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Review Article

Exploring Genetic Variation and Therapeutic Properties of *Moringa oleifera*: Progress and Future Potential for Crop Improvements

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ABSTRACT

Among 13 species of *Moringa, Moringa oleifera* is the most extensively explored and cultivated species around the world. This species is considered a miracle plant due to some impressive properties that are worth mentioning, apart from their drought-resistant and fast-growing characteristics. Nevertheless, the rapidly growing population has raised concerns for global food security for improved yield and nutritious crops. In this review, we describe the varieties and ecotypes of *M. oleifera* evidence from different countries associated with their genetic variability and phytochemical properties. Genetic variation happens due to several factors but is mainly caused by a variety of geographical factors resulting in the formation of subspecies, races, and ecotypes. The variation pattern of *Moringa* can be acknowledged through molecular screening and not limited to morphological and phytochemical features instead. Molecular markers such as Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR), Inter-Simple Sequence Repeat (ISSR), and Amplified Fragment Length Polymorphisms (AFLP) can be a useful tool in genetic diversity to indicate the presence of intraspecific variation of *M. oleifera* at molecular level. The phytochemical variability of *M. oleifera* according to their potent compounds has also been conversing with respect to their plant parts. Both molecular and phytochemical evidence provided can be utilized for the future development of this crop with improved yield and quality characters.

Keywords: Genetic variability, Miracle tree, Molecular markers, *Moringa oleifera*, Phytochemicals.

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INTRODUCTION

Moringa oleifera is a plant species of dicots that derives from the family Moringaceae and belongs to *Moringa* genus. *Moringa oleifera* Lamarck shares the same genus with other 12 species.[1] The thirteen species in this genus includes *M. arborea, M. hildebrandtii, M. borziana, M. ruspoliana, M. concanensis, M. rivae, M. drouhardi, M. longituba, M. ovalifolia, M. peregrina, M. oleifera, M. pygmaea, M. stenopetala* are found worldwide.[2] Of these species, only 3 species, *Moringa stenopetala, Moringa peregrina* and *Moringa concanensis* are extensively cultivated apart from *Moringa oleifera.* The *M. oleifera* Lam tree (synonym: *M. pterygosperma* Gaerth) comes from Kingdom Plantae and classified under Division Magnoliphyta and Class Magnoliopsida.[3] This species further classified under Brassicales order and belongs to the single genus family of Moringaceae.

M. oleifera is referred based on the characteristics of the parts of the tree such as drumstick tree, ben oil tree or horseradish tree.[4] The name horseradish was earned for its strong taste of the roots, drumstick because of the appearance of the *Moringa* pod and ben oil due to oil present in the seed.[5,6] It is commonly referred as a Miracle Tree as well owing to its impressive health benefits.[7,8] This tree goes by many other names in the different communities of various region of world. *Moringa oleifera* is commonly called as kelor tree,[9] Shagara al Rauwaq,[10] Sohanjna,[6] Shigru,[11] Bagaruwa, Rimimaka,[12] 'Nébéday', mother's best friend, malunggay and benzolive tree.[13] In the family of Moringaceae, *M. oleifera* is one of the most naturalized and widely spread *Moringa* species.[14,15] This plant's history of origin is India but now has been indigenous to many locales in the Southwestern Africa, Arabia, Southeast Asia, Philippines, Cambodia, South America, ranges of India, Sri Lanka, Caribbean Islands and Madagascar.[3,13,16]

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Moringa is a cross-pollinated tree hence high heterogeneity in form and yield is expected.[17] The tree is true diploid with a chromosomal number of 2n = 28.[14] This tree is a thriving perennial plant that grows in tropical climate. *Moringa* tree can grow up to a height of 5 to 10 m and prefers a rainfall range of 250 mm to 3000 mm.[3,18] This plant is drought-resistant and can withstand several days without water. So, they are capable of adapting and growing in various range of environments that includes humid regions, hot dry areas and regions with asymmetry rainfall.[3] This deciduous tree is usually found abundantly in wild, close to the sandy beds of streams and rivers and can be grown in household gardens as well.[6,18] *Moringa* trees grown in household can easily flourish without regular fertilizer requirement. Although, *Moringa* tree tolerates soