Molecular markers assisted characterization of the genus *Ocimum*

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❖ **Abstract** :

The *Ocimum* spp. commonly known as Basil, the most popular economically important herb or shrub is known for its immense value credited to its biological activity, medicinal properties, and higher safety than synthetic medication. The existence of morphological diversity, variability in biochemical constituent composition within the genus *Ocimum,* its taxonomic classification as well as the phylogenetic relationship, is still questionable. Differentiation of most taxa is based on the leaf morphology and colour, which frequently depends on environmental conditions leading to ambiguity in the classification. The chemical constituents of Basil are highly complex, containing an enormous type of biologically active phytochemicals with variable proportions among varieties or even plants within the same field. Therefore, the accurate characterization of *Ocimum* and the determination of genetic variation among different species of *Ocimum* are important. For the characterization of *Ocimum*, DNA-based molecular markers (RADP, AFLP, ISSR, etc.) have been used profusely since it is a very sensitive and effective technology. The discovery of Polymerase Chain Reaction (PCR) made the innovation of DNA-based markers easier. Among these techniques, Random Amplified Polymorphic DNA (RAPD) has been broadly used for the detection of intraspecific and interspecific genetic diversity as this approach requires no previous knowledge of the genome that is being analyzed. This review work is an attempt to assess the status of metabolite profiling and molecular markers assisted characterization of genus *Ocimum.*

❖ **Keywords :**
Ocimum, DNA, Biological, Molecular, RAPD Markers, Diversity.

Some of the traditional herbs known for aromatic, culinary, medicinal, ornamental, and many other aesthetic properties in the world belong to the family Lamiaceae. The genus *Ocimum* belongs to the subfamily Ocimoideae or Nepetoideae of the Lamiaceae family and contains over 65 different species and varieties. (Rajni et al., 2016). The name 'Tulsi' is obtained from the ‘Sanskrit' word which means ‘Matchless one' (Ghosh 1995, Simpson and Conner 1986). These plants are highly variable and hold a wide range of genetic diversity at intra and interspecies levels. Nine species of *Ocimum* viz. *O. sanctum* L, *O. basilicum* L, *O. gratissimum* L, *O. kilimandscharicum* Gürke*, O. micranthum* L*, O. americanum* L, *O. citriodorum* L, *O. campechianum Miller (Mill.), O. minimum* L are found in India, three of which (*O. americanum* L, *O. citriodorum L, O. minimum* L) are exotic (Upadhyay et al., 2015).Many bioactive molecules have been found in Ocimum sp. The amount of substances produced depends on the nature of the soil, harvesting processing, and storage. Some of the important essential oil constituents reported from *Ocimum* species include linalool, linalyl acetate, geraniol, citral, camphor, eugenol, methyl eugenol, methyl chavicol, methyl cinnamate, thymol, etc., which are of immense value in the perfumery and cosmetic industries (Balyan and Pushpan-

gadan 1988) and also shown to have antibacterial activity. While research on commercial natural products has advanced, the herbal drug industry faces significant issues of adulteration and misidentification of plants (Sarwat et al., 2012). It is sometimes noticed that the wild *Ocimum sanctum* has been adulterated with its morphologically similar relative *Vitex negundo*. The second plant has the same morphological characters such as the colour of the flower, size, and shape of leaves as the first one (Prakash et al., 2013). The key reasons for drug replacement are lack of knowledge of genuine sources, the similarity in morphological characters, negligent collection by herbal collectors and suppliers, lack of availability of indigenous drugs, and sometimes high manufacturing costs for these drugs (Sarwat et al., 2012).Research shows that high levels of both morphological and chemical variability exist within the *Ocimum* sp due to interspecific hybridization, polyploidy, and the existence of chemotypes or chemical races that do not differ notably in morphology (Simon et al., 1990). Hence it is important to characterize the genus at a molecular level for solving the taxonomic problem. Molecular or DNA-based markers are extensively used and it is advantageous over other conventional methods. The primary advantages of using molecular markers are (1) availability of a huge number of markers (2) generally unchanged by environmental influences (Sarwat et al., 2016). The capability of a molecular marker depends upon its ability to detect the total amount of polymorphism in the samples being analysed (Leela et al., 2009). Restriction fragment length polymorphism (RFLPs) is one of the preliminary molecular marker approaches utilized for identifying variations in a DNA sequence by restriction digestion of DNA with the help of restriction enzymes. Later many other methods are developed. A more modern way of detecting individual variations in DNA nucleotides is with the help of Single Nucleotide Polymorphism (SNPs).

Description of the molecular markers (DNA based marker)

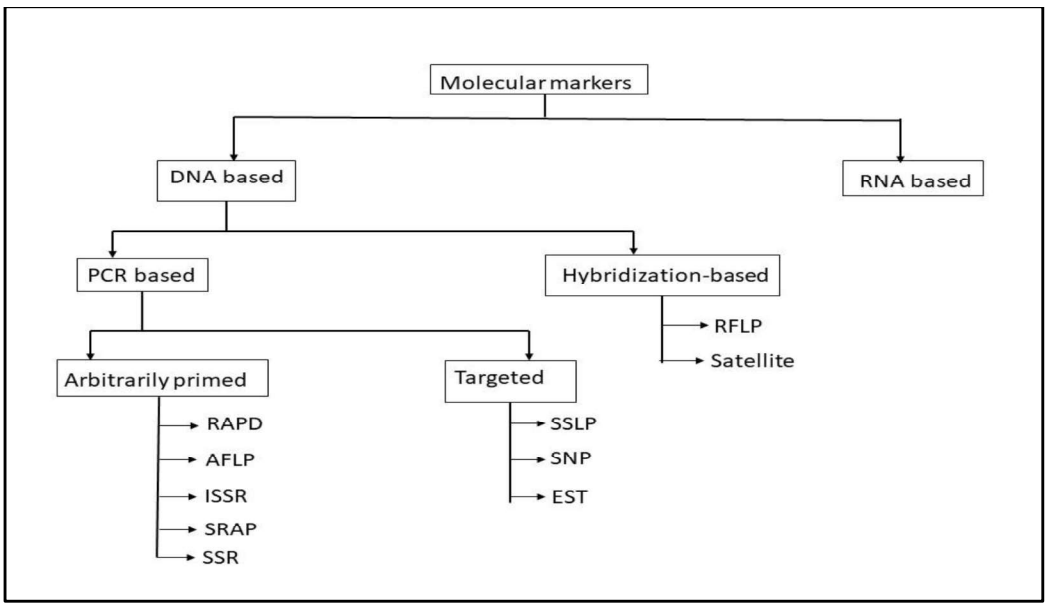

Fig 1: Classification of Molecular markers.

DNA marker can be elucidated as a particular segment of DNA that is representative of the differences at the genome level. DNA markers should not be treated as regular genes; instead, they should be viewed as constant genome landmarks. Various types of DNA markers are present and based on their method of detection. They can be broadly classified into two classes (fig 1), one is hybridization-based (such as RFLP) and another one is PCR-based (such as RAPD). DNA profiles can be seen by hybridizing with restriction enzyme processed DNA fragments of known sequence to a labelled probe. PCR-based markers include amplification of particular DNA sequences using randomly chosen nucleotide sequences known as primers and a DNA polymerase enzyme. These amplified fragments are separated using agarose gel electrophoresis and the banding patterns are identified by different techniques such as staining and autoradiography (Sarwat et al., 2011). The DNA markers may also be described as dominant or codominant. This category is based on whether these markers can distinguish between homozygous and heterozygotes. Codominant markers specify size variation where dominant markers are analysed by their presence or absence. An ideal DNA marker should have certain criteria like:

i. Genome specific
ii. Evenly distributed in the whole genome
iii. Can differentiate between homozygotes and heterozygotes
iv. High level of polymorphism
v. Free of environmental factors
vi. Reproducible

SL No.	Type	Example
a)	The first-generation molecular markers	e.g., RFLPs, RAPDs, and their modifications
b)	The second-generation molecular markers	e.g., AFLPs and their modified forms
c)	The third-generation molecular markers	e.g., ESTs

Table 1: Based on the development of these approaches in the last three decades, molecular markers have been classified into three classes (adopted from Gupta et al., 2001)

Types : These are some molecular markers and their modified forms:

1. RFLP (Restriction fragment length polymorphism)
2. RAPD (Random amplification of polymorphic DNA)
3. AFLP (Amplified fragment length polymorphism)
4. ISSR (Inter Simple Sequence Repeats)
5. SSR (Simple Sequence Repeats) or STMS (Sequence Tagged Microsatellite Markers)
6. SNP (Single nucleotide polymorphism)
7. SRAP (Sequence-related amplified polymorphism)
8. SSLP (Simple Sequence Length Polymorphisms)
9. EST (Expressed Sequence Tag)

Based on the development of these approaches in the last three decades, they have been classified into three classes (Gupta et al., 2001) shown in table 1. Different molecular markers can be used as detection systems for genetic variations by using genomic DNA for genetic analysis, among them the RAPD approach is one of the earliest modifications of PCR used for genome scanning (Welsh and McClelland, 1990; Williams et al., 1990). The procedure used in RAPD is shown in fig 2.

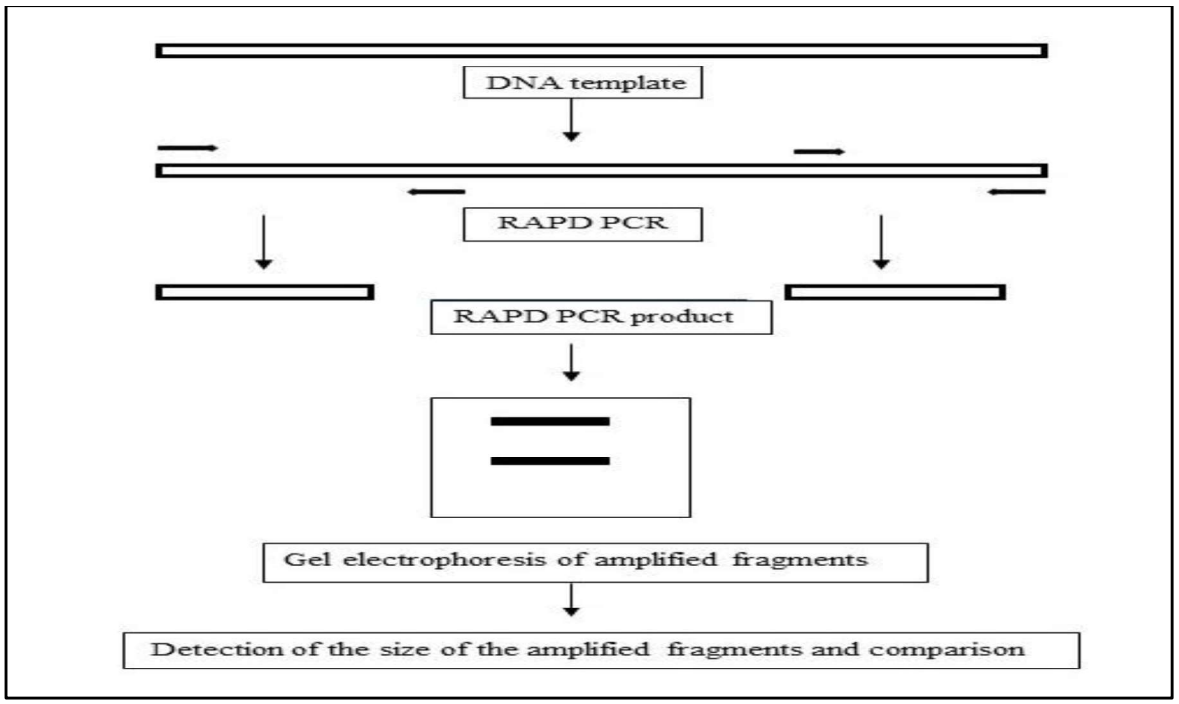

Fig 2: Steps involved in RAPD mapping: The DNA templates are used for PCR with RAPD random primers.

