, alpha-ethenyldecahydro-2-hydro                |
| 30         | 16.651       | 187453    | 0.45   | (3aR,4R,7R)-1,4,9,9-Tetramethyl-3,4,5,6,7,8-hexahydro-2               |
| 31         | 16.822       | 938511    | 2.24   | Santalol, E-cis,epi-beta,-                                           |
| 32         | 16.915       | 104923    | 0.25   | 2-FURANMETHANOL, 5-ETHENYL-TETRAHYDRO-A                              |
| 33         | 17.169       | 498732    | 1.19   | 4,8-DIMETHYL-3,8-NONADIEN-2-ONE                                       |
| 34         | 17.367       | 245089    | 0.58   | ACETYL CEDRENE                                                        |
| 35         | 17.609       | 152712    | 0.36   | 1H-Benzocyclohepten-7-0L, 2,3,4,4a,5,6,7,8-octahydro-1,1                |
| 36         | 17.941       | 346720    | 0.83   | Neophytadiene                                                         |
| 37         | 18.129       | 184873    | 0.44   | 4,6,6,7,8,8-HEXAMETHYL-1,3,4,6,7,8-HEXAHYDROC                         |
| 38         | 18.212       | 185266    | 0.44   | 7-ACETYL-1,1,3,4,4,6-HEXAMETHYL TETRALIN                           |
| 39         | 19.882       | 666930    | 1.59   | Ethylene brassylate                                                  |
| 40         | 20.105       | 66589     | 0.16   | CYCLODODECASILXOXANE, TETRACOSAMETHYL-                              |
| 41         | 22.495       | 86863     | 0.21   | SILICONE OIL                                                          |
| 42         | 23.572       | 75245     | 0.18   | SILICONE OIL                                                          |
| 43         | 24.252       | 1011139   | 2.41   | Bis(2-ethylhexyl) phthalate                                           |
| 44         | 26.522       | 11434137  | 27.25  | Squalene                                                              |
oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-1, (all-E)-. It is actually an intermediate in triterpenoid biosynthesis pathway which is produced from squalene by the enzyme squalene mono-oxygenase (E.C. 1.14.14.17) (Fig. 5). Another major triterpene and anti-inflammatory compound detected by our GC-MS analysis is lupeol, which is present in high amount (17.26 %) and famous for its wide range of bioactivities. Methyl commate B (C\textsubscript{31}H\textsubscript{50}O\textsubscript{3}) is also an anti-inflammatory triterpene (Table 2) present in CJLE, which is actually a methyl ester of comic acid B. Methyl esterification may occur due to exposure of sample to methyl alcohol during extraction. Both lupeol and methyl commate B are biosynthesized in the same pathway where degradation of squalene occurs. Furthermore, step by step enzymatic reactions involved in the formation of

Table 1 GC-MC result of Camellia japonica leaf extract (Continued)

| Peak index | R. time | Area   | Area% | Name                                                                 |
|------------|---------|--------|-------|----------------------------------------------------------------------|
| 45         | 27.528  | 178555 | 0.43  | CB-86                                                                 |
| 46         | 27.664  | 230045 | 0.55  | SOLANESOL                                                            |
| 47         | 27.796  | 523171 | 1.25  | Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)\textsubscript{1,} (all-E)-        |
| 48         | 30.688  | 2100440| 5.01  | Vitamin E                                                            |
| 49         | 36.935  | 1787264| 4.26  | METHYL COMMATE B                                                     |
| 50         | 45.960  | 7241879| 17.26 | Lupeol                                                               |

