RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Towards comprehension of complex chemical evolution and diversification of terpene and phenylpropanoid pathways in *Ocimum* species

Priyanka Singh, Raviraj M. Kalunke, Ashok P. Giri*

Plant Molecular Biology Unit, Division of Biochemical Sciences, CSIR-National Chemical Laboratory, Pune 411008, Maharashtra, India

***Corresponding author: Ashok P. Giri**

Tel.: +91-2025902710; Fax: +91-2025902648

E-mail: ap.giri@ncl.res.in

Abstract

Ocimum species present a wide array of diverse secondary metabolites possessing immense medicinal and economic value. The importance of this genus is undisputable and exemplified in the ancient science of Chinese and Indian (Ayurveda) traditional medicine. Unlike several other plant species of *Artemisia*, *Salvia, Catharanthus*, *Taxus*, *Mentha*, etc. that are largely exploited, detailed characterization and identification of important metabolites from *Ocimum* species remained unexplored. Till date, most of the analyzed *Ocimum* species are predominantly rich in either phenylpropanoids or terpenoids. Metabolite data suggests domination of a unique set of signature compounds in all species. However, molecular pathways leading to the production, accumulation and metabolism of these compounds are poorly understood. Past few years have witnessed an upsurge in our understanding of the complex and intricately woven secondary metabolic pathways. Such information is generated through systematic analysis and correlation of metabolite profiling with transcriptomics data sets from different *Ocimum* species. The present review is aimed at integrating our current knowledge to understand the active secondary metabolic pathways, the key players in flux regulation including external stimuli, differential gene expression, transcription factors, microRNAs, enzyme promiscuity, etc. Extensive analysis of available data identifies events that may have contributed to evolve *Ocimum* species rich with specific set of metabolites, thus, shedding light on pathway diversification. We believe that a better understanding of the multi-level regulation of intermediates and metabolites will help us harness the inherent diversity of *Ocimum* species optimally.

1. Introduction

Genus *Ocimum* belonging to family Lamiaceae comprises between 50 to150 species.[1] The difference in the estimates of species number is partly attributed to reasons like taxonomic revisions and generic description of the genus amongst others. It was first described by Linnaeus in 1753 in the book *Species Plantarum*.[2] The name *Ocimum basilicum* was derived from the Greek word *Okimon* (smell) and *basilikon* (royal), referring to its royal fragnance. While in India the *Ocimum* plant is considered sacred and worshipped, in other parts of the world it is hailed as the "queen of herbs" because of its strong aromatic appeal and culinary usage. With the establishment of ancient medicinal practises in India (Ayurveda) and China (Traditional Chinese Medicine), *Ocimum* was recognised as a medicinal herb with great healing powers.

Main centres of diversity for *Ocimum* include tropical and subtropical regions of Africa, India and South America.[3] With the exception of *O. tenuiflorum* and *O. gratissimum* that are indegenous to India, most species are native to Africa and found in wild population.[4] Although *Ocimum* species are known to abound in medicinally important metabolites, only few species have been thoroughly profiled. Our knowledge about most other species remains limited. All species are identifiable by the presence of a large amount of signature metabolite(s) along with several other metabolites in relatively minute quantities. The diversity of metabolites produced by *Ocimum* plants is indeed enormous. Specific functions and/or necessity for production of such diverse and complex chemical compounds by the plant remain elusive. Interestingly, what we know is certain *Ocimum* species are either "terpenoid-rich" or "phenylpropanoid-rich". However, factors determining the direction of flux are largely unknown. Terpenoids are formed from the mevalonic acid (MVA) pathway in the cytoplasm and the methylerythritol phosphate (MEP) pathway in the plastid.[5] Phenylpropanoid pathway starts with the amino acid phenylalanine and

eventually results in the formation of phenylpropenes such as eugenol, chavicol, anethole etc., along with intermediates for biosynthesis of lignin, rosmarinic acid, anthocyanins etc. These pathways have been well characterized in related genera including *Salvia*, *Mentha* and *Lavandula* [6-12] but not in such details in any *Ocimum* species. However, with the influx of next generation sequencing data[13, 14] along with metabolomics, proteomics and phylogeny studies,[15-19] now it seems possible to gain a deeper insight into the perplexing diversity. The present review aims at providing a comprehensive overview of the evolutionary, environmental and internal factors that may have resulted in pathway diversification and extensive chemical evolution across *Ocimum* species.

1.1 Importance of studying genus *Ocimum*

The unequivocal importance of genus *Ocimum* was established more than 5,000 years back with the advent of ancient traditional medicinal practises in India and China. Thereafter, there have been several reports of important bioactivities of *Ocimum* species; tissue extracts and metabolites there in (**Table 1**).[20-81] Although most species in this genus are associated with some or the other bioactivity, the exact compound or group of compounds, responsible for the said bioactivity remains elusive in most cases (**Table 1**). Basil also finds extensive application in the food, flavor, and fragnance industry, and the essential oil serves as a major source of economic wealth to the country. The plant is easy to grow and propagate, and adapts well to extreme environmental conditions including high precipitation, long dry spells and high temperature. Some species are capable of vegetative propagation through stem cuttings like *O. kilimandscharicum*, which makes commercial cultivation less tedious and more cost effective. Several *Ocimum* species grow as wild plants in various parts of the world. Since there has been no significant domestication of this wild medicinal plant, its genetic diversity has been preserved

in nature, making the system more interesting to explore. Furthermore, presence of different basil types/cultivars rich in diverse metabolites provides a unique system for studying secondary metabolic pathways. In addition, glandular trichomes accord the opportunity to study the biosynthesis and regulation of these pathways at the level of a single cell. *Ocimum* thus presents an attractive system to explore, particularly from the point of view of secondary metabolism.

1.2 Overview of extensive diversity within genus *Ocimum*

Although genus *Ocimum* boasts of 50 – 150 species, metabolite data for very few species is available. For example, *O. obovatum* and *O. labiatum* are well tested for therapeutic properties (**Table 1**); however, their chemical composition remains unknown. *Ocimum* species abound in diverse secondary metabolites including terpenoids, phenylpropanoids, rosmarinic acid, flavonoids and phenolics. **Figure 1** shows representative examples of structurally diverse classes of secondary metabolites found across genus *Ocimum*. These mainly include monoterpenes (example, camphor, eucalyptol, α-pinene, β-ocimene, terpinolene), sesquiterpenes (example, farnesene, β-caryophyllene, germacrene D) and phenylpropanoids (example, eugenol, eugenol methyl ether, chavicol, methyl chavicol, methyl cinnamate). Few metabolites like germacrene D and β-caryophyllene are commonly found across most species in the genus; however, others like camphor and eugenol have a specie- specific distribution **(Table 2)**. Higher terpenes (C20 and above) and alkaloids have not been well charaterized from any *Ocimum* species. Since most *Ocimum* species have not been profiled for their metabolites, the possibility that the genus represents much more diversity than what we percieve now is realistic. As mentioned previously, each species is characterized by a distinct metabolic fingerprint and presence of a signature compound(s) as the major fraction. Although metabolite profiling *via* conventional techniques such as gas chromatography (GC) has been routinely employed, advanced analytical techniques

including liquid chromatography (LC), mass spectrometry (MS) and nuclear magnectic resonance (NMR) have not been reported, which help in gaining a better understanding of the global distribution of metabolites and pathway intermediates. Till now only 12 *Ocimum* species have been analysed for their essential oil composition (**Fig. 2, Table 2**). Overall, they can be classified as having (i) high phenylpropanoid content, (ii) high terpenoid content, and (iii) similar/comparable amounts of phenylpropnoids and terpenoids. High phenylpropanoid content group contains about 60 to 90% phenylpropanoids and includes *O. gratissimum*,[82] *O. tenuiflorum*[83] and *O. selloi*.[84] High terpenoid containing group, includes *O kilimandscharicum*,[85] *O. minimum,*[86] *O. basilicum,*[87] *O. americanum,*[88] *Ocimum* × *citriodorum*[89] and *O. lamiifolium*[90] contain approximately 40 to 75% terpenoids. The third group includes *O. campechianum,*[91] O. *micranthum*[92] and *O. canum*[93] which show approximately equal amount of phenylpropanoids and terpenoids. Interestingly, terpenoids unlike phenylpropanoids, show a universal presence in varying amount in all *Ocimum* species. **Table 2** gives a comprehensive list of species-wise metabolite distribution.

Signature compounds known in *Ocimum* species are as follows: camphor in *O. kilimandscharicum* (56%), citral in *O. americanum* (47%), eugenol in *O. gratissimum* (82%) and *O. micranthum* (47%), eugenol methyl ether in *O. tenuiflorum* (62%), linalool in *O. basilicum* (48%), methyl chavicol in *O. selloi* (93%) and *O. canum* (53%), geranyl acetate in *O. minimum* (70%), sabinene in *O. lamiifolium* (33%) and geranial in *Ocimum* × *citriodorum* (43%) (**Fig. 2, Table 2**). In plant kingdom, metabolite diversity is commonly found at the level of family or genus, but such vivid diversity at the level of species and subtypes (within species) makes genus *Ocimum* occupy a special niche in nature.

2. Potential evolutionary events influencing metabolite diversity *via* pathway modulation

Ocimum genome has evolved as a result of dramatic series of events including polyploidy, aneuploidy, chromosomal duplications/translocations/deletions etc., [16, 94, 95] which led to unprecedented diversification of species in Africa, India and South America. The ability to cross-pollinate and hybridize further led to the emergence of subtypes within species, which were capable of interbreeding and producing hybrids. For example, *Ocimum*×*citriodorum* is a hybrid between *O. americanum* and *O. basilicum* and has a strong lemony scent.[18] *O. americanum* originated from *O. canum* and *O. basilicum*.[96] The African blue basil subtype (*O. kilimandscharicum*) is evolved as a hybrid between *O. kilimandscharicum* and *O. basilicum* and abounds in camphor, linalool and eucalyptol. Interestingly, the hybrids display significantly different metabolite profile than their parents including new metabolites that are not found in the parents, indicating co-dominance, epistatis or interaction of genes.[89] As reported in several other plant genera, ploidy levels also affect essential oil production, resulting in a greater accumulation of essential oils in polyploids than that in diploids.[95, 97-99] All these events taken together might have led to greater genetic diversity and continuous expansion of gene pool, yielding new species/subtypes/varieties over a short period.

During the course of evolution, there may have been events that led to terpenoid and phenylpropanoid pathway diversification across different *Ocimum* species. It is interesting to note that species abounding in phenylpropanoids also have an active terpenoid pathway and vice versa. This suggests that all species evolved from an ancestor, which harbored active genes for both the pathways. However, differential expression and regulation of pathway genes determined the final chemical composition in each species.[5, 100] Other factors like plant habit may also have

influenced the selection of one pathway over the other. For example, it has been suggested that the sanctum group has evolved to produce phenolic compounds because of its perennial woody habit, whereas the basilicum group has evolved to produce terpenoid-rich compounds owing to its annual herbaceous habit.[4] Evolution of gene coding regions also had a profound impact on the diversity of *Ocimum* species metabolites. For example, *O. basilicum* fenchol synthase and myrcene synthase, and geraniol synthase and linalool synthase are 95% and 81% similar, respectively; however, they catalyse the formation of very different products. These genes most probably evolved as a result of gene duplication events and acquired mutations leading to functional differentiation, [100] eventually contributing to metabolite diversity. Few pathway genes involved in the biosynthesis of selected metabolites have been reported and characterized from *Ocimum* and few other genera (**Table 3**). Genes like eugenol synthase involved in catalysing the final step of eugenol production has been well charaterized (**Table 3**). However, most genes present upstream in the eugenol biosynthetic pathway remain functionally uncharacterized despite availability of huge transcriptomic databases. Genes from camphor biosynthesis pathway have been well characterized from related genera like *Salvia* and *Lavandula*, however, there are no reports from genus *Ocimum* (**Table 3**). Modifying enzymes like chavicol and eugenol O-methyltransferases also have been well characterized (**Table 3**). Information about transcription factors responsible for controlling biosynthesis of these metabolites and the transporter proteins responsible for long distance transport from source to sink tissue in *Ocimum* species also remains scarce. Genes reported from yet another important category of compounds, flavones and flavonoids, have been listed in **Table 3**. Overall, information about the biosynthesis, transport and storage of these metabolites, at the genetic level is very scarce and need to be further probed. Several other factors during species diversification and naturalization in other parts of the world

have been discussed briefly, which help us in explaining the mystery behind the complex chemical evolution and pathway diversification.

3. Factors regulating secondary metabolite flux and chemical diversity in *Ocimum* species

Metabolite diversity observed at the level of species in genus *Ocimum* is dependent on several internal and external factors (**Fig. 3**). Some of the known factors responsible for regulating terpenoid and phenylpropanoid pathways are discussed.

3.1 Differential gene expression of enzymes in phenylpropanoid and terpenoid pathways

Gene expression plays an important role in diverting metabolic flux toward either the terpenoid or the phenylpropanoid pathway.[5, 13,100] In particular increased expression of terminal enzymes in the terpenoid pathway and reduced expression of phenylpropanoid entry point enzymes such as phenylalanine ammonia-lyase (PAL) has been observed in *O. basilicum var.* SD, rich in terpenoids. In another variety, *O. basilicum var.* EMX, however, the expression level of general phenylpropanoid pathway enzymes, PAL and 4-coumarate-CoA ligase (4CL) was found to be significantly higher corresponding to higher phenylpropanoid content.[5] These results were supported by next generation sequencing data of *O. tenuiflorum* and *O. basilicum.*[14] *O. tenuiflorum* rich in phenylpropanoids, shows much higher expression of general phenylpropanoid pathway enzymes including PAL, cinnamate-4-hydroxylase (C4H) and 4CL, reads per kilobase per million (RPKM) = 91.47, 34.53 and 9.52 respectively; compared to *O. basilicum* rich in terpenoids, RPKM = 11.3, 11.83 and 5.65 respectively. In *O. basilicum*, however, the entry point enzymes of the MEP pathway, representing the cytosolic pathway for terpenoid synthesis, including 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) was more (RPKM = 50.58) compared to *O. tenuiflorum* (RPKM = 15.69).[14] Thus, overexpressing the

entry point enzymes at major metabolic branching points also helps in directing the flux towards either phenylpropanoid or terpenoid pathway.[122-124] Evidently, differential expression of enzymes strategically present at pathway branch points might play a crucial role in determining flux regulation (**Fig. 4**).

3.2 Enzyme promiscuity

One of the major reasons for metabolite diversity observed in *Ocimum* species is the promiscuity of terpene synthases. These enzymes are capable of accepting a substrate and yielding a major product as well as multiple side products. For instance, Iijima *et al* characterized eight terpene synthases from three cultivars of *O. basilicum.*[100, 114] *In vitro* recombinant protein assays using geranyl diphosphate (GPP) as substrate for putative monoterpene synthases and farnesyl diphosphate (FPP) as substrate for putative sesquiterpene synthases was performed. Terpinolene synthase gave terpinolene as the major product and α-pinene, limonene and an unidentified monoterpene as the side products. Fenchol synthase produced fenchol and limonene as major products and α-pinene and an unidentified monoterpene as the side products. Cadinene synthase produced γ-cadinene as the major product and muurola 3, 5-diene as the side product. Selinene synthase produced selinene as the major product and β-elemene and nerolidol as side products. In contrast, myrcene synthase and geraniol synthase exclusively produced myrcene and geraniol as end products.[100] In another study by Major *et al*, using bornyl diphosphate synthase (producing camphene as the side product), it was proven that electrostatically guided dynamics determined end product formation.[110] Current evidence suggests that enzyme promiscuity may play an important role in contributing to the diversity across *Ocimum* species.

3.3 Transcription factors

Transcriptional regulation of secondary metabolism in plants for flavonoids (particularly anthocyanins), alkaloids (including nicotine, indole alkaloids and benzylisoquinolines) and terpenoids has been widely reported.[125-127] Recently, PAP1 transcription factor was shown to enhance the production of both terpenoids and phenylpropanoids in rose plant.[116] Deep sequencing of *O. tenuiflorum* and *O. basilicum* revealed the presence of 40 transcription factor families including MYB, WRKY, bHLH, HB, NAC, bZIP etc. which are known regulators of secondary metabolism in plants.[14] A recent study performed using the red and green forma of *O. tenuiflorum* suggested light-mediated regulation of anthocyanin accumulation.[128] It was observed that when red forma seedlings grown under natural lighting conditions, were transferred to a special greenhouse which cuts off the UV-A and UV-B radiation, the leaves turned green within 20 days. Further investigation revealed the role of transcription factors, bHLH and WD40, in downregulating the terminal enzymes of anthocyanin biosynthesis including flavonone-3′-hydroxylase, leucoanthocyanidine dioxygenase and dihydroflavonol reductase, responsible for red coloration. In another study by Misra *et al*, transcription factors belonging to APETALA2/Ethylene responsive factor (ERF), WRKY, plant homeo domain (PHD) and zinc finger families were upregulated in methyl jasmonate (MeJa)-treated *O. basilicum* plants, suggesting their possible role in regulating secondary metabolism in *Ocimum* species.[115] Thus, available data suggests transcription factors are also key regulators of terpenoid and phenylpropanoid pathway in *Ocimum* species and provide a more stringent control over the direction of flux.

3.4 Post-translational modifications

Post-translational modifications including phosphorylation, ubiquitination and arginine monomethylation of phenylpropanoid and terpenoid pathway enzymes such as

phosphoglucomutase, glucose-6-phosphate isomerase, phosphoglycerate mutase, PAL and chavicol O-methyl transferase (CVOMT) were observed in basil glandular trichomes. Post translation modifications help in explaining situations where the mRNA level does not match with the metabolite or protein level. For example, the enzyme CVOMT is responsible for methylating chavicol. *O. basilicum var.* SD produces negligible amount of methylchavicol. However, the mRNA and protein levels for this enzyme were found to be very high. In contrast, very little enzyme activity and metabolites were detected. It was observed that this enzyme was ubiquinated providing a valid explanation for the discrepencies in mRNA, protein, enzyme activity and metabolite level. Ubiquitination leads to a rapid degradation of CVOMT post translation,[5] resulting in decreased formation of methyl chavicol. In another example, PAL, catalyzing the first committed step in phenylpropanoid biosynthesis, is phosphorylated in *O. basilicum var*. SD, rich in monoterpenes; however, other basil varieties (SW, MC, and EMX-1), rich in phenylpropanoids, lack PAL phosphorylation.[5] It has been reported earlier that phosphorylation results in the reduction of PAL activity.[129,130] Above examples suggest that post translation modifications provide an additional regulatory step in determining the expression of key enzyme activities in secondary metabolic pathways in *Ocimum* species.

3.5 Presence of isozymes

Phenylpropanoid pathway produces substrates for synthesis of several important secondary metabolites. PAL, C4H and 4CL catalyse the initial few steps leading to the formation of coumaryl CoA. Latter represents a branching point, from which different end products including phenylpropenes, lignins, flavonoids and rosmarinic acid can be synthesized. Thus, 4CL represents a crucial step in pathway regulation and diversification. In recent work by Rastogi *et al*, it was reported that *O. basilicum* 4CL has 5 different isoforms.[103] RNAi experiments

involving the silencing of a specific isoform, Oba4CL, led to a reduced production of phenylpropanoids without affecting lignin and sinapic acid content. Thus, only one of the isoforms of 4CL was involved in the synthesis of phenylpropenes. This also represents the commitment of a specific isoform of an enzyme to a specific biosynthetic pathway at a very initial step. Presence of such pathway-committed isoforms keeps the pathway finely tuned and delicately balanced in basil.

3.6 External factors

Being species native to the tropics, *Ocimum* plants are always subjected to severe environmental conditions including excessive heat, rainfall, humidity, dryness etc. Adaptability, thus, is the key to survival. It has been reported that external environmental factors, including the type of light, radiation, season, geographic conditions etc., influence essential oil composition. Same *Ocimum* specie show altered metabolic profile under different environmental factors. Red and blue shading conditions in *O. selloi* showed decline in level of phenylpropanoids and elevated level of in comparison with plants grown in full light.[84] Plants grown under blue shading had more number of metabolites than plants subjected to full light and red shading. Decreased accumulation of methyl chavicol was observed in plants cultured under colored netting, accompanied by an increase in α-copaene, germacrene D and bicyclogermacrene content.[84] This suggests a chemical defense strategy of plants against less favorable growth conditions. Similar kind of study was performed with *O. basilicum* cultivated in soil covered by colored mulches which demonstrated that size and aroma of leaves as well as the concentration of soluble phenols greatly improved.[131] Seasonal variation of essential oil composition was observed in *O. basilicum* and *O. tenuiflorum*.[83] To show the effect of geographic conditions on essential oil composition, *O. gratissimum* and *O. campechianum* were grown in Chocó Department (Columbia) and

Ecuador region that resulted in different chemical composition.[91] Similarly, *O. basilicum* and *O. gratissimum* grown in Benin, Cameroon, Congo and Gabon vary in chemical composition.[90] *O.gratissimum* plants grown in Columbia showed altered metabolite profile as compared with those grown in Europe.[91,132-135] This data indicates external factors including climatic conditions and geographical variations might be influencing the chemical profile of *Ocimum* species.

3.7 Developmental and tissue specific regulation

During cinnamic acid and methylcinnamate (MC) formation from phenylalanine, activity of two enzymes, PAL and S-adenosyl-L-methionine: cinnamate carboxyl methyltransferase (SAM:CCMT) shows an important regulatory control point.[136] In different developemental stages of *O. basilicum*, the relation between MC content, PAL and SAM:CCMT activity was examined. SAM: CCMT activity showed correlation with MC content in young leaves.[136] Likewise, eugenol-O-methyltransferase (EOMT) is responsible for methylation of eugenol to form methyleugenol in one of the final steps of phenylpropanoid pathway. The expression pattern of EOMT positively correlated with the amount of eugenol/isoeugenol and methyleugenol in different developmental stages of all the analyzed chemotypes.[137] Along with development-specific regulation of metabolite accumulation, some metabolites in *Ocimum* species also show tissue-specific regulation. For example, analysis of trichome, leaf, stem and root shows a strong association between eugenol content and Ob4CL expression in *O. basilicum*.[103]

3.8 MicroRNA mediated regulation

Based on *O. basilicum* EST data set, the function of miRNAs and their targets was predicted using *in silico* approach.[138] Four miRNA families miR164c, miR5658, miR414 and miR5021 were evaluated for their potential targets. These miRNA families showed regulatory role during

stress-metabolite response. Although this study was based upon computational evaluation, further *in planta* experimentation is required to determine the critical role of miRNAs during secondary metabolism in *Ocimum* species.[138]

4. Future perspective

Ocimum acts as a reservoir of several important secondary metabolites found in nature, thereby making it a very attractive system to explore. Although the genome of *Ocimum* has not yet been sequenced, the recent influx of next generation sequencing data of various tissues such as trichomes and leaves, has helped us in understanding various factors that are responsible for regulating the formation of phenylpropanoids and terpenoids in *Ocimum* species. Using the current information, we can genetically engineer *Ocimum* species to overexpress the desired metabolites by redirecting the metabolite flux.[139-142] This knowledge can also be used for breeding new chemotypes producing interesting spectra of essential metabolites. Since these metabolites impart flavor and aroma, and possess medicinal properties, they can be heterologously expressed in plants, which are routinely used raw in our diet, such as tomato, thereby increasing their flavor and nutritive value. The expression of *O. basilicum* α-zingiberene synthase under the control of polygalacturonase promotor led to the unexpected accumulation of 15 sesquiterpenes and 10 monoterpenes, which were not present in the non-transformed fruit.[143] In a separate study, the expression of *O. basilicum* geraniol synthase under the same promoter led to the accumulation of geraniol and its derivatives, which had profound impact on tomato flavor as well as aroma.[144] Moreover, expressing terpene synthase genes from *Ocimum* in food crops will impart greater resistance against pathogens and pests. Till date, it is not well established whether there is a cross talk between the phenylpropanoid and terpenoid pathways. The glandular trichomes present in several *Ocimum* plants provide a very exciting isolated

single-celled system to unravel the exchange, if any, of upstream intermediates between these two pathways. Thus, *Ocimum* species find useful applications in industrial, culinary, medicinal as well as scientific research areas, asserting their important position in the plant kingdom.

5. Conclusions

Phenylpropanoid and terpenoid pathways in genus *Ocimum* have evolved as a result of various evolutionary, environmental and molecular events. Understanding the regulatory checkpoints in these pathways is a step closer towards efficiently harnessing this plant system. We believe that genes for both terpenoid and phenylpropanoid biosynthesis are present in all *Ocimum* species. However, several factors including genetic background, habit, ploidy levels, hybridization, differential gene expression, transcriptional and post translational modifications, isozymes etc. have played a major role in metabolic pathway diversification making *Ocimum* species either terpene- or phenylpropanoid- rich. The presence of many terpene synthases in single species and each one's ability to synthesize diverse metabolites from a single sustrate has further complicated the chemical evolution process. Most reviews on *Ocimum* have concentrated only on the medicinal benefits and industrial uses of this genus. To our knowledge, this is the first review that attempts to provide cumulative information about the potential reason/s for the complex chemical evolution and discusses terpenoid and phenylpropanoid pathway diversification events across *Ocimum* species.