Molecular marker-assisted characterization of the genus Ocimum

Molecular or DNA-based markers have been utilized extensively for studying genetic relationships in different species of *Ocimum*. Interspecific hybridization and polyploidy are the major events (Harley et al., 1992) that occurred within the genus *Ocimum* and created taxonomic confusion. PCR based techniques such as RAPD (Williams et al., 1990), provide an excellent way to produce numerous molecular markers that can be used to identify genetic relationship and to allow revaluation of the classification. RAPD markers have been utilized effectively in separating chemotypes of *O. gratissimum* (Vieira et al., 2001). Vieira et al., (2011) pointed out that volatile oils and flavonoids could differentiate the accessions of *O. gratissimum* in a similar manner to the molecular markers. Molecular markers have been found to be correlated with the presence of certain volatile oil. Eugeno, geraniol, and thymol were the primary essential oils, while xantomicrol and cirsimaritin were the most important flavones found in *Ocimum gratissimum* (Singh et al., 2003). A chemotype having primary essential oil and flavone (geraniol, xanthomicrol, isothymusin, cirsimaritin and luteolin) was found when analyzed by RAPD markers in a genetically distant accession from the other groups.

There were 96.47% polymorphic RAPD bands and 98.17% polymorphic ISSR bands in a sample of 17 *Ocimum* genotypes belonging to 5 different species (*O. basilicum, O. americanum, O. sanctum, O. gratissimum, and O. polystachyon*) (Patel et al., 2015). RAPD and ISSR markers have amplified several distinct species-specific alleles and the greatest number of distinct alleles was found in the case of *O. Sanctum* in both marker systems (Patel et al., 2015). Similarly, Lal et al., 2012 performed an ISSR study of 6 *Ocimum* species and found them to be 100% polymorphic. Comparative RAPD analysis of *O. sanctum*, *O. basilicum*, *O. gratissimum,* and *O. americanum* showed amplified fragments ranging in size from 250 bp – 2500 bp and 60 % polymorphism. Species-specific bands for *O. sanctum* aiding in the authentication of the species were found. Among the ten ISSR primers tested, bands in the size range of 250 bp to 1 Kb, and among *O. sanctum*, *O. basilicum,* and *O. gratissimum* 40% polymorphism was found using ISSR markers were obtained (Sarwat et al., 2016). RAPD and ISSR techniques are sensitive, accurate, and are structured tools for genomic analysis in the species of *Ocimum* and have been used alongside with traditional morphological and biochemical markers. Major differences were noticed in the morphological characters except for *O. x africanum* and *O. basilicum*. The cluster created from the morphological information indicated two distinct groups viz. *basilicum* group and sanctum group. There was not much difference between morphologically related species viz. *O. x africanum and O. basilicum* in chemical analysis but molecular analysis such as RAPD analysis demonstrated that *O. x africanum* and *O. basilicum* are distinct species. Thus, the combined study of morphological features, biochemical and molecular markers is the best possible method for confirming taxonomic description (Chowdhury et al., 2017). The species-specific markers amplified can be turned into simple PCR-based sequence characterized amplified region (SCAR) markers which will allow the screening of an enormous number of samples and populations from a different region (Yao et al., 2008). Some scientists have confirmed the ability of the AFLP method to examine genetic distances between *O. basilicum* L. varieties and to check the occurrence of any association between genetic relationship, essential oil composition, and morphological character (Labra et al., 2004). Bilai et al., (2020) screened 20 accessions of *O. gratissimum*, *O. sanctum* and *O. basilicum* collected from different places. Varieties were analysed through RAPD and ISSR primers to find out the extent of molecular characterization. PCR amplification of the polymorphic regions using a single primer generates multiple amplification products which can be utilized as a dominant multilocus marker system for the study of genetic diversity among organisms. These PCR amplified samples were subjected to Sanger sequencing. Some of the markers used for the characterization of different species of *Ocimum* have been summarized in table 2.