Fig. 1 GC-MS chromatogram of Camellia japonica leaf extract
Table 2  Type of compounds present in Camellia japonica leaf extract and anti-inflammatory compounds in it

| Peak index | Camellia japonica leaf compounds | Type             | Area% |
|------------|---------------------------------|------------------|-------|
| 1          | Eucalyptol (Juergens et al. 2003) | Monoterpene      | 1.18  |
| 2          | Linalyl acetate (Peana et al. 2002) | Monoterpene      | 0.66  |
| 3          | 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl acetate (Yang et al. 2014) | Monoterpene      | 0.43  |
| 4          | 3-cyclohexene-1-methanol, \(\alpha,\alpha,\alpha,4\)… (Held et al. 2007) | Monoterpene      | 1.79  |
| 5          | Epiglobulol (Jayaprakash et al. 2019) | Sesquiterpene    | 0.31  |
| 6          | 1 h-cycloprop[e]azulene, 1A,2,3,4,4A,5,6,7B-oct… (Rajput et al. 2018) | Sesquiterpene    | 1.89  |
| 7          | Caryophyllene (Fernandes et al. 2007) | Sesquiterpene    | 1.32  |
| 8          | Isoledene | Sesquiterpene | 1.32 |
| 9          | Seychellene (Raharjo et al. 2017) | Sesquiterpene    | 0.22  |
| 10         | Neoalloccimene (Pravdich-Neminskaya and Kachkov 1978) | Sesquiterpene    | 0.14  |
| 11         | 1H-3a,7-Methanoazulene, 2,3,6,8a-hexahydro-1,4,9,9-t | Sesquiterpene    | 0.16  |
| 12         | 1H-3a,7-Methanoazulene, octahydro-1,9,9-trimethyl-4-met | Sesquiterpene    | 0.16  |
| 13         | Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8 (Tambe et al. 1996) | Sesquiterpene    | 0.34  |
| 14         | (1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo[8.1.0]undeca | Sesquiterpene    | 0.28  |
| 15         | (1AR,3AS,7S,7AS,7BR)-1,1,3a,7-Tetramethyldecahydro-1H- or maaliol (Sah et al. 2012) | Sesquiterpene    | 0.79  |
| 16         | 1H-Cycloprop[E]azulene-7-ol, decahydro-1,1,7-trimethyl-4-m or (+)-spathulenol (do Nascimento et al. 2018) | Sesquiterpene    | 0.61  |
| 17         | 1,2-benzenedicarboxylic acid, diethyl ester (Singh et al. 2012) | Phthalate ester | 5.11  |
| 18         | Epicurzerenone (Makabe et al. 2006) | Sesquiterpene    | 1.81  |
| 19         | 1,1,4,7-Tetramethyldecahydro-1H-cycloprop[e]azulene-4,7 | Sesquiterpene    | 0.98  |
| 20         | Trideuteriothymi 10-epoxy-7-ethyl-3,11-dim | Sesquiterpene    | 1.17  |
| 21         | Methyl (3-oxo-2-pentylcyclopentyl)acetat… (Dang et al. 2008) | Sesquiterpene    | 1.83  |
| 22         | 2-penten-1-ol, 5-(2,3-dimethyltricyclo[2.2.1.02... (Bommareddy et al. 2019) | Sesquiterpene    | 1.3   |
| 23         | Patchouli alcohol (Li et al. 2011) | Sesquiterpene    | 3.49  |
| 24         | 1-Naphthalenepropanol, \(\alpha,\beta\)-ethyldecahydro-2-hydroxy… (Tsai et al. 2018) | Diterpene       | 0.61  |
| 25         | (3AR,4R,7R)-1,4,9,9-Tetramethyl-3,4,5,6,7,8-hexahydro-2H… or cypereneone (Gupta and Shaw 2009) | Sesquiterpene    | 0.45  |
| 26         | Santalol, E-cis,epi-\beta.- | Sesquiterpene    | 2.24  |
| 27         | 2-furanmethanol, 5-ethenyltetrahydro-a | Monoterpene      | 0.25  |
| 28         | Acetyl cedrene | Sesquiterpene    | 0.58  |
| 29         | 1H-Benzocyclohepten-7-ol, 2,3,4,4A,5,6,7,8-octahydro-1,1, … or widdrol (Jin et al. 2015) | Sesquiterpene    | 0.36  |
| 30         | Neophydiene (Bhardwaj et al. 2020) | Sesquiterpene    | 0.83  |
| 31         | 4,6,6,7,8-hexamethyl-1,3,4,6,7,8-hexahydroxy | Sesquiterpene    | 0.44  |
| 32         | 7-acetyl-1,1,3,4,4,6-hexamethyl tetralin | Sesquiterpene    | 0.44  |
| 33         | Ethylene brassylate (Kim et al. 2006) | Sesquiterpene    | 1.59  |
| 34         | Cyclododecasiloxane, tetracosamethyl- (Kumar et al. 2018) | Sesquiterpene    | 0.16  |
| 35         | Bis(2-ethylhexyl) phthalate (Mohammed et al. 2014) | Phthalate ester | 2.41  |
| 36         | Squalene (Fernando et al. 2018) | Triterpene       | 27.25 |
| 37         | Cb-B6 | Cannabinoid | 0.43 |
| 38         | Solanesol (Yan et al. 2015) | Triterpene-alcohol | 0.55 |
| 39         | Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,1 | Triterpene       | 1.25  |
| 40         | Vitamin E (Jiang 2014) | Tocopherols | 5.01 |
| 41         | Methyl commate B (Arora and Kumar 2018) | Triterpene       | 4.26  |
| 42         | Lupeol (Saleem 2009) | Triterpene       | 17.26 |