6. Abbreviations

MVA	Mevalonic acid
MEP	Methylerythritol phosphate
GC	Gas chromatography
LC	Liquid chromatography

MS	Mass spectrometry
NMR	Nuclear magnetic resonance
PAL	Phenylalanine ammonia-lyase
4CL	4-Coumarate-CoA ligase
C4H	Cinnamate-4-hydroxylase
RPKM	Reads per kilobase per million
DXR	1-deoxy-D-xylulose 5-phosphate reductoisomerase
GPP	Geranyl diphosphate
FPP	Farnesyl diphosphate
UV-A	Ultraviolet-A
UV-B	Ultraviolet-B
ERF	Ethylene responsive factor
PHD	Plant homeo domain
MeJa	Methyl Jasmonate
CVOMT	Chavicol O-methyl transferase
MC	Methyl cinnamate
SAM	S-adenosyl-L-methionine
CCMT	Cinnamate carboxyl methyltransferase
EOMT	Eugenol-O-methyltransferase
Ob4CL	Ocimum basilicum 4-Coumarate-CoA ligase

Acknowledgement

PS acknowledges support from Council of Scientific and Industrial Research (CSIR), New Delhi for research fellowship. This research work is supported by CSIR under XII five-year plan network project (BSC0124) to CSIR- National Chemical Laboratory.

References

1. J.E. Simon, J. Quinn and R.G. Murray, in *Advances in new crops*, ed. J. Janick and J.E. Simon, Timber Press, Portland, 1990, Basil: A source of essential oils, 484.
2. C. Linnaeus, in *Species Plantarum*, Laurentii Salvii, Holmiae (Stockholm), 1753, *Ocimum*, 597.
3. P.D. Cantino, R.M. Harley and S.J. Wagstaff, in *Advances in Labiate Science*, ed. R. M. Harley and T. Reynolds, Royal Botanic Gardens, Kew, 1992, Genera of Labiatae: Status and Classification, 502.
4. M. K. Khosla, *Ind J. Genet.*, 1995, **55**, 71.
5. Z. Xie, J. Kapteyn and D.R. Gang, *Plant J.*, 2008, **54**, 349.
6. D. Yang, X. Du, X. Liang, R. Han, Z. Liang, Y. Liu, F. Liu and J. Zhao, *PLoSOne*, 2012, **7**, e46797.
7. A. Marchev, C. Haas, S. Schulz, V. Georgiev, J. Steingroewer, T. Bley and A. Pavlov, *Biotechnol. Lett*, 2014, **36**, 211.
8. G.W. Turner and R. Croteau, *Plant Physiol*, 2004, **136**, 4215.
9. R. B. Croteau, E. M. Davis, K. L. Ringer and M. R. Wildung, *Naturwissenschaften,* 2005, **92,** 562.
10. R. Rios-Estepa, G. W. Turner, J. M. Lee, R. B. Croteau and B. M. Lange, *PNAS*, 2008, **105**, 2818.
11. A. Lane, A. Boecklemann, G. N. Woronuk, L. Sarker and S. S. Mahmoud, *Planta*, 2010, **231,** 835.
12. G. Woronuk, Z. Demissie, M. Rheault and S. Mahmoud, *Planta Med.*, 2011, **77**, 7.

13. D. R. Gang, J. Wang, N. Dudareva, K. H. Nam, J. E. Simon, E. Lewinsohn and E. Pichersky, *Plant Physiol.*, 2001, **125**, 539.

14. S. Rastogi, S. Meena, A. Bhattacharya, S. Ghosh, R. K.Shukla, N.S. Sangwan, R. K. Lal, M. M. Gupta, U. C. Lavania, V. Gupta, D. A. Nagegowda and A. K. Shasany, *BMC Genomics*, 2014, **15**, 588.

15. F. Bast, P. Rani and D. Meena, *Scientific World J.*, *2014*, **2014,** doi:10.1155/2014/847482

16. K. Carović-Stanko, Z. Liber, V. Besendorfer, B. Javornik, B. Bohanec, I. Kolak and Z. Satovic, *Plant Syst.Evol.*, 2010, **285**, 13.

17. A. J. Paton, D. Springate, S. Suddee, D. Otieno, R. J. Grayer, M. M. Harley, F. Willis, M. S. J. Simmonds, M. P. Powell and V. Savolainen, *Mol. Phylogenet. Evol.*, 2004, **31**, 277.

18. A. Paton and E. Putievsky, *Kew Bull.*, 1996, **51**, 509.

19. A. P. Singh, S. Dwivedi, S. Bharti, S. Srivastava, V. Singh and S. P. S. Khanuja, *Euphytica*, 2004, **136**, 11.

20. V. T. de Lima, M. C. Vieira, C. A. Kassuya, C. A. Cardoso, J. M. Alves, M. A. Foglio, J. E. de Carvalho and A. S. Formagio, *Phytomedicine*, 2014, **21**, 1298.

21. J. Monga, M. Sharma, N. Tailor and N. Ganesh, *Pharm. Biol.*, 2011, **49**, 428.

22. P. Singh, R. H. Jayaramaiah, P. Sarate, H. V. Thulasiram, M. J. Kulkarni and A. P. Giri, *PloS One*, 2014, **9**, e104377.

23. R. V. Sarin, S. Narwal and P. A. Bafna, *J. Ethnopharmacol.*, 2013, **148**, 223.

24. D. Runyoro, O. Ngassapa, K. Vagionas, N. Aligiannis, K. Graikou and I. Chinou, *Food Chem.*, 2010, **119**, 311.

25. B. O. Owuor, J. O. Ochanda, J. O. Kokwaro, A. C. Cheruiyot, R. A. Yeda, C. A. Okudo and H. M. Akala, *J. Ethnopharmacol.*, 2012, **144**, 779.

26. H. Song, P. Kumar, G. Arivazhagan, S. I. Lee, H. M. Yoon, I. H. Kim, H. J. Kwon, J. M. Kim and F. L. Hakkim, *J. Plant Biotechnol*, 2012, **39**, 146.

27. F. L. Hakkim, G. Arivazhagan and R. Boopathy, *J. Med. Plant. Res*, 2008, **2**, 250.

28. E. J. Kweka, F. W. Mosha, A. Lowassa, A. M. Mahande, M. J. Mahande, C. P. Massenga, F. Tenu, E. E. Lyatuu, M. A. Mboya and E. A. Temu, *Parasit. Vectors*, 2008, **1**, doi:10.1186/1756-3305-1-42.

29. P. Kapewangolo, J. J. Omolo, R. Bruwer, P. Fonteh and D. Meyer, *J. Inflamm.,* 2015*,* **12**, doi:10.1186/s12950-015-0049-4.

30. E. Lulekal, J. Rondevaldova, E. Bernaskova, J. Cepkova, Z. Asfaw, E. Kelbessa, L. Kokoska and P. Van Damme, *Pharm. Biol.*, 2013, **52**, 614.

31. D. Damtie and Y. Mekonnen, *Afr. J. of Pharm. Pharmacol*, 2015, **9**, 91.

32. F. F. Dube, K. Tadesse,G. Birgersson, E. Seyoum, H. Tekie, R. Ignell and S. R. Hill, *Malar. J.*, 2011, **10**, doi: 10.1186/1475-2875-10-375.

33. M. J. Mukazayire, J. C. Tomani, C. Stevigny, J. C. Chalchat, F. Conforti, F. Menichini and P. Duez, *Food Chem.*, 2011, **129**, 753.

34. W. Mequanint, E. Makonnen and K. Urga, *J. Ethnopharmacol.*, 2011, **134**, 32.

35. M. J. Mukazayire, V. Allaeys, P. B. Calderon, C. Stévigny, M. J. Bigendako and P. Duez, *Exp. Toxicol. Pathol.*, 2010, **62**, 289.

36. E. Makonnen, A. Debella, D. Abebe and F. Teka, *Phytother. Res.*, 2003, **17**, 1108.

37. Y. Naidoo, C. T. Sadashiva, N. Kasim, A. Nicholas and G. Naidoo, *J. Essent. Oil Bear. Pl.*, 2014, **17**, 142.

38. S. Parasuraman, S. Balamurugan, P. V. Christapher, R. R. Petchi, W. Y. Yeng, J. Sujithra and C. Vijaya, *Pharmacogn. Res.*, 2015, **7**, 156.

39. F. L. Hakkim, C. G. Shankar and S. Girija, *J. Agric.Food Chem.*, 2007, **55**, 9109.

40. B. Sarkar, D. Kumar, D. Sasmal and K. Mukhopadhyay, *Nat. Prod. Res*., 2014, **28**, 1393.

41. P. Phanthong, P. Lomarat, M. T. Chomnawang and N. Bunyapraphatsara, *Science Asia*, 2013, **39**, 472.

42. M. Stefan, M. M. Zamfirache, C. Padurariu, E. Trută and I. Gostin, *Open Life Sci.*, 2013, **8**, 600.

43. K. Karthikeyan, P. Gunasekaran, N. Ramamurthy and S. Govindasamy, *Pharm. Biol.*, 1999, **37**, 285.

44. F. M. Mahomoodally, A. H. Subratty, A. Gurib-Fakim and M. I. Choudhary, *BMC Complement. Altern. Med.*, 2012, **12**, doi:10.1186/1472-6882-12-165.

45. R. C. Saxena, R. Singh, P. Kumar, M. P. S. Negi, V. S. Saxena, P. Geetharani, J. J. Allan and K. Venkateshwarlu, *Evid. Based Complement. Alternat. Med*., 2011, **2012**, doi:10.1155/2012/894509.

46. P. Sudha, S. S. Zinjarde, S.Y. Bhargava and A.R. Kumar, *BMC Complement. Altern. Med.*, 2011, **11**, doi:10.1186/1472-6882-11-5.

47. O. Chokechaijaroenporn, N. Bunyapraphatsora and S. Kongchuensin, *Phytomedicine*, 1994, **1**, 135.

48. R. Yucharoen, S. Anuchapreeda and Y. Tragoolpua, *Afr. J. Biotechnol*., 2013, **10**, 860.

49. G. Kaur, A. Bali, N. Singh and A. S. Jaggi, *An. Acad. Bras. Ciênc.*, 2015, **87**, 417.

50. B. T. Aluko, O. I. Oloyede and A. J. Afolayan, *Pak. J. Biol. Sci.*, 2013, **16**, 22.

51. A. N. Yamada, R. Grespan, A. T. Yamada, E. L. Silva, S. E. Silva-Filho, M. J. Damião, M. M. de Oliveira Dalalio, C. A. Bersani-Amado and R. K. N. Cuman, *Am. J. Chin. Med.*, 2013, **41**, 913.

52. S. Thaweboon and B. Thaweboon, *Southeast Asian J. Trop. Med. Public Health*, 2009, **40**, 1025.

53. U. Szymanowska, U. Złotek, M. Karas and B. Baraniak, *Food Chem.*, 2015, **172**, 71.

54. N. P. Yadav, R. Khatri, D. U. Bawankule, A. Pal, K. Shanker, P. Srivastava, A. K. Gupta and D. Chanda, *Planta Med.*, **75**, doi:10.1055/s-0029-1234397.

55. S. J. Inbaneson, R. Sundaram and P. Suganthi, *Asian Pac. J.Trop. Med.,* 2012, **5**, 103.

56. D. Beatović, D. Krstić-Milošević, S. Trifunović, J. Šiljegović, J. Glamočlija, M. Ristić, and S. Jelačić, *Rec. Nat. Prod.*, 2015, **9**, 62.

57. L. Vlase, D. Benedec, D. Hanganu, G. Damian, I. Csillag, B. Sevastre, A. C. Mot, R. Silaghi-Dumitrescu and I. Tilea, *Molecules*, 2014, **19**, 5490.

58. B. Kaurinovic, M. Popovic, S. Vlaisavljevic and S. Trivic, *Molecules*, 2011, **16**, 7401.

59. I. H. N. Bassolé, A. Lamien-Meda, B. Bayala, S. Tirogo, C. Franz, J. Novak, R. C. Nebié and M. H. Dicko, *Molecules*, 2010, **15**, 7825.