Sl. No.	Name of the species	Type of molecular markers	Percentage of polymorphism	Nature of diversity detected	References
1	*O. sanctum*	RAPD	13.84 to 3.07%	Genetic	Chikkaswamy et al., 2013
		RAPD, ISSR, SSR	100%	Genetic	Lal et al., 2012
		ISSR	98.17%	Genetic	Patel et al., 2015
2	*O. tenuiflorum*	SRAP, RAPD,	97%	Genetic	Chen et al., 2013

		ISSR			
		AFLP	44.7%	Molecular char-acterization	Carovic´-Stanko et al., 2011
		ISSR	99.585%	Genetic	Amit et al., 2016
3	*O. basilicum*	RAPD	92.31%	Interspecies as-sociation	Sairkar et al., 2012
		AFLP	44.7%	Genetic diversity	Carovic´-Stanko et al., 2011
		RAPD, ISSR, SSR	100%	Genetic	Lal et al., 2012
		RAPD	13.84to 3.07%	Genetic	Chikkaswamy et al., 2013
		ISSR	80%	Genetic	Gupta et al., 2021
		SCoT	84.5%	Genetic	Gupta et al., 2021
		ISSR	86%	Genetic	Alves et al., 2019
		ISSR	98.17%	Genetic	Patel et al., 2015
4	*O. gratissimum*	AFLP	44.7%	Interspecific	Carovic´-Stanko et al., 2011
		RAPD	92.31%	Genetic	Sairkar et al., 2012
		RAPD, ISSR, SSR	100%	Genetic	Lal et al., 2012
		RAPD, ISSR, SRAP	97%	Genetic	Chen et al., 2013
		RAPD	13.84 to 3.07%	Genetic	Chikkaswamy et al., 2013
		ISSR	98.17%	Genetic	Patel et al., 2015
		ISSR	99.585%	Genetic	Amit et al., 2016
5	*O. killimandscharicum*	AFLP	44.7%	Interspecific	Carovic´-Stanko et al., 2011
		RAPD	13.84 to 3.07%	Genetic	Chikkaswamy et al., 2013
		RAPD	92.31%	Genetic	Sairkar et al., 2012
		ISSR	99.585%	Genetic	Amit et al., 2016
6	*O. africanum*	AFLP	44.7%	Interspecific	Carovic´-Stanko et al., 2011
		ISSR	99.585%	Genetic	Amit et al., 2016
7	*O. canum*	RAPD	92.31%	Interspecies as-sociation	Sairkar et al., 2012
8	*O. campechianum*	AFLP	44.7%	Interspecific	Carovic´-Stanko et al., 2011
9	*O. micranthum*	RAPD	92.31%	Interspecies as-sociation	Sairkar et al., 2012
10	*O. viride*	AFLP	44.7%	Interspecific	Carovic´-Stanko et al., 2011
		ISSR	99.585%	Genetic	Amit et al., 2016
11	*O. polystachyon*	AFLP	44.7%	Interspecific	Carovic´-Stanko et al., 2011

		RAPD, ISSR, SSR	100%	Genetic	Lal et al., 2012
		ISSR	98.17%	Genetic	Patel et al., 2015
12	*O. americanum*	AFLP	44.7%	Interspecific	Carovic´-Stanko et al., 2011
		RAPD, ISSR, SSR	100%	Genetic	Lal et al., 2012
		RAPD, SRAP, ISSR	97%	Genetic	Christina et al., 2014
		ISSR	98.17%	Genetic	Patel et al., 2015
		ISSR	99.585%	Genetic	Amit et al., 2016
13	*O. basilicum* var. *basilicum*	AFLP	66.7%	Intraspecific & genetic	Carovic´-Stanko et al., 2011
		RAPD	95%	Intraspecific & genetic	Chen et al., 2013
		ISSR	97%		
		SRAP	93%		
		RAPD	44.83%	Intraspecific & genetic	Ibrahim et al., 2013
		AFLP	85.18%	Intraspecific & genetic	Carovic´-Stanko et al., 2010
14	*O. basilicum* var. *minimum*	AFLP	85.18%	Intraspecific & genetic	Carovic´-Stanko et al., 2010
		RAPD	95%	Intraspecific & genetic	Chen et al., 2013
		ISSR	97%		
		SRAP	93%		
15	*O. basilicum* var. *thyrsiflorum*	AFLP	66.78%	Intraspecific & genetic	Carovic´-Stanko et al., 2011
		RAPD	95% polymorphism	Intraspecific & genetic	Chen et al., 2013
		ISSR	97%		
		SRAP	93%		
16	*O. basilicum* var. *difforme*	AFLP	85.18%	Intraspecific & genetic	Carovic´-Stanko et al., 2010
17	*O. basilicum* var. *purpurascens*	AFLP	85.18%	Intraspecific & genetic	Carovic´-Stanko et al., 2010
		RAPD	95%	Intraspecific genetic diversity	Chen et al., 2013
		ISSR	97%		
		SRAP	93%		
		AFLP	66.7%	Intraspecific & genetic	Carovic´-Stanko et al., 2011
		ISSR	99.585%	Genetic	Amit et al., 2016
18	*O.* × *africanum*	ISSR	97.29%	Genetic	Kurnia et al., 2020