Anti-inflammatory compounds are written in bold. References against each anti-inflammatory compound are given in round brackets.
Lupeol and methyl commate B (Figs. 6 and 7) are described in the “Discussion” section.

**Sesquiterpenoids**
Patchouli alcohol (3.49%), the major sesquiterpene present in this leaf extract, is a germacrene and synthesized from FPP by the enzyme patchoulol synthase (E.C. 4.2.3.70) (Fig. 8) (https://www.genome.jp/kegg/pathway.html). In the same pathway, nineteen other sesquiterpenes of the plant are also produced from FPP by different enzymes.

**Monoterpenoids**
Total of four monoterpene compounds have been found in our GC-MS result. Among them, the higher amount of monoterpene is terpinyl acetate or 3-cyclohexene-1-methanol, \( \text{\(\alpha\),\(\alpha\),4-trimethyl-}, \text{acetate (1.79\%) which is the acetate ester of alpha-terpineol synthesized from GPP by the enzyme alpha-terpineol synthase (E.C. 4.2.3.112). Another major monoterpene eucalyptol (1.18\%) follows the same pathway which is synthesized from GPP by the enzyme 1,8-cineole synthase (E.C. 4.2.3.108). Linalyl acetate (0.66\%) is also a monoterpene which is the acetate ester of linalool. Biosynthesis of linalool by linalool synthase enzyme (E.C. 4.2.3.26) is also established in monoterpenoid biosynthesis pathway which is shown in Fig. 9.

**Vitamin E, sclareol, and solanesol**
According to our GC-MS result, vitamin E (5.01\%), more specifically alpha-tocopherol, is one of the major anti-inflammatory compound which is synthesized in ubiquinone and other terpenoid quinone biosynthesis pathway. Sclareol (0.61%; a diterpene) and solanesol (0.55%; a nonaprenol), two anti-inflammatory compounds (Table 2), are present in minute amounts in CJLE, but being lone representatives of their respective chemical groups and having relations with earlier mentioned terpenoids regarding biosynthesis pathways, they are included in this study. Like other terpenoids, both

---

**Major chemical groups in Camellia japonica leaf**

![Diagram showing the percentage distribution of various chemical groups in Camellia japonica leaf extract](https://www.genome.jp/kegg/pathway.html)

**Fig. 2** Anti-inflammatory compounds of *Camellia japonica* leaf extract. C1-C50 = GC-MS detected compounds’ peak numbers in order (Table 1)

**Fig. 3** Types of compounds on the basis of their biosynthesis in *Camellia japonica* leaf extract; majority of triterpenoids in *Camellia japonica* leaf
alpha-tocopherol (Fig. 10), sclareol, and solanesol (Fig. 11) are biosynthesized from IPP and DMAPP derived precursors in plastids through MEP metabolic pathway which is described in the “Discussion” section.