60. K. R. Durga, S. Karthikumar and K. Jegatheesan, *Afr. J. Plant Sci.*, 2010, **4**, 163.

61. P. A. Ntonga, N. Baldovini, E. Mouray, L. Mambu, P. Belong and P. Grellier, *Parasite*, 2014, **21**, doi:10.1051/parasite/2014033.

62. K. H. Al-Ali, H. A. El-Beshbishy, A. A. El-Badry and M. Alkhalaf, *Pak. J. Biol. Sci.*, 2013, **16**, 1744.

63. A. K. Qamar, A. Dar, B. S. Siddiqui, N. Kabir, H. Aslam, S. Ahmed, S. Erum, S. Habib and S. Begum, *Lett. Drug Des. Discov.*, 2010, **7**, 726.

64. M. Govindarajan, R. Sivakumar, M. Rajeswary and K. Yogalakshmi, *Exp. Parasitol.*, 2013, **134**, 7.

65. K. Murugan, P. Murugan and A. Noortheen, *Bioresour. Technol.*, 2007, **98**, 198.

66. B. S. Siddiqui, H. A. Bhatti, S. Begum and S. Perwaiz, *J. Ethnopharmacol.*, 2012, **144**, 220.

67. K. S. Bora, S. Arora and R. Shri, *J. Ethnopharmacol.*, 2011, **137**, 1360.

68. A. Umar, G. Imam, W. Yimin, P. Kerim, I. Tohti, B. Berké and N. Moore, *Hypertension Res.*, **33**, 727.

69. S. Amrani, H. Harnafi, D. Gadi, H. Mekhfi, A. Legssyer, M. Aziz, F. Martin- Nizard and L. Bosca, *J. Ethnopharmacol.*, 2009, **125**, 157.

70. I. de Almeida, D. S. Alviano, D. P. Vieira, P. B. Alves, A. F. Blank, A. H. C. Lopes, C. S. Alviano and S. R. Maria do Socorro, *Parasitol. Res.*, 2007, **101**, 443.

71. L. C. Chiang, L. T. Ng, P. W. Cheng, W. Chiang and C. C. Lin, *Clin. Exp. Pharmacol. Physiol.*, 2005, **32**, 811.

72. Y. H. Chen, Y. W. Chiu, J. C. Shyu, C. C. Tsai, H. H. Lee, C. C. Hung, J. M. Hwang, J. Y. Liu and W. H. Wang, *Chinese J. Physiol.*, 2015, **58**, 55.

73. D. C. Gontijo, L. C. Fietto and J. P. V. Leite, *Rev. Bras. Plantas Med.*, 2014, **16**, 874.

74. G. H. Bénédicta, K. Kpoviessia, S. D. S. Kpoviessia, E. Y. Ladekan, F. Gbaguidi, M. Frédérichd, M. Moudachiroub, J. Quetin-Leclercqc, J.C. Accrombessia and J. Beroc, *J. Ethnopharmacol.*, 2014, **155**, 1417.

75. S. K. Mahapatra and S. Roy, *Asian Pac. J.Trop. Med.,* 2014, **7**, S391.

76. Y. W. Chiu, P. Y. Chao, C. C. Tsai, H. L. Chiou, Y. C. Liu, C. C. Hung, H. C. Shih, T. J. Lai and J. Y. Liu, *Am. J. Chin. Med.*, 2014, **42**, 833.

77. R. K. Joshi, *Indian J. Pharm. Sci.*, 2013, **75**, 457.

78. A. Katara, C. K. Pradhan, P. Singh, V. Singh and M. Ali, *J. Essent. Oil Bear. Pl.*, 2013, **16**, 283.

79. E. F. F. Matias, F. A. V. Santos, J. M. F. Silva, C. E. S. Souza, S. R. Tintino, G. M. M. Guedes, C. R. Medeiros, M. F. B. Braga, T. S. Almeida, J. G. M. Costa, I. R. A. Menezes and H. D. M. Coutinho, *Afr. J. Microbiol. Res.*, 2012, **6**, 1902.

80. M. O. Nkiko and J. T. Bamgbose, *Portugaliae Electrochimica Acta*, 2011, **29**, 419.

81. K. S. Bora, R. Shri and J. Monga, *Pharm. Biol.*, 2011, **49**, 175.

82. A. C. M. de Santana, G. S.Pereira, C. M. Boaventura, A. P. T. Uetenabaro, L. C. D. B. Costa and R. A. de Oliveira, *Revista Brasileira de Farmacognosia*., 2014, **24**, 524.

83. S. R. Vani, S. F. Cheng and C. H. Chuah, *Am. J. Appl. Sci.*, 2009, **6**, 523.

84. L. C. Costa, J. E. Pinto, E. M. Castro, E. Alves, L. F. Rosal, S. K. Bertolucci, P. B. Alves and T. S. Evangelino, *J.Essent. Oil Res.*, 2010, **22**, 34.

85. S. K.Singh, A. Anand, S. K. Verma, M. A. Siddiqui, A. Mathur and S. Saklani, *Int. J. curr. Pharm. Res.,* 2011, **3**, 40.

86. M. Özcan and J. C. Chalchat, *Czech J. Food Sci.*, 2002, **20**, 223.

87. D. Ioannidis, B. Lynda and C. B. Johnson, *Ann. Botany*, 2002, **90,** 453.

88. R. Parida, S. I. Sandeep, B. K. Sethy, S.Sahoo and S. Mohanty, *Int J Pharm Pharm Sci,* 2014, **6,** 7.

89. A. S. D. Costa, M. D. F. Arrigoni-Blank, M. A. A. P. D. Silva, M. F. Alves, D. D. A. Santos, P. B. Alves and A. F. Blank, *Scientific World J*., 2014, **2014**, doi:10.1155/2014/824594

90. F. Tchoumbougnang, P. A. Zollo, F. Avlessi, G. A. Alitonou, D. K. Sohounhloue, J. M. Ouamba and C. Menut, *J. Essent. Oil Res.*, 2006, **18**, 194-199.

91. N. P. Benitez, E. M. M. León and E. E. Stashenko, *J. Chromatogr. Sci.,* 2009, **47**, 800.

92. G. Sacchetti, A. Medici, S. Maietti, M. Radice, M. Muzzoli, S. Manfredini, E. Braccioli and R.Bruni, *J. Agric. Food Chem.*, 2004, **52**, 3486.

93. J. C. Nascimento, L.C. Barbosa, V. F. Paula, J. M. David, R. Fontana, L. A. Silva and R. S. França, *Anais da Academia Brasileira de Ciências*, 2011, **83**, 787.

94. O. U. Edet and P. O. Aikpokpodion, *Am. J. Plant Sci.*, 2014, **5**, 126.

95. R. B. Bose and J. K. Choudhury, *Caryologia*, 1962, **15**, 435.

96. P. Pushpangadan and S. N. Sobti, *Cytologia*, 1982, **47**, 575.

97. T. Zderkiewicz, *Acta Agrobot.*, 1971, **24**, 121.

98. H. Dijkstra and G. J. Speckmann, *Euphytica*, 1980, **29**, 89.

99. E. K. Janaki Ammal and S. N. Sobti, *Curr. Sci.*, 1962, **31**, 387.

100. Y. Iijima, R. Davidovich-Rikanati, E. Fridman, D. R. Gang, E. Bar, E. Lewinsohn and E. Pichersky, *Plant Physiol.*, 2004, **136**, 3724.

101. G. V. Louie, T. J. Baiga, M. E. Bowman, T. Koeduka, J. H. Taylor, S. M. Spassova, E. Pichersky and J. P. Noel, *PloS One*, 2007, **2**, e993.

102. I. Aragüez, S. Osorio, T. Hoffmann, J. L. Rambla, N. Medina-Escobar, A. Granell, M. A. Botella, W. Schwab and V. Valpuesta, *Plant Physiol.*, 2013, **163**, 946.

103. S. Rastogi, R. Kumar, C. S. Chanotiya, K. Shanker, M. M. Gupta, D. A. Nagegowda and A. K. Shasany, *Plant Cell Physiol.*, 2013, doi:10.1093/pcp/pct073.

104. L. Medina-Puche, F. J. Molina-Hidalgo, M. Boersma, R. C. Schuurink, I. López-Vidriero, R. Solano, J. M. Franco-Zorrilla, J. L. Caballero, R. Blanco-Portales and J. Muñoz-Blanco, *Plant Physiol.*, 2015, **168**, 598.

105. D. R. Gang, N. Lavid, C. Zubieta, F. Chen, T. Beuerle, E. Lewinsohn, J. P. Noel and E. Pichersky, *Plant Cell*, 2002,**14**, 505.

106. E. Lewinsohn, I. Ziv-Raz, N. Dudai, Y. Tadmor, E. Lastochkin, O. Larkov, D. Chaimovitsh, U. Ravid, E. Putievsky, E. Pichersky and Y. Shoham, *Plant Sci*., 2000, **160**, 27.

107. R. Croteau and F. Karp, *Arch. Biochem. Biophys*., 1979, **198**, 512.

108. M. L. Wise, T. J. Savage, E. Katahira and R. Croteau, *J. Biol. Chem.*, 1998, **273**, 14891.

109. D. A. Whittington, M. L. Wise, M. Urbansky, R. M. Coates, R. B. Croteau and D. W. Christianson, *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 15375.

110. D.T. Major and M. Weitman, *J. Am. Chem. Soc.*, 2012, **134**, 19454.

111. R. Croteau, C. L. Hooper and M. Felton, *Arch. Biochem. Biophys.*, 1978, **188**, 182.

112. L. S. Sarker, M. Galata, Z. A. Demissie and S. S. Mahmoud, *Arch. Biochem. Biophys.*, 2012, **528**, 163.

113. R. Croteau and F. Karp, *Arch. Biochem. Biophys.*, 1977, **179**, 257.

114. Y. Iijima, D. R. Gang, E. Fridman, E. Lewinsohn and E. Pichersky, *Plant Physiol.*, 2004, **134**, 370.

115. R. C. Misra, P. Maiti, C. S. Chanotiya, K. Shanker and S. Ghosh, *Plant Physiol.*, 2014, **164**, 1028.

116. M. M. B. Zvi, E. Shklarman, T. Masci, H. Kalev, T. Debener, S. Shafir, M. Ovadis and A. Vainstein, *New Phytol.*, 2012, **195**, 335.

117. A. K. Upadhyay, A. R. Chacko, A. Gandhimathi, P. Ghosh, K. Harini, A. P. Joseph, *et al*., *BMC Plant Biol*., 2015, **15**, 212.

118. A. Berim, J. Park and D. R. Gang, *Plant J.*, 2014, **80**, 385.

119. A. Berim and D. R. Gang, *Phytochem.*, 2013, **92**, 33.

120. A. Berim and D. R. Gang, *J. Biol. Chem.*, 2013, **288**, 1795.

121. A., Berim, D. C. Hyatt and D. R. Gang, *Plant Physiol.*, 2012, **160**, 1052.

122. N. J. Bate, J. Orr, W. T. Ni, A. Meromi, T. Nadlerhassar, P. W. Doerner, R. A. Dixon, C. J. Lamb and Y. Elkind, *Proc. Natl Acad. Sci.*, 1994, **91**, 7608.

123. L. Carretero-Paulet, I. Ahumada, N. Cunillera, M. Rodriguez-Concepcion, A. Ferrer, A. Boronat and N. Campos, *Plant Physiol.*, 2002, **129**, 1581.

124. M. Rodriguez-Concepcion, *Phytochem. Rev.*, 2006, **5**, 1.

125. Z. X. Yu, J. X. Li, C. Q. Yang, W. L. Hu, L. J. Wang and X. Y. Chen, *Mol. Plant*, 2012, **5**, 353.

126. S. Mishra, V. Triptahi, S. Singh, U. J. Phukan, M. M. Gupta, K. Shanker and R. K. Shukla, *PLoS ONE*, 2013, **8**, e52784

127. M. A. Naoumkina, X. He and R. A. Dixon, *BMC Plant Biol*, 2008, **8**, doi: 10.1186/1471-2229-8-132.

128. P. Vyas, I. Haque, M. Kumar and M. Mukhopadhyay, *Acta Physiol. Plant.*, 2014, **36**, 2091.

129. E. G. Allwood, D. R. Davies, C. Gerrish, B. E. Ellis and G. P. Bolwell, *FEBS Lett.*, 1999, **457**, 47.

130. G. P. Bolwell, *Phytochemistry*, 1992, **31**, 4081.

131. J. H. Loughrin and M. J. Kasperbauer, *J. Agr. Food Chem.*, 2001, **49**, 1331.

132. M. G. de Vasconcelos, A. A. Craveiro, F. J. Abreu, M. I. L. Machado and J.W. Alencar, *Fitoterapia,* 1999*,* **70,** 32.

133. C. M. Murbach, M. Ortiz and M. Costa. *J. Ethnopharmacol.,* 2006*,* **105,** 161.

134. E. Guenther, in *The Essential oils*., van Nostrand Co. Inc., New York, USA, Volume III, V, 1995, 427–428

135. R. Hiltunen and Y. Holm, *Basil the genus Ocimum*, Harwood Academic Publishers, 1999.

136. Z. Hao, Biosynthesis of methylcinnamate in *Ocimum basilicum* L., Purdue University, 1998.

137. I. K. Renu, I. Haque, M. Kumar, R. Poddar, R. Bandopadhyay, A. Rai and K. Mukhopadhyay, *Mol. Biol. Rep.*, 2014, **41**, 1857.

138. N. Singh and A. Sharma, *Gene*, 2014, **552**, 277.

139. B. M. Lange and A. Ahkami, *Plant Biotech. J.*, 2013, **11**, 169.

140. N. Dudareva, A. Klempien, J. K. Muhlemann and I. Kaplan, *New Phytol.*, 2013, **198**, 16.

141. M. J. Fischer, S. Meyer, P. Claudel, M. Bergdol and F. Karst, *Biotechnol. Bioeng.*, 2011, **108**, 1883.

142. N. Misawa, *Curr. Opin. Biotechnol.*, 2011, **22**, 627.

143. R. Davidovich-Rikanati, E. Lewinsohn, E. Bar, Y. Iijima, E. Pichersky and Y. Sitrit, *Plant J.*, 2008, **56**, 228.

144. R. Davidovich-Rikanati, Y. Sitrit, Y. Tadmor, Y. Iijima, N. Bilenko, E. Bar, B. Carmona, E. Fallik, N. Dudai, J. E. Simon, E. Pichersky and E. Lewinsohn, *Nature Biotechnol.*, 2007, **25**, 899.

Table 1: Bioactivities of *Ocimum* species tissue extracts/purified compound(s)

sp.	Bioactivity	Extract/Compound	Dose and/or activity	Organism/ cell line/ assay	
Ok	Free radical scavanging[20]	Leaf essential oil, camphor, mixture of 1,8-cineole and limonene	Essential oil, GI50= 8.31 µg/mL Camphor, IC50=12.56 µg/mL Limonene + 1,8-cineole, IC50=23.25 µg/mL	DPPH free- radical scavenging assay	GC–MS[20]
	Anticancer[20, 21]	Leaf essential oil;[20] 50% alcoholic aqueous leaf extract[21]	Essential oil via hydrodistillation, GI50= 31.90 mg/mL[20] 50% alcoholic aqueous leaf extract, dose = 200 mg/kg p.o.[21]	Human ovarian cancer cell line[20]; C(57)BL and Swiss albino mice injected intradermally with $B_{10}H_{16}$ metastatic melanoma cell line.[21]	GC–MS[20]
	Anti-inflamatory[20]	Leaf essential oil, camphor, mixture of 1,8-cineole and limonene	Reduction in total leucocyte migration= 82 ± 4% (30 mg/kg essentia oil), 95 ± 4% (100 mg/kg of essential oil), 83 ± 9% (camphor), 80 ± 5% (1,8-cineole + limonene)	Carrageenan-induced pleurisy in mice	GC–MS[20]
	Insecticidal[22]	DCM leaf extract, camphor, limonene and β-caryophyllene	Dose =10, 100 and 1000 ppm incorporated in artificial diet	*Helicoverpa armigera* (second instar larvae)	GC-MS
	Antidiarrhoel[23]	Aqueous leaf extract	100, 200 and 400 mg/kg , p.o.	Castor-oil induced diarrhoea model, castor oil induced enteropooling assay in rats; charcoal meal/intestinal motility test in mice	Nil
	Antimicrobial[24]	Essential oil, borneol, bornyl acetate, camphor, caryophyllene oxide, 1,8-cineole, limonene, linalool, α-pinene, β-pinene, spathulenol	MIC, essential oil (1.55-3.35), borneol (1.25-4.50), bornyl acetate (1.75-4.88), camphor (1.33-3.24), caryophyllene oxide (0.073 to >6.4), 1,8- cineole (2.0-9.5), limonene (>20), linalool (0.25 to >20), α-pinene (2.0-15.0), β-pinene (9.75 to >20), spathulenol (1.35 to >20)	*S. aureus, S. epidermidis, S. mutans, S. viridan, E. coli, E. cloacae, K. pneumoniae, P. aeruginosa, C. albicans, C. tropicali, C. glabrata.*	GC-MS

			(*values in mg/mL)		
	Antiplasmodial[25]	DCM plant extract	Extract, IC50 (CQ resistant clone) = 1.547±0.226 µg/mL Extract, IC50 (CQ sensitive clone) = 0.843±0.123 µg/mL	SYBR Green I fluorescence assay (MSF assay) against *Plasmodium falciparum* (CQ resistant and sensitive clone)	Nil
	Antioxidant[26,27]	Methanolic extracts of leaves,[26, 27] and callus[26]	Extract, dose = 1 mg/mL[26, 27]	Ferric reducing antioxidant power (FRAP) assay[26]; Iron (III) reduction, β-carotene-linoleic acid bleaching, DPPH, superoxide anion free radical scavenging assay[27]	HPLC[27]
	Radioprotective[21]	50% alcoholic aqueous leaf extract	Extract, dose = 200 mg/kg p.o.	Mice irradiated by ^{60}Co source in the cobalt therapy unit	Nil
	Mosquito repellent[28]	Plant essential oil, dry plant material	20% plant essential oil solution prepared in glycerine and acetone, and burning 1 kg of dry plant material; protection efficiency (PE), Essential oil = 89.75% (*Anopheles arabiensis*) and 90.50% (*Culex quinquefasciatus*)	Field trials (community study) using *A. arabiensis, A. gambiae* and *C. quinquefasciatus*	Nil
Olb	Antioxidant[29]	Ethanolic leaf extract, labdane (isolated diterpenoid)	Extract, IC50 = 13 ± 0.8 (DPPH assay), 53.62 ± 0.57 (FRAP assay), 47.32 ± 0.76 (CUPRAC assay) and 54.86 ± 1.28 µg/mL (AAPH); Labdane diterpenoid demonstrated minimal to no activity	DPPH, FRAP, Cupric reducing antioxidant capacity (CUPRAC) and crocin bleaching assay (AAPH)	NMR
	Anti-inflammatory[29]	Ethanolic leaf extract, labdane diterpenoid	Extract, dose = 25µg/mL Labdane diterpenoid, dose = 50 µM[29]	Cytometric bead array (CBA) technique[29]	NMR[29]
Ola	Antimicrobial[24,30, 31]	Essential oil extract; Ethanolic extract of various plant parts; methanol, aqueous and n-hexane extracts	MIC= 1.75-4.90 mg/mL[24]; MIC (all extracts) < 512 µg/mL[30]; MIC= 10 - 50 mg/mL[31]	*S. aureus, S. epidermidis, S. mutans, S. viridan, E. coli, E. cloacae, K. pneumoniae, P. aeruginosa, C. albicans, C. tropicalis, C. glabrata*;[24] *B. cereus, C. perfringens, L. monocytogenes, E. faecalis, S. aureus, S. pyogenes, S. epidermidis;*[30]*S. aureus, E. coli,*	GC-MS[24]

				P. aeruginosa, S. boydii	
	Mosquito-repellent[32]	Volatiles from fresh, dried and smoking dried leaves	β-ocimene (14%) strongly repelled female mosquitoes	*Anopheles arabiensis, Aedes aegypti,*	GC-MS
	Antioxidant[27,33]	Plant essential oils and methanolic extracts[27]	Methanolic extract, dose=1 mg/mL;[27] Methanolic extract, IC50 = 8.6 ± 0.7 (DPPH assay); 12.8 ± 0.8 (linoleic acid assay);[33] Essential oil, IC50 = 27.5 ± 1.4 (DPPH assay); 46.1 ± 2.3 (linoleic acid assay) µg/mL[33]	Iron (III) reduction, β-carotene- linoleic acid bleaching, DPPH, superoxide anion free radical scavenging assay;[27] DPPH and linoleic acid peroxidation assay[33]	HPLC[27], GC-MS[33]
	Anti-inflammatory[34]	Aqueous and ethanolic leaf extracts	400, 600 and 800 mg/kg body weight	carrageenin, histamine, and serotonin induced mice paw edema.	Nil
	Hepatoprotective[35]	Aqueous and methanolic leaf extracts	1 and 2 mg/mL	Aqueous extracts tested on CCl4 -treated guinea pigs by using barbiturate induced sleep modification; methanolic extracts tested in vitro using precision cut liver slices (PCLS) against acetaminophen-induced hepatotoxicity	
	Analgesic[36]	Aqueous and ethanolic plant extracts	400, 600 and 800 mg/kg body weight	Tail-flick, hot-plate and tail-pinch assay in albino male mice	Nil
Oo	Anitmicrobial[37]	Leaf essential oil	MIC= 50-200 µg/mL	*Escherichia coli, Staphylococcus aureus, Klebsiella spp, Pseudomonas* spp., *Proteus* spp.	GC-MS
Ot	Antidiabetic[38]	60% ethanolic leaf extract	Extract, dose = 250 and 500 mg/kg body weight	Male Wistar albino rats	Nil
	Anti-hyperlipidemic[38]	60% ethanolic leaf extract	Extract, dose = 250 and 500 mg/kg body weight	Male Wistar albino rats	Nil
	Anti-oral toxicity effect[38]	Hydroalcoholic leaf extract	5- 2000 mg/kg body weight	Male Wistar albino rats	Nil
	Antioxidant[39]	Methanolic extracts of leaf, inflorescence, stem and callus	Extract, dose = 50-1000 µl	DPPH, hydroxyl radicals, hydrogen peroxide, chelating ferrous iron and ferric ion reducing potential assay	Reverse phase HPLC

	DNA damage protective[40]	Anthocyanin extracts	5, 10 and 20 µg/ml	Oxidative DNA damage induced via H_2O_2 and UV using pUC19 plasmid	UPLC
	Antibacterial[41,42]	Essential oil	MIC (essential oil) = 25-100 µg/mL;[41] MIC (essential oil) = 0.364 mg/mL (*S. aureus*), 0.728 mg/mL (*E. coli*)	*E. coli, S. enteritidis, S. typhimurium, S. typhi, S. flexneri, B. cereus, S. aureus*;[41] *E. coli* and *S. aureus*[42]	
	Anticancer[43]	Aqueous and ethanolic leaf extracts	50- 400 µg/mL	Sarcoma-180, HFS-1080 cell lines; swiss albino wistar mice	Nil
	Antiglycation[44]	Methanolic and water extracts and their fractions (DCM, ethyl-acetate, *n*-butanol, water)	IC50 values for different fractions ranged from 21.01 ± 2.06 to 68.29 ± 1.68 µg/mL; concentrations tested= 250 to 2000 µg/mL	measuring inhibition of advanced glycation end products by flurometry	Nil
	Antistress[45]	OciBest (whole plant extract in gelatin capsules)	1200 mg of actives per day	Self-evaluation by participants using symptom rating scale after 0, 2, 4, 6 week of trial period	Nil
	α-amylase inhibitory[46]	Isopropanol extract	IC50= 8.9 µg/mL	Porcine pancreatic α-amylase (PPA) inhibition assays	GC-MS
	Mosquito repellent[47]	Plant essential oil	EC50= 133 ppm; EC90= 240 ppm	*Aedes aegypti*	GC-MS
	Antiherpes[48]	Methanol and DCM extracts	Therapeutic index (TI), DCM extract = 10.003 (after HSV-2G adsorption); TI for methanol extracts = 1.644, 2.473 and 29.395 before,during and after HSV-2G adsorption	African Green Monkey (GMK) cells infected with Herpes Simplex Virus (HSV)	Nil
	Ameliorative potential[49]	Methanol extracts, Saponin- rich fraction	100 and 200 mg/kg p.o.	Wistar albino rats	HPTLC
Oa	Free Radical Scavenging[50]	Ethanol, butanol and ethyl-acetate extracts from leaves	50-300 µg/mL	DPPH-, ABTS-, hydrogen peroxide-, nitric oxide-, hydroxyl radical- scavenging assay	

	Anti-inflammatory Activity[51]	Essential oil, linalool, 1,8-cineole	50-300 mg/kg body weight	Zymosan-induced arthritis and paw edema in female balb/c mice	GC-MS
	Anti-herpes[48]	Methanol and DCM extracts	Therapeutic index (TI) for DCM extracts = 1.865, 2.623 and 7.04 before,during and after HSV-2G adsorption; TI for methanol extracts = 2.345 and 27.357, during and after HSV-2G adsorption	African Green Monkey (GMK) cells infected with Herpes Simplex Virus (HSV)	Nil
	Antimircobial[52]	Plant essential oil	MIC = 0.04% v/v (for S. mutans, L. casei and C. albicans); MCC = 0.08%, 0.3% and 0.08% v/v (for S. mutans, L. casei and C. albicans resp.)	*Streptococcus mutans, Lactobacillus casei, Candida albicans*	Nil
Oba	Antiherpes[48]	Methanol and DCM extracts	Therapeutic index (TI) for DCM extracts = 1.835 and 1.817, during and after HSV-2G adsorption; TI for methanol extracts = 1.563 and 2.176, during and after HSV-2G adsorption	African Green Monkey (GMK) cells infected with Herpes Simplex Virus (HSV)	Nil
	Anti-inflammatory [53,54]	Whole plants;[53] Ethanol-water (25%) extract of leaves[54]	Abiotic elicitors (aqueous solution), dose = 10^{-6}M (jasmonic acid), 10^{-6}M (arachidonic acid), 10^{-2}M (β-aminobutyric acid) sprayed on 21-day old plants;[53] 4 mg extract per day for five days[54]	Lipoxygenase and cyclooxygenase inhibitory assay in leaves treated with abiotic elicitors;[53] Swiss albino mice[54]	
	Antiplasmodial[55]	Plant ethanolic extracts (leaf, stem, root,flower)	IC50=43.81-78.69 µg/mL	*Plasmodium falciparum*	Nil
	Antioxidant and Antimicrobial[56-60]	Essential oil extracted *via* hydrodistillation;[56] plant extracts prepared using ethanol, butanol,	DPPH assay, IC50 = 0.03 to >100 µg/mL, Antimicrobial assay, MIC = 0.009-23.48 µg/mL;[56]IC50 = 124.95 µg/mL (DPPH assay), 25.69 (µmol Trolox/mg plant material (TEAC assay), 18.84% (HAPX assay);[57]	DPPH assay for antioxidation, *B. cereus*, *M. flavus*, *S. aureus*, *E. faecalis*, *E. coli*, *P. aeruginosa*, *S. typhimurium*, *L. monocytogenes*, *A. fumigatus*, *A. niger*, *A. versicolor*. *A. ochraceus*, *P. funiculosum*, *P. ochrochloron*, *T. viride* tested for antimicrobial activity;[56] DPPH,	GC-MS[56,59]; HPLC-MS[57]

	chloroform,water, ethyl acetate;[58] essential oil, linalool, eugenol[59] acetone and ethanol extracts[60]	IC50 = 8.17-24.91 µg/mL (neutralization of DPPH radical), 6.92-25.45 µg/mL (neutralization of NO radical), 10.61-17.21 µg/mL (neutralization of superoxide radical), 17.93-71.42 µg/mL (neutralization of hydrogen peroxide radical);[58] MIC = 60-100 µg/0.1 mL (acetone extract), 20-60 µg/0.1 mL (ethanol extract)[60]	Trolox equivalent antioxidant capacity (TEAC) ,hemoglobin ascorbate peroxidase activity inhibition (HAPX) and electron paramagnetic resonance (EPR);[57]assay for neutralization of DPPH, NO, superoxide and hydrogen peroxide radicals;[58], *E. coli, E. aerogenes,E. faecalis, L. monocytogenes, P. aeruginosa, S. enterica, S. typhimurium,S. dysenteriae, S. aureus*[59] *E. coli, K. pneumonia, S. aureus, P. aeruginosa and Proteus sp*.[60]	
Antimalarial[61]	Leaf essential oil	IC_{50} = 21.0 ± 4.6 µg/mL	*Plasmodium falciparum*	GC-MS
Anticancer[62,63]	Plant methanolic extract;[62] petroleum ether soluble and insoluble methanolic extracts, ursolic acid	Dose = 20-320 µg/mL;[62] Ursolic acid, LC_{50}= 18.6 µg/mL	Cytotoxic activity against MCF-7 cells;[62] Sulforhodamine B assay using HT-144, MCF-7, NCI-H460, SF-268 cell lines, immuno-fluorescence microscopy for studying effect on cytoskeleton and nuclei of MCF-7 cells	Nil
Larvicidal activity[64,65]	Leaf essential oil[64]	LC_{50}= 9.75-14.1 ppm;[64] LC_{50} = 3.734% (first instar larvae),4.154% (second instar larvae), 4.664% (third instar larvae), 5.124% (fourth instar larvae), 5.449% (pupae)[65]	*Culex tritaeniorhynchus, Aedes albopictus* and *Anopheles subpictus*;[64] *Aedes aegypti*[65]	GC–MS[64]
Antituberculosis[66]	Methanolic extract of leaves, fruits and flowers; bacilicin	Dose = 6.25 µg/mL, inhibition = 8-49%	Microplate Alamar Blue Assay (MABA)	
Preventing ischemia, reperfusion-induced cerebral damage and motor dysfunctions[67]	Ethyl-acetate extract of leaves	100 and 200 mg/kg p.o.	Swiss albino mice	Nil
Antihypertensive effects[68]	Aqueous plant extract	100, 200 and 400 mg/kg per day orally	Two kidney one clip Goldblatt model for renovascular hypertension in Wistar rats	Nil

	Vasorelaxant and anti-platelet aggregation[69]	Aqueous plant extract	0.5 g/kg body weight for 10 weeks	Female wistar rats	HPLC
	Antigiardial activity[70]	Plant essential oil, linalool, eugenol	Dose = 2 mg/mL (essential oil), 300 µg/mL (linalool), 850 µg/mL (eugenol)	*Giardia lamblia*	GC-MS
	Antiviral[71]	Aqueous and ethanolic plant extracts, apigenin, linalool, ursolic acid	Ursolic Acid, EC50 = 6.6, 4.2, 0.4, 0.5 mg/L (against HSV-1, ADV-8, CVB-1 and EV-71 resp.); Apigenin, EC50 = 9.7, 11.1, 7.1, 12.8 mg/L (against HSV-2, ADV-3, Hepatitis B surface antigen, Hepatitis B 'E' antigen resp.); Linalool, EC50 = 16.9 mg/L (against ADV-II)	Herpes viruses (HSV), Adenoviruses (ADV), Hepatitis B virus, coxsackievirus B1 (CVB1) and Enterovirus 71 (EV71)	Nil
Og	Protection of liver from oxidative stress and fibrosis[72]	Polyphenol extract	Dose = 0 to 12 mg/kg body weight for 8 weeks	CCl_4-induced liver fibrosis in wistar rats	HPLC
	Antioxidant and Antimutagenic[73]	Leaf aqueous extract	Antioxidant activity, IC_{50} = 83.0 µg/mL	DPPH assay for antioxidant activity; antimutagenic activity evaluated using *Salmonella typhimurium* (TA98 and TA100) strains using the Salmonella/microsome test	
	Antitrypanosomal and antiplasmodial[74]	Crude ethanol extract, essential oil and pure compounds	IC50 (antitrypanosomal activity) = 1.29 to >100 µg/mL IC50 (antiplasmodial activity) = 41.92-76.92 µg/mL	*Trypanosoma bruceibrucei,Plasmodium falciparum*	GC-MS
	Free radical scavenging and antioxidant[75]	Aqueous extract, methanol extract and eugenol	EC_{50} = 242.47-254.33 µg/mL (DPPH assay); 10.47-46.33 µg/mL (hydroxyl radical scavenging activity); 14.17-37.88 µg/mL (nitric oxide scavenging activity) and 50.92-92.26 µg/mL (antioxidant activity).	DPPH assay, hydroxyl radical and nitric oxide scavenging assay, ferric thiocyanate (FTC) method, reducing power determination	HPLC, FTIR, NMR

Prevention against Liver Fibrosis[76]	Aqueous leaf extract	doses = 0 to 40 mg/kg body weight) for 8 weeks.	CCl_4-induced liver fibrosis in wistar rats	Nil
Antimicrobial[77,78,79]	Plant essential oil;[77,78] eugenol, methyl eugenol;[77] hexane and methanol extracts alone and in combination with aminoglycosides[79]	MIC=0.18-3.75 mg/mL;[77] dose, essential oil = 10, 50 , 100 mg/mL;[78]	*S. aureus*, *S. epidermidis*, *S. faecalis*, *M. flavus*, *M. luteus*, *B. subtilis*, *E. coli*, *K. pneumonia*, *S. marcescens*, *P. vulgaris*, *P. mirabilis*, *P. aeruginosa*, *S. typhimurium*, *E. aerogenes*, *A. niger*, *A. fumigatus*, *P. chrysogenum*;[77] *B. cereus*, *S. flexineri*,*C. albicans*[78]; *E. coli* and *S. aureus* (clinical and standard strains)[79]	GC-MS
Corrosion Inhibition[80]	Seed extract	Dose, extract = 4% – 10% (v/v)	Gravimetric methods	Nil
Cerebroprotection[81]	Ethanolic plant extract	150 or 300 mg/kg body weight p.o.	Male wistar rats	HPLC

*Ok (*O. kilimandscharicum*), Olb (*O. labiatum*), Ola (*O. lamiifolium*), Oo (*O. Obovatum*), Ot (*O. tenuiflorum*), Oa (*O. americanum*), Oba (*O. basilicum*), Og (*O. gratissimum*), GI50 (growth inhibition at 50%), IC50 (half maximum inhibitiory concentration), EC50 (half maximal effective concentration), LC50 (median lethal concentration), p.o. (oral administration), MIC (minimum inhibitory concentration), GC-MS (gas chromatography – mass spectrometry), DCM (dichlorormethane), TI (therapeutic index), HPLC (High performance liquid chromatography), HPTLC (High performance thin layer chromatography), FTIR (Fourier transform infrared spectroscopy), NMR (Nuclear magnetic resonance), UPLC (Ultra performance liquid chromatography)

Table 2: Species- wise distribution of terpenes and phenylpropanoids in *Ocimum*

spp.	Chemical composition		
	Monoterpene (%)	**Sesquiterpene (%)**	**Phenylpropanoids (%)**
Ot[83]	Camphene (0.79), borneol (2.74)	Germacrene (4.9), β-elemene (1.59), β-caryophyllene (8.7), farnesol (4.19), fanesene (11.27)	Methyl eugenol (62.29), eugenol (2.79).
Os[84]	Nil	β- Caryophyllene (2.04), germacrene- D (1.3), bicyclogermacrene (1.2), pathulenol (1.30).	Methyl chavicol (93.2), methyl eugenol (0.6)
Og[82]	Sabinene (0.31), myrcene (0.30), (Z)-ocimene (11.88), (E)-ocimene (0.77), trans-4-thujanol (0.44), terpinen-4-ol (0.44).	Copaene (0.29), bourbonene (0.43), (E)-caryophyllene (0.88), germacrene-D (2.23), cadinene (0.24), caryophyllene oxide (0.21).	Eugenol (82)
Ok [85]	α-Pinene (1.23), camphene (7.32), β -myrcene (1.58), L-phellandrene (0.26), α-terpinene (0.33), p-cymene (0.62), limonene (13.56), 1,8-cineole (0.85), β-ocimene (2.00), γ-terpinene (0.88), trans-sabinene hydrate (0.49), α-terpinolene (1.33), linalool (1.7), cis-sabinene hydrate (0.47), camphor (56.07), 4-terpineol (3.5), myrtenol (1.24).	Trans-caryophyllene (0.33), germarcrene D (0.43)	Nil
Om[86]	α-Thujene (0.002), α-pinene (0.004), sabinene (0.01), β-pinene (0.003), myrcene (0.013), α-terpinene (0.003), limonene (0.002), β-phellandrene (0.003), eucalyptol (0.01), trans-β-ocimene (0.006), γ-terpinene (0.13), cis-linalool oxide (0.01), trans-linalool oxide (0.03), neo-allo-ocimene (0.013), plinol (0.022), terpinen-4-ol (2.352), α-terpineol (0.022), *n*-octyl acetate (0.007), nerol (0.034), linalyl acetate (0.194), geraniol (0.07), geranyl acetate	α-Copaene (0.028), α-cubebene (0.12)	Eugenol (0.126), chavicol (0.224).