Table 3 : Some of the markers used for the characterization of different species of *Ocimum* have been summarized

The Status of *Ocimum* sp metabolite profiling

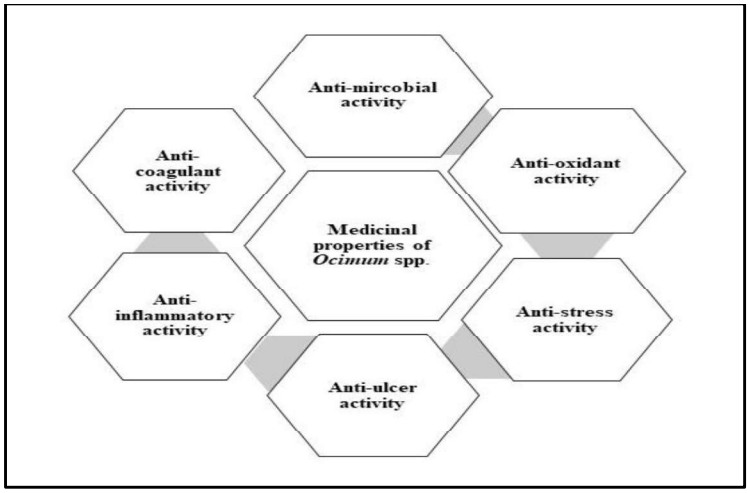

Fig3: Showing the medicinal properties of *Ocimum* spp.

Among some of the astonishing herbs, the genus *Ocimum* is ranked highest for having enormous medicinal potentialities such as antioxidant, antimicrobial, anti-inflammatory activity, etc. is shown in Fig:3. Basil plants can grow well in different soils and climatic conditions and are suitable for the cultivation of rich loam through to poor laterite , salty-alkaline soil and medium acidic soil (Pushpangadan and Bradu 1995). *Ocimum* species contains exclusive metabolites such as phenylpropanoids, monoterpenoids, sesquiterpenoids, and volatile oils, the content and amount of these metabolites depend upon environmental factors, the developmental stage of the plant, harvesting season (Gurav et al., 2021). The leaf volatile oil (Kelm et al., 2000) contains eugenol (1- hydroxy-2- methoxy -4- allylbenzene), euginal, urosolic acids (Shishodia et al., 2003), carvacrol, limatrol, carophyllene, methyl carvicol while the seed volatile oil has fatty acids and sitosterol. In addition, the seed mucilage contains some levels of sugars and the anthocyanins are present in green leaves (Pattanayak et al., 2010). The leaf volatiles (terpenes and phenylpropenes) are synthesized and sequestered in glandular hairs present on the leaves, also known as peltate trichomes, which are the characteristics of Lamiaceae family members (Iijima et al., 2004, Tissier et al., 2012, Chandra et al., 2020).Diversity in the Secondary Metabolite composition & uses of *Ocimum* sp is shown in table 4.