Figure 12 is designed as a brief diagram on biosynthesis pathway of all the abovementioned CJLE compounds found in the GC-MS result.

**Discussion**

Biosynthesis pathways are studied to investigate origin, precursors, intermediates, and breakdown products of different compounds, probable pathways of newly reported compounds having similar established structures, reactions, and relations between several compounds in individual plant bodies. In our research, we have used GC-
MS, one of the preliminary and first-step technique of metabolomics, to analyze the terpenoids present in *Camellia japonica*. Then studies on their biosynthesis pathways were carried out where several interactions and interrelations came out along with designs of probable biosynthesis pathways of unestablished metabolomes. Wang et al. (2013), in their studies on biosynthesis pathway of terpenoid, proposed involvement of three different stages; firstly, the generation of CS isopentyl di-phosphate (IPP) precursor and its double bond isomer dimethylallyl di-phosphate (DMAPP); secondly, the generation of direct precursors like geranyl diphosphate or GPP (for monoterpenes), farnesyl diphosphate or FPP (for triterpenes and sesquiterpenes), and geranylgeranyl diphosphate or GGPP (for diterpenes and tocopherols) (Fig. 4); and thirdly, biosynthesis and modification of terpenes via oxidation-reduction, acylation, glycosylation, and other reactions. In general, biosynthesis of sesquiterpene and triterpene takes place in the cytosol part of the cell while monoterpenes, tocopherols, and solanesol are synthesized inside the plastid just like chlorophylls and carotenoids in higher plants.

Triterpenes, in general, are produced from the precursor FPP which is derived from two IPP molecules and one DMAPP (McGarvey and Croteau 1995) by farnesyl diphosphate synthase through mevalonate pathway as shown in Fig. 4. Both squalene (27.25%) and lupeol (17.26%) are two abundant most compounds in this leaf extract which imparts anti-inflammatory activity to it. Squalene is produced from FPP by the enzyme squalene synthase (E.C. 2.5.1.21) (Fig. 5). In plants, squalene is metabolized by the enzyme squalene mono-oxygenase (E.C. 1.14.14.17) to produce (3S)-2,3-epoxy-2,3-dihydro-squalene or oxidosqualene (Wang et al. 2010) which is also present in our result. It then enters into pentacyclic triterpenoid biosynthesis pathway. Oxidosqualene can be converted into pentacyclic triterpenes like lupeol, alpha-amyrrin, beta-amyrrin, and several potentially bio-active phytosterols, an amazing number of structurally diverse backbones, including over hundred identified in plants (Wang et al. 2010) by oxidosqualene cyclase enzymes. Lupeol is a pharmacologically active pentacyclic and lupine type of triterpene. According to Saleem (2009), lupeol exhibits various pharmacological activities.

**Fig. 6** Lupeol biosynthesis and degradation (common biosynthesis pathway for any pentacyclic triterpene) (compound structures source: https://pubchem.ncbi.nlm.nih.gov)