	(69.48), bornyl acetate (0.095), myrtenyl formate (0.03), carvacrol (0.043), exo-2-hydroxycineole acetate (0.018)		
Oba[87]	α-Pinene (1.9), sabinene (1.9), β-pinene (3.3), β-myrcene (3.0), D-limonene (1.6), 1,8-cineole (22.6), β-phellandrene (0.1), β-cis-Ocimene (0.5), β-linalool (47.6), camphor (0.8), α-terpineol (1.2)	α-Ylangene (0.76), β-cubebene (0.16), α-cis-bergamotene (0.76), α-trans-bergamotene (4.8), cis-muurola- 4(14), 5-diene (2)	Eugenol (0.9)
Oa[88]	Linalyl alcohol (2.03), β-citral (36.58), citral (47.18), nerol acetate (0.32)	Caryophyllene (1.05), α-bergamotene (0.94), humulene (0.51), germacrene D (1.4), α-selinene (0.37), bicyclogermacrene (0.92), β-elemene (0.24), α-bulnesene (0.46), cis-α-bisabolene (2.67), (-)-spathulenol (0.31), β-selinenol (0.19).	Nil
Oci×*Cit*[89]	Nerol (5.04), neral (33.0), geraniol (1.6), geranial (43.0)	Nil	Nil
Ola[90]	α-Thujene (0.3), α-pinene (0.6), sabinene (33.8), β-pinene (2.2), myrcene (2.8), α-phellandrene (0.5), α-terpinene (2.0), p-cymene (2.2), β-phellandrene (4.0), limonene (1.0), (Z)-β-ocimene (17.2), (E)-β-ocimene (3.0), γ-terpinene (1.6), terpinolene (1.9), linalool (2.0), *cis*-p-menth-2-en-1-ol (0.5), *trans-p*-menth-2-en-1-ol (0.3), terpinen-4-ol (8.4), p-cymen-8-ol (0.8), α-terpineol (0.2)	α-copaene (0.2), β-bourbonene (0.3), β-caryophyllene (5.6), α-humulene (0.1), (E)-β-farnesene (1.0), germacrene-D (1.1), γ-cadinene (0.1), δ-cadinene (0.2)	Nil

Oc[91]	Camphene (0.4), α-pinene (0.2), sabinene (0.1), β-pinene (0.8), limonene (0.3), β-myrcene (0.2), 1.8-cineole (3.3), linalool (2.9), borneol (0.5), α-terpineol (0.3)	β-Bourbonene (9.5), α-copaene (1.9), *trans*-β-caryophyllene (7.8), α-guaiene (5.6), *cis*-β-farnesene (0.5), sesquisabinene (0.2), α-humulene (2.8), γ-muurolene (0.3), germacrene D (10.1), β-selinene (1.4), bicyclogermacrene (3.4), α-bulnesene (7.1), γ-cadinene (0.5), δ-cadinene (2.0), β-sesquiphellandrene (0.4), caryophyllene oxide (0.4), spathulenol (0.4), epi-α-muurolol (2.0), T-muurolol (0.7), 11- selinen-4-α-ol (1.1).	Eugenol (9.0), methyl eugenol (12)
Omi[92]	R-Pinene (0.24), camphene (0.07), α-pinene (0.75), myrcene (0.26), 1,8-cineole, (5.35), cis-ocimene (2.69), trans-ocimene (0.35), linalool (1.49), allo-ocimene (2.42), borneol (0.14), mentha1,5-dien-8-ol (0.33), R-terpineol (0.45), neral (0.06).	Elemene isomer (0.63), α-elemene (4.17), elemene isomer (0.63), α-elemene (9.06), α-caryophyllene (11.94), R-bergamotene (0.13), R-humulene (2.4), germacrene (0.13), α-selinene (0.86), bicyclogermacrene (2.9), spathuleno (1.15), caryophyllene oxide (1.23).	Eugenol (46.55)
Oca[93]	R-Pinene (0.2), β-pinene (0.32), β-myrcene (0.18), 1,8-cineole (4.98), γ -terpinene (0.21), linalool (26.37), camphor (0.41), borneol (0.17), trans-β-terpineol (0.17), terpinen-4-ol (1.64), endo fenchyl acetate (0.21), bornyl acetate (0.64).	β- Elemene (0.29), trans-caryophyllene (0.5), trans-α-bergamotene (1.7), germacrene (0.52), bicyclogermacrene (0.6), γ -cadinene (0.38), cedrol (0.22), cadinol (1.18).	Methylchavicol (52.71), eugenol (2.6)

***** Ot (*O. tenuiflorum*), Os (*O. selloi)*, Og (*O. gratissimum)*, Ok (*O. kilimandscharicum*), Om (*O. minimum*), Oba (*O. basilicum)*, Oa (O. *americanum*), Oci×cit (*Ocimum× citriodorum*), Ola (*O. lamiifolium*), Oc (*O. campechianum*), Omi (*O. micranthum,* Oca (*O. canum)* (Parenthesis values indicate relative percentage of respective metabolite)

Table 3: Genes involved in biosynthesis of major secondary metabolites in *Ocimum* species

Compound	Gene	Reaction catalysed	Method of characterization
Eugenol	Eugenol synthase (EGS) (*Ocimum basilicum*)[101]	Coniferyl acetate to eugenol	Three dimensional structure determination *viz.* protein x-ray crystallography and *in vitro* mutagenesis studies suggest that reaction proceeds via formation of quinone-methide intermediate followed by reduction; involving conserved residue Lys-132.
	Eugenol synthase (EGS) (*Fragaria ananassa*)[102]		Cloning, functional characterization and expression of FaEGS1a and FaEGS1b (catalysing formation of eugenol); and FaEGS2 (catalysing formation of eugenol and also isoeugenol with lower catalytic efficiency)
	Coumaryl CoA Ligase (4CL) (*Ocimum tenuiflorum*)[103]	Hydroxycinnamic acids to coenzyme A (CoA) esters	Transient silencing of 4CL gene leads to reduction in eugenol accumulation, however, lignin and sinapic acid content remained unaffected, indicating involvement of a specific isoform of 4CL in eugenol biosynthesis which is different from those involved in lignin biosynthesis.
	R2R3-MYB transcription factor (EOBII) (*Fragaria ananassa*)[104]	Transcription factor regulating structural genes in phenylpropanoid pathway	Identification and functional characterization of FaEOBII in strawberry fruit receptacles, responsible for regulating eugenol biosynthesis by interaction with FaMYB10
Eugenol methyl ether	Eugenol O-methyl transferase (EOMT) (*Ocimum basilicum*)[105, 106]	Eugenol to eugenol methyl ether	Recombinant protein expression and characterization in *E. coli*, molecular modelling based on crystal structure of IOMT and site directed mutagenesis suggested single amino acid difference being responsible for substrate specificity in EOMT and CVOMT
Methyl chavicol	Chavicol O-methyl transferase (CVOMT) (*Ocimum basilicum*)[105,106]	Chavicol to methyl chavicol	Linking O-methyltransferase activity with developemental timing and chemotype of *O. basilicum* through enzyme assays

Camphor	bornyl diphosphate synthase (BPPS) (S*alvia officinalis*)[107-110]	Geranyl diphosphate to bornyl diphosphate	Partial purification and characterization of BPPS from soluble enzyme preparations of young leaves; demonstration of GPP as preferred substrate for cyclization
			Characterization and functional expression of recombinant BPPS
			X-ray crystal structure determination using Multiwave anomolous dispersion (MAD) to 2.0Å resolution, modelling with substrates, intermediates and mechanistic implications on terpene cyclization
			Molecular dynamics and multidynamic free energy simulations reveal bornyl cation to be an important enzyme induced bifurcation point; electrostatic steering by diphosphate moiety in active site guides the formation of primary product (BPP)
	Borneol dehydrogenase (BDH) (*Salvia officinalis*)[111]	Borneol to camphor	Partial purification and characterization of BDH from soluble enzyme extracts prepared using young leaves
	Borneol dehydrogenase (BDH) (*Lavandula intermedia*)[112]		Cloning, functional characterization and determination of tissue-specific expression of LiBDH
Eucalyptol (1,8- cineole)	1,8-cineole synthetase (S*alvia officinalis*)[113]	Neryl diphosphate to 1,8-cineole	Partial purification and characterization of cineole synthetase from soluble enzyme extracts prepared using young leaves
Linalool	Linalool synthase (LIS) (*O. basilicum*)[100]	GPP to linalool	Cloning and expression of full length cDNA in *E.coli* followed by characterization via enzyme assays
Terpinolene	Terpinolene synthase (TES) (*O. basilicum*) [100]	GPP to terpinolene (as major product) and α-pinene and limonene (as side products)	Cloning and expression of full length cDNA in *E.coli* followed by characterization via enzyme assays
Fenchol	Fenchol synthase (FES) (*O. basilicum*) [100]	GPP to fenchol (as major product) and α-pinene and limonene (as side products)	Cloning and expression of full length cDNA in *E.coli* followed by characterization via enzyme assays
Myrcene	Myrcene synthase (MES) (*O. basilicum*) [100]	GPP to myrcene	Cloning and expression of full length cDNA in *E.coli* followed by characterization via enzyme assays

Cadinene	Cadinene synthase (CDS) (*O. basilicum*) [100]	FPP to γ-cadinene (as major product) and Muurola 3,5-diene (as side product)	Cloning and expression of full length cDNA in *E.coli* followed by characterization via enzyme assays
Selinene	Selinene synthase (SES) (*O. basilicum*) [100]	FPP to α & β- selinene (as major product) and β-elemene and nerolidol (as side product)	Cloning and expression of full length cDNA in *E.coli* followed by characterization via enzyme assays
Zingiberene	Zingiberene synthase (ZIS) (*O. basilicum*) [100]	FPP to α-zingiberene (as major product) and α-bergamotene, nerolidol, β-farnesene and β-bisabolene (as side product)	Cloning and expression of full length cDNA in *E.coli* followed by characterization via enzyme assays
Germacrene-D	Germacrene D synthase (GDS) (*O. basilicum*) [100]	FPP to Germacrene D	Cloning and expression of full length cDNA in *E.coli* followed by characterization via enzyme assays
Geraniol	Geraniol synthase (GES) (*O. basilicum*)[114]	GPP to geraniol	Cloning, expression and functional charaterization of the enzyme followed by RNA gel- blot analysis revealing exclusive expression of GES in trichomes and not in leaves
Amyrin (triterpene)	2,3-oxidosqualene cyclase (AS1 and AS2) (*O. basilicum*)[115]	2,3-Epoxy-2,3-dihydrosqualene to α/β-amyrin	Cloning and expression of ObAS1 and ObAS2 in *Saccharomyces cerevisiae* strain BY4741 under GAL1 promoter; ObAS1 was identified as α-amyrin synthase, while ObAS2 produced both α- and β- amyrins
General phenyl propanoid pathway	Production of anthocyanin pigment 1 (PAP1) (*A. thaliana*)[116]	Transcriptional regulator of floral scent	Introduction of PAP1 transcription factor from *A. thaliana* into *Rosa hybrida* (Rose) altered the colour and scent profile of transgenic plants resulting from an increase in metabolic flux through terpenoid and phenylpropanoid pathways
	p-coumaroyl shikimate 3'-hydroxylase (CS3'H) (*O. tenuiflorum*)[14]	p-Coumaroyl 5-O-shikimate to caffeoyl 5-O-shikimate	*de novo* sequencing of transcriptome

	Caffeic acid O-methyl transferase (COMT) (*O. basilicum*)[14]	Caffeate to ferrulate	*de novo* sequencing of transcriptome
	Caffeic acid O-methyl transferase (COMT) (*O. tenuiflorum*)[14]	Caffeate to ferrulate	*de novo* sequencing of transcriptome
	Cinnamyl alcohol dehydrogenase (CAD) (*O. tenuiflorum*)[14]	Cinnamyl alcohol to cinnamyldehyde	*de novo* sequencing of transcriptome
	Cinnamyl alcohol dehydrogenase (CAD) (*O. basilicum*)[14]	Cinnamyl alcohol to cinnamyldehyde	*de novo* sequencing of transcriptome
	Cinnamate-4-hydroxylase (C4H) (*O. tenuiflorum*)[14]	Cinnamic acid to 4-coumaric acid	*de novo* sequencing of transcriptome
	Cinnamate-4-hydroxylase (C4H) (*O. basilicum*)[14]	Cinnamic acid to 4-coumaric acid	*de novo* sequencing of transcriptome
Flavonoid pathway	Chalcone synthase (CHS) (*O. tenuiflorum*)[117]	Conversion of 4-coumaroyl-CoA and malonyl-CoA to naringenin chalcone	*de novo* sequencing of transcriptome
	Flavone 8-hydroxylase (F8H) (*O. basilicum*)[118, 119]	Hydroxylation of salvigenin	Cloning and expression of recombinant protein ObF8H-1 in yeast and followed by characterization via enzyme assays
	2-Oxoglutarate-dependent flavone demethylase (*O. basilicum*)[120]	Accumulation of 7-O-demethylated flavone nevadensin	Enzyme assays using trichome protein extracts
	Flavonoid O-methyltransferase (FOMT) (*Ocimum basilicum*)[121]	6- and 4'-O-methylation of flavones	Cloning and expression of full length cDNA in *E. coli* followed by characterization via enzyme assays