Sl. No.	Name	Predominant chemical constituent			Uses	Reference
		Monoterpenoids	Sesquiterpenoids	Phenylpropanoids		
1.	*O. tenuiflorum*	Linalool, 1,8-Cineole	β-Bisabolene, β-Caryophyllene	Eugenol, Methyl eugenol	Antibiotic, anti-inflammatory	Upadhyay et al., 2015
2.	*O. basilicum*	Borneol, Menthone, Camphor, Linalyl acetate.	Bergamotene, Farnesene, Bisabolene, Bicyclosesquiphellandren, Muurolol, Cadinol.	Anethole, Methyl chavicol, Methyl eugenol, Methyl cinnamate.	Anti-spasmodic, anti-asthamatic, expectorant, hepatoprotective, antipyretic	Mandoulakani et al., 2017
3.	*O. gratissimum*	Bornyl acetate, p-Cymene, γ-	α-Bulnesene, , α-Humulene, α-Farnesene	Thymol, trans-Methyl	Neuralgia, cephalalgia, and anti-fertility	Singh, E et al., 2003.

		Terpinene Geraniol, Ocimene.		isoeugenol		
4.	*O. kilimand-scharicum*	1,8-Cineole, Cam-phor,Linalool	γ-Cadinene, β-Selinene	Methyl chavicol, Eugenol,Methyl eugenol	Insecticidal, mosquito repel-lent, spasmolytic and antibacterial	Carovic´-Stanko et al., 2011.
5.	*O. americanum*	Camphor, 1,8-Cineole, Citral	β-Bisabolene, Caryophyllene	Anisole, Methyl cinnamate Eugenol, Methyl chavicol	Expectorant, analgesic	Padalia et al., 2017
6.	*O. cam-pechianum*	1,8-Cineole	β-Caryophyllene, Sabinene	Methyl eugenol, Eugenol	Act as an absor-bent, flavoring agent	Ali et al., 2021
7.	*O. canum*	p-Cymene, Camphor, Linalool, Limonene, Terpineol	β- Pinene	Eugenol, Thymol, Methyl chavicol, Methyl eugenol	Flavoring agent, coloring agent, lubricant, manu-facturing chemi-cal	Silva et al., 2018
8	Ocimum sanc-tum	1,8-Cineole	Bisabolene, Caryophyllene	Eugenol, ursolic acid, rosmarinic acid	Analgesic, anti-septic, Fragrance ingredients	Khare et al., 2007
9.	*O. micranthum*	-	Caryophyllene β-Caryophyllene,	Eugenol, Methyl cinnamate	Flavoring agent, fungicide, fluid property modu-lator	Gurav et al., 2021
10.	*O. minimum*	Geranyl ace-tate, Linalool	- -	Methyl chavicol, Methyl cinnamate	Anti-cancer, anti-asthmatic, anti-emetic, dia-phoretic	Gurav et al., 2021
11.	*O. africanum*	Citral	-	Methyl chavicol	Antibacterial, anticoagulant activity, relief of minor ache	Ali et al., 2021

Table 4: Diversity in the Secondary Metabolite composition & uses of *Ocimum* sp

Conclusion :

Plants are a valuable resource for a variety of products that are important for human needs.Concerning medicine, the use of plant-based materials dates back to ancient civiliza-tions. Medicinal plants are of huge importance to human health. Medicinal plants have played an significant role in the therapeutic needs of people. Some of these plants are not only used as spices and food but also serve as natural sources for the research and development of new medicines.About one-third of the currently available medicines come from natural products of plant origin. Although herbal therapies have great potential in advancing modern medical treatments, research is still lagging (especially compared to the interest in developing syn-thetic drugs for commercial use). This may be partly because conventional plant drug discov-ery methodologies can be slow and expensive. Nonetheless, there may be utilized to increase research in the area of medicinal plants. The literature and resources available in this field are

often scattered, making it difficulttouse the information available on medicinal plants. The drugs which are already in use to treat infectious diseases are of concern because drug safety remains an enormous global issue.

Future Prospects :

Morphological diversity and analysis of molecular markers may help to recognize genetic diversity between different basil species and varieties, assist in improving plants, and establish a well-organized way to maintain the genetic resources of basil varieties.

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