**Fig. 7** Probable biosynthesis pathway of commic acid B and methyl commate B (compound structures source: https://pubchem.ncbi.nlm.nih.gov)
against inflammation (Table 2), cancer, arthritis, diabetes, heart diseases, renal toxicity, and hepatic toxicity. Lupeol has been extensively studied for its inhibitory effects on inflammation under in vitro and in animal models where it showed positive responses (Saleem 2009). Lupeol synthase (E.C. 5.4.99.41), an oxidosqualene cyclase, is a multifunctional enzyme that forms lupeol (Fig. 6) and other triterpene alcohols (like beta-amyrin). Sequence analysis suggests that lupeol synthase diverged from cycloartenol synthase after plants diverged from fungi and animals for which fungi and animals do not synthesize lupeol (Herrera et al. 1998), and presence of
squalene, oxidosqualene, and lupeol together as major compounds has already proved the presence of lupeol synthase in this plant. According to Huang et al. (2012), lupeol then enters into betulinate biosynthesis pathway where after a series of oxidation process it converts into betulinate; \( \text{C}_{30}\text{H}_{47}\text{O}_3 \) (parent compound betulinic acid; \( \text{C}_{30}\text{H}_{48}\text{O}_3 \)) by the enzyme lupeol-28-monooxygenase (E.C. 1.14.14.126). According to Saleem (2009), different fruits (olive, mango, and Japanese pear), aloe leaves, elm plant, and ginseng oil are the major source of lupeol. Lupeol was reported in Camellia seed oil (Akihisa et al. 1997) but there is no previous report that discloses the presence of lupeol in the leaves of Camellia japonica. Methyl commate B is another major triterpene found in CJLE. Unfortunately, there is no report on biosynthesis pathway for methyl commate B or comic acid B but the structure of comic acid B (\( \text{C}_{30}\text{H}_{48}\text{O}_3 \)) is very much close to other pentacyclic triterpene acids like betulinic acid (lupeol derived), ursolic acid (alpha-amyrin derived), and oleanolic acid (beta-amyrin derived) which have the same chemical formula, that is \( \text{C}_{30}\text{H}_{48}\text{O}_3 \), and most of them are seen potentially medicinal in nature. According to the terpenoid biosynthesis pathway, these \( \text{C}_{30}\text{H}_{48}\text{O}_3 \) pentacyclic triterpene acids or active \( \text{C}_{30}\text{H}_{47}\text{O}_3 \) (ionized) are usually produced from squalene derived pentacyclic triterpene phytosterols or secondary alcohol (\( \text{C}_{30}\text{H}_{50}\text{O} \)) by oxidoreductase enzymes which are functionally same and termed as triterpene C-28 oxidase (E.C. 1.14.14.126). According to our interpretation, the same reactions should be followed in comic acid B formation from its specific phytosterol precursor. However, methyl commate B is not derived from lupeol as it is not a lupine type of triterpene but an ursane (Thomas and Willhalm 1964). Established triterpene biosynthesis pathways and their interactions have helped us to design a probable pathway for comic acid B synthesis (Fig. 7) where a squalene derived \( \text{C}_{30}\text{H}_{50}\text{O} \) phytosterol (the precursor comic acid B) must be present as a precursor just like other triterpenes. But, in our GC-MS result, it is either absent or not detected as a precursor, thus, Camellia japonica must be subjected for further research to find responsible genes and enzymes behind biosynthesis of this compound.

After triterpene, the second most major chemical group in this plant extract is sesquiterpene including twenty different compounds, which are again produced from the precursor FPP following the mevalonate pathway. After terpenoid backbone biosynthesis and before squalene synthesis, FPP gets involved in the sesquiterpenoid biosynthesis pathway where it converts into a number of anti-inflammatory sesquiterpenes (Table 2) including patchouli alcohol.

Four monoterpene compounds have also been found in our GC-MS result as described earlier. Monoterpenes in
Fig. 11 Pathways involved in sclareol (diterpenoid biosynthesis) and solanesol biosynthesis. IPP, isopentyl diphosphate; DMAPP, dimethylallyl diphosphate; GGPP, geranyl geranyl diphosphate; SPP, solanesyl diphosphate (compound structures source: https://pubchem.ncbi.nlm.nih.gov)

Fig. 12 Single diagram showing biosynthesis of major CJLE compounds. IPP, isopentyl diphosphate; DMAPP, dimethylallyl diphosphate; FPP, farnesyl diphosphate; GGPP, geranyl-geranyl diphosphate; GPP, geranyl diphosphate; PP, diphosphate
plants are biosynthesized from GPP which is produced by the enzyme geranyl pyrophosphate synthase (E.C. 2.5.1.1) from one IPP and one DMAPP molecule which further produces monoterpenes by GPP diphosphate-lyases (Fig. 4) inside the plastid (Davis and Croteau 2000).