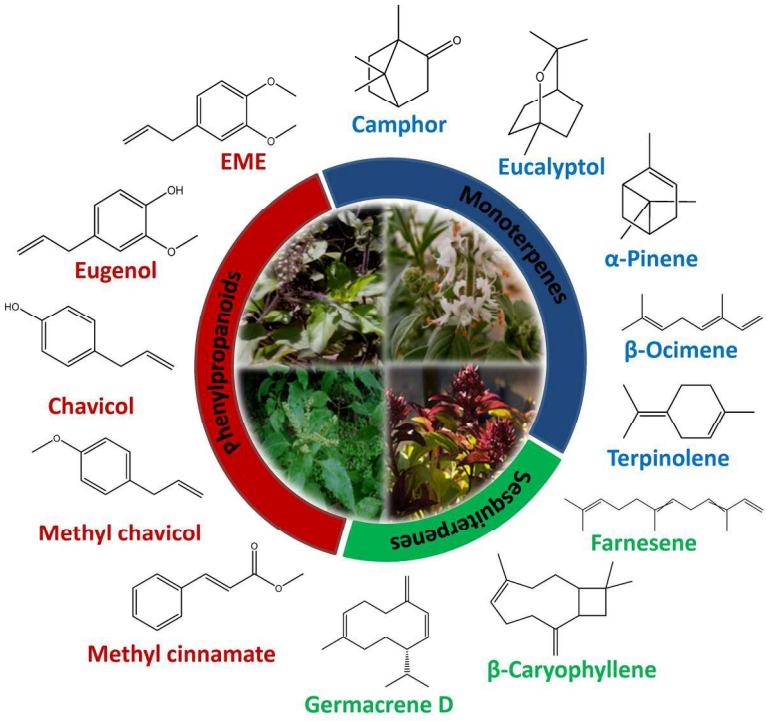
Figure legends

Figure 1: Representative examples of structurally diverse classes of secondary metabolites *viz*. monoterpenes, sesquiterpenes and phenylpropanoids found across genus *Ocimum*

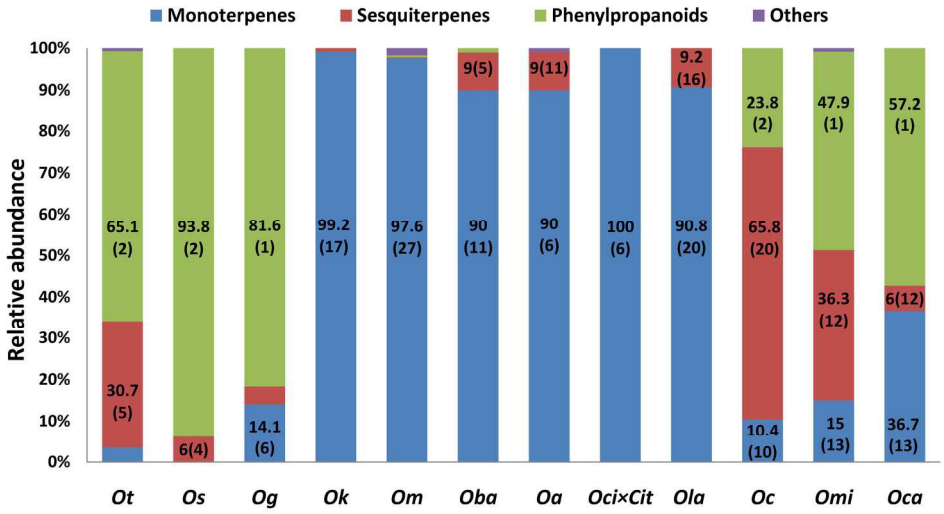
Figure 2: Overview of diversity across *Ocimum* species. Numbers in parenthesis indicates number of compounds; numbers outside parenthesis indicate percentage of metabolite in total volatile fraction. (*Ot*, *O. tenuiflorum*; *Os*, *O. selloi*; Og, *O. gratissimum*; *Ok*, *O. kilimandscharicum*; *Om*, *O. minimum*; *Oba*, *O.basilicum*; *Oa*, *O. americanum*; *Oci x Cit*, *Ocimum x Citriodorum*; *Ola*, *O. lamiifolium*; *Oc*, *O. campechianum*; *Omi*, *O. micranthum*; *Oca*, *O. canum*)

Figure 3: Factors responsible for chemical diversity; and terpenoid and phenylpropanoid pathway diversification in *Ocimum* species

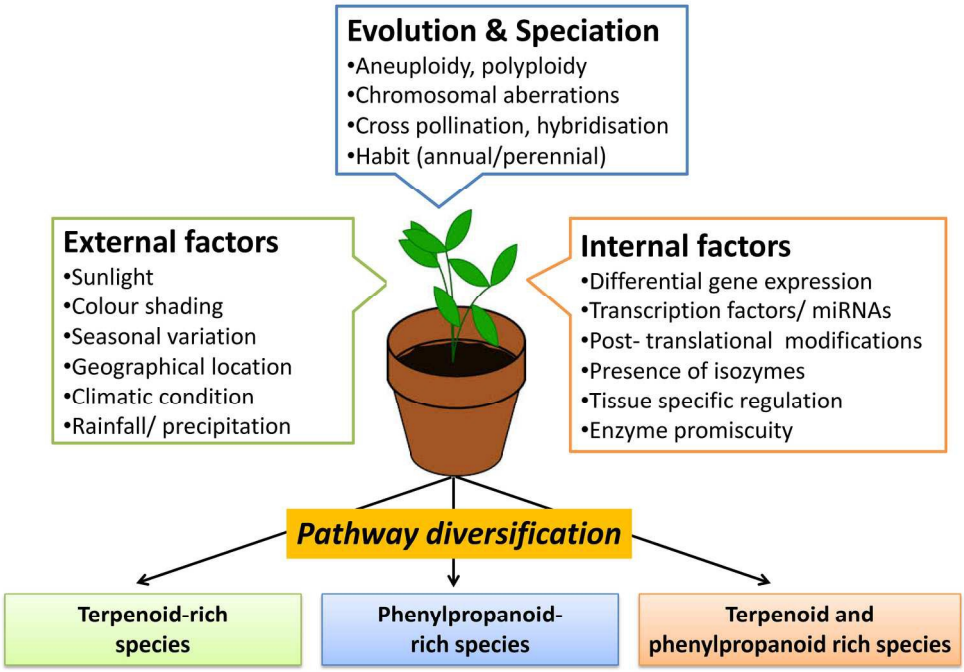
Figure 4: Major regulatory checkpoints in phenylpropanoid and terpenoid pathways. Enzymes potentially governing the direction of flux have been marked in red. (PEP, Phosphoenol pyruvate; G3P, Glyceraldehyde-3-phosphate; DXP, 1-deoxy-D-xylulose 5-phosphate; MEP, Methylerythritol phosphate; MVA, Mevalonic acid; IPP, Isopentenyl pyrophosphate; DMAPP, Dimethylallyl pyrophosphate; GPP, General phenylpropanoid pathway; DAHPS, 3-Deoxy-D-arabinoheptulosonate 7-phosphate synthase; PAL, Phenylalanine ammonia-lyase; C4H, Cinnamate-4-hydroxylase; 4CL, 4-Coumarate-CoA ligase; DXS, 1-deoxy-D-xylulose 5-phosphate synthase; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; MTPS, Monoterpene synthases; STPS, Sesquiterpene synthases; DTPS, diterpene synthases; TTPS; Triterpene synthases)



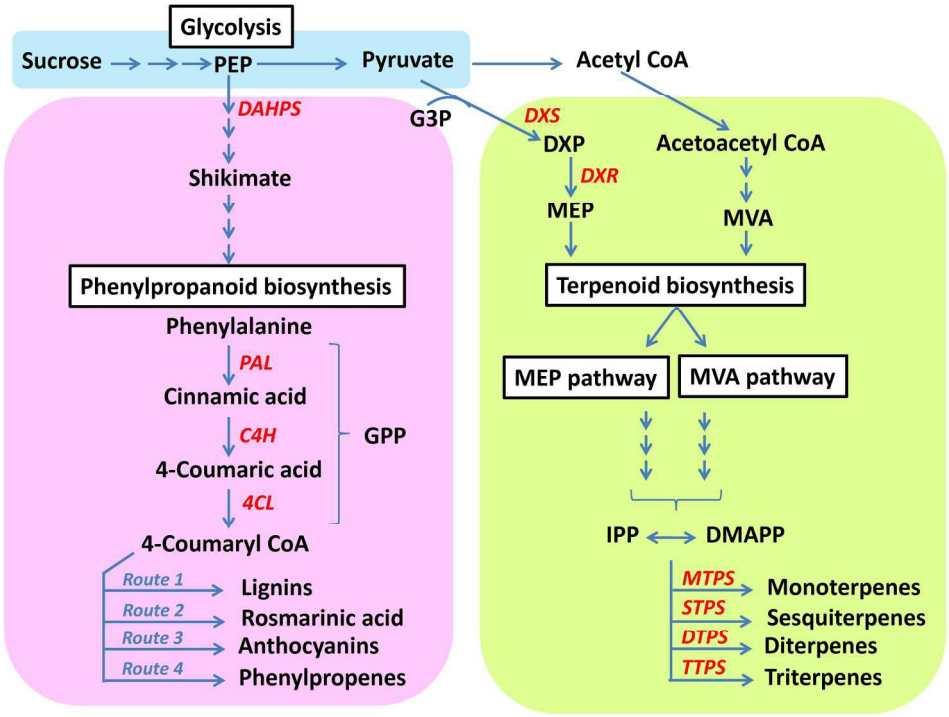
190x142mm (300 x 300 DPI)



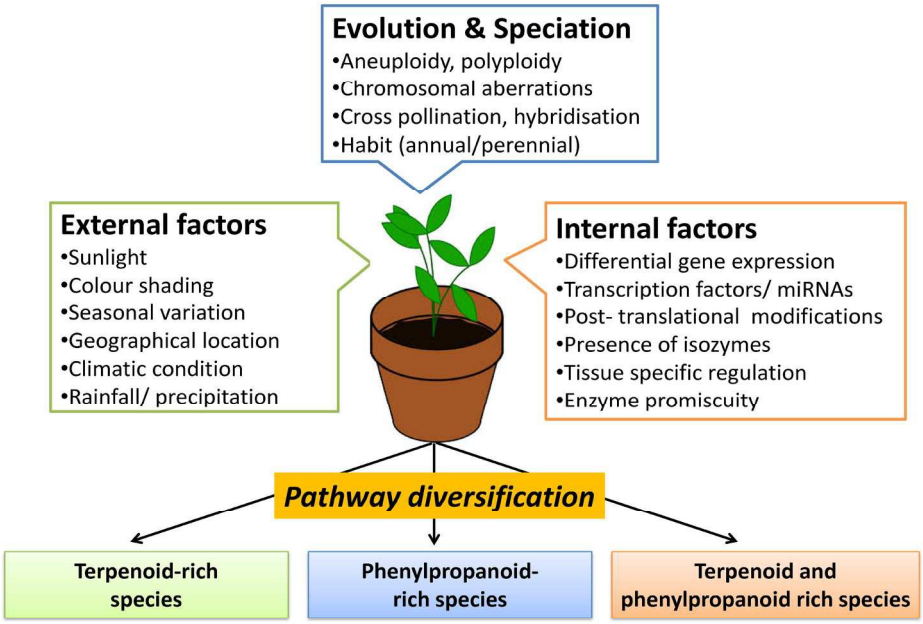
190x142mm (300 x 300 DPI)



190x142mm (300 x 300 DPI)



190x142mm (300 x 300 DPI)



Textual abstract: Evolution of chemical diversity and diversification of terpene and phenylpropanoid pathway in *Ocimum* species

190x142mm (300 x 300 DPI)