After FPP and GPP, GGPP is the main precursor in this plant as it converts into vitamin E, another major compound detected in CJLE. This precursor, itself is synthesized from IPP and DMAPP (W’ang et al. 2013) like other terpenoid precursors which proves IPP and DMAPP as important factors that help us to understand interrelations between different terpenes over their biosynthesis. Moreover, enzyme geranyl diphosphate synthase (E.C. 2.5.1.29) forms GGPP which further converts into phytol diphosphate or PPP (Fig. 4) by enzyme geranyl reductase (E.C. 1.3.1.83) to synthesize vitamin E. After completing a series of reactions, tocopherol or vitamin E is synthesized where 2-methyl-6-phytylbenzoquinol (E.C. 2.5.1.115) and 2,3-dimethyl-6-phytylbenzoquinol (E.C. 2.1.1.295) take part in order as intermediates (Fig. 10). Another pathway of tocopherol biosynthesis is reported in plants where the same enzymatic reactions occur and GGPP converts directly into vitamin E without forming PPP (https://www.genome.jp/kegg/pathway.html). Biosynthesis of chlorophyll, carotenoids, plastoquinone, ubiquinone, solanesol, and di-terpenoid like scareol is also initiated by the same precursor GGPP. GGPP converts into copal-8-ol diphosphate (E.C. 4.2.1.133) which further produces scareol (E.C. 4.2.3.141) as shown in Fig. 11. Solanesol, another terpenoid (a non-cyclic terpene alcohol) detected in CJLE, is synthesized from GGPP following the same three-stage terpenoid biosynthesis pathway of Wang et al. (2013). According to Yan et al. 2017, solanesyl diphosphate (SPP), the precursor of solanesol and plastoquinone, is synthesized from IPP, DMAPP, GPP, FPP, and GGPP by solanesyl diphosphate synthase (Fig. 4).

Some phthalate esters were also detected but they are already reported as chemical contaminants from laboratory plastic tools (Reid et al. 2007), thus, further research is needed to establish their origin in this extract.

Moreover, natural products are still the most successful source of biologically active lead compounds in drug discovery (Atanasov et al. 2015) and there are some cases where the crude extract is more active than the isolated pure compound, e.g., the extract of Artemisia annua has more potent antimalarial properties than its pure natural product, artemisinin (De Donno et al. 2012) and our CJLE can be an example of this hypothesis too as we have found many anti-inflammatory compounds from different chemical groups where not only a few of triterpenes in higher amounts are responsible but also a number of compounds from sesquiterpene, monoterpene, tocopherol, diterpene biosynthesis pathways are there which also exhibits the same property.

Conclusion
Ongoing anti-inflammatory assays, other experiments, and gene sequencing studies are needed to confirm this metabolite-based pathway study and to find out the key enzymatic genes behind the synthesis of those anti-inflammatory terpenoids found in CJLE. Herbal medications are becoming popular day by day because of their relatively few side effects, and our GC-MS analysis suggests not only as an ornamental plant but also this plant should be cultivated vigorously in this region to use the leaves as anti-inflammatory herbal formulations. Moreover, twenty-nine anti-inflammatory compounds with a share of more than eighty-three percent area in Camellia japonica leaf extract; large peaks of metabolites leading by bioactive triterpenoids like squalene and lupeol (which can be isolated to use in pharmaceutical industries as this research has shown their abundance in Camellia japonica leaf); abundance of novel anticancer and anti-inflammatory medicinal compound lupeol with 17.26% peak area; and design of unexplored biosynthesis pathway of common plant component methyl comminate B through metabolomics; are the key outcomes and highlights of the study.

Abbreviations
CJLE: Camellia japonica leaf extract; DMAPP: Dimethylallyl diphosphate; FPP: Farnesyl diphosphate; GC-MS: Gas chromatography-mass spectrometry; GGPP: Geranylgeranyl diphosphate; GPP: Geranyl diphosphate; IPP: Isopentyl diphosphate; SPP: Solanesyl diphosphate; PPP: Phytol diphosphate

Acknowledgements
Not applicable

Authors’ contributions
All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by SM, AG, and MB. The first draft of the manuscript was written by SM and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding
Not applicable

Availability of data and materials
All data analyzed during this study are included in this article.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 5 June 2020 Accepted: 14 August 2020
Published online: 27 August 2020

References
Akihisa T, Yasukawa K, Kimura Y, Si T, Yamanouchi S, Tamura T (1997) Triterpene alcohols from camellia and sasanqua oils and their anti-inflammatory effects. Chem Pharm Bull 45(12):2016–2023
Arosa S, Kumar G (2018) Screening of bioactive compounds from leaf of Cenchrus ciliaris L. from Thar region of Rajasthan, India. Int J Pharm Sci Res 9(5):1878–1885
Atanassov AG, Waltenberger B, Pletscher-Wenzig EM, Linder T, Wawrosch C, Uhrin P, Temml V, Wang L, Schwaiger S, Heiss EH, Rollinger JM (2015) Discovery and resupply of pharmacologically active plant-derived natural products: a review. Biotechnol Adv 33(8):1582–1614
Bhardwaj M, Sali VK, Mani S, Vasanthi HR. Neophytadiene from Turbinaria ornata suppresses LPS-induced inflammatory response in RAW 264.7 Macrophages and Sprague Dawley Rats. Inflammation. 2020 Jan 24:1-4.
Bommareddy A, Brozeina S, Seiergaard J, Landis T, Hughes T, Mabuy E, Knopp A, VanVier AL, Dwivedi C (2018) Medicinal properties of alpha-santalol, a naturally occurring constituent of sandalwood oil. Nat Prod Res 33(4):527–534
Dang HT, Lee HJ, Yoo ES, Hong J, Baob C, Choi JS, Jung JH (2008) New jasmonate analogues as potential anti-inflammatory agents. Bioorg Med Chem 16(24): 10228–10235
Das S, Vasudeva N, Sharma S (2014) Chemical composition of ethanol extract of Macrotysluma uniflorum (lamb) Verdc. Using GC-MS spectroscopy. Organ Med Chem Lett 4(1):9
Davis EM, Croteau R. Cyclization enzymes in the biosynthesis of monoterpenoids, sesquiterpenes, and diterpenes. In Biosynthesis 2000 (pp. 53-95). Springer, Berlin, Heidelberg.
De Donno A, Grassi T, Proietti M, Peroni G, Passafaro E, Magni ML, Denisov AS, Spadoni M, Sun Y, Beretta A, Fabbro A, Varró E (2015) In vitro cytotoxic and anti-inflammatory activity of Artemisia annua herbal tea and artemisinin. J Ethnopharmacology 170(1):144–150
De Konemann E. Trees and shrubs (Botanica). 2019.
Jin S, Yun HJ, Jeong HY, Oh YN, Park HJ, Yun SG, Kim BW, Kwon HJ (2015) Anti-inflammatory and antioxidative effects of Camellia japonica on human corneal epithelial cells and experimental dry eye: in vivo and in vitro study. Invest Ophthalmol Vis Sci 56(2):1196–1207
Li YC, Xian YF, Ip SP, Su ZR, Su JY, He JJ, Xie QF, Lai XP, Lin ZX (2011) Anti-inflammatory activity of patchouli alcohol isolated from Pogostemon Herba in animal models. Fitoterapia. 82(8):1295–1301
Lim TK, Camellia Japonica. Edible Medicinal and Non Medicinal Plants 2014 (pp. 764-776). Springer, Dordrecht.
Makabe H, Maru N, Kubawara A, Kamo T, Hirota M (2006) Anti-inflammatory sesquiterpenes from Curcuma zedoaria. Nat Prod Res 20(7):680–685
Marcon XC, Bost JW, Marcon A (2010) Natural anti-inflammatory agents for pain relief. Surgical Neurol Int 1
McGavrey DJ, Croteau R (1995) Terpenoid metabolism. Plant Cell 7(10):1019
Mohammed MS, Ahmed WJ, Khalid HS, Mahmoud AM, Garelnabi EA (2014) Dh-2′-ethyhexyl phthalate and stigmastanol with anti-inflammatory effect from Cyperus rotundus. Int J Pharm Chem Biol Sci 4(3):453–459
Nomoda R, Hanashiro K, Yasumoto T (2006) Camelannol, a novel antioxidant glycoside from the leaves of Camellia japonica. Biosci Biotechnol Biochem 70(10): 1995–1998
Peana AT, D’Aquila PS, Panin F, Serra G, Pippia P, Moretti MD (2002) Anti-inflammatory activity of linalool and linalyl acetate constituents of essential oils. Photomedicine. 9(8):721–726
Pravdich-Nemirovskaya TV, Kachkov AP (1978) Effect of Allo-oicocene on the healing of experimental soft tissue wounds. Bull Exp Biol Med 85(1):57–60
Rajput MS, Rathore D, Dahiya R (2018) Anti-inflammatory potential of c-fenchol and α-gurjunene: an in vitro study. Panacea J Pharm Pharm Sci 7(3):129–135
Reid AM, Brougham CA, Fogarty AM, Roche JJ (2007) An investigation into possible sources of phthalate contamination in the environmental analytical laboratory. Int J Environ Anal Chem 87(2):125–133
Sah SP, Mathela CS, Chopra K (2012) Valeriana wallichii DC. (maiolchymot etype): antinociceptive studies on experimental animal models and possible mechanism of action. Pharmacologia. 3432–437
Saleem M (2009) Lupeol, a novel anti-inflammatory and anti-cancer dietary triterpene. Cancer Lett 285(2):109–115
Singh R, Dar SA, Sharma P (2012) Antibacterial activity and toxicological evaluation of semi purified hexane extract of Unica dolce leaves. Res J Med Plants 6(2):123–135
Tambly Y, Tsuchiya H, Honda G, Ikehijo Y, Tanaka S (1996) Gastric cytoprotection of the non-steroidal anti-inflammatory sesquiterpene, β-caryophyllene. Planta Med 62(5):469–470
The Kyoto Encyclopedia of Genes and Genomes database https://www.genome.jp/kegg/pathway.html. Accessed on 20 May 2020.
The PubChem. https://pubchem.ncbi.nlm.nih.gov. Accessed on 18 May 2020.
Thomas AF, Willhalm B (1964) The triterpenes of Commiphora IV mass spectra and organic analysis V mass spectroscopic studies and the structure of comnic acid a and B. Tetrahedron Lett 54(3):317–318
Tsai SW, Hsieh MC, Li S, Lin SC, Wang SP, Lehamn CW, Lien CZ, Lin CC (2018) Therapeutic potential of sclareol in experimental models of rheumatoid arthritis. Int J Mol Sci 19(5):1515
Wang L, Fang X, Yang C, Li J, Chen X (2013) Biosynthesis and regulation of secondary terpenoid metabolism in plants. Scienta Sinica Viteae.43(12):1030–1046
Wang Z, Yeats T, Han H, Jetter R (2010) Cloning and characterization of oxidosqualene cyclases from Kalanche hosemandentiana enzymes catalyzing up to 10 rearrangement steps yielding friedelin and other triterpenoids. J Biol Chem 285(39):29703–29712
Yan N, Liu Y, Gong D, Du Y, Zhang H, Zhang Z (2015) Solaneros: a review of its resources, derivatives, bioactivities, medicinal applications, and biosynthesis. Phytochemistry. Rev 14(3):403–417
Yan N, Liu Y, Zhang H, Du Y, Liu X, and Zhang Z. 2017. Solaneros biosynthesis in plants. Molecules, 22(4), p.510.
Yang H, Zhao R, Chen H, Jia P, Bao L, Tang H (2014) Bornyl acetate has an anti-inflammatory effect in human chondrocytes via induction of IL-11. JUMB Life 66(12):854–859
Yoon IS, Park DH, Kim JE, Yoo JC, Bae MS, Oh DS, Shim JH, Choi CY, An KW, Kim EI, Kim GY (2017) Identification of the biologically active constituents of Camellia japonica leaf and anti-hyperuricemic effect in vitro and in vivo. Int J Mol Med 39(6):1613–1620

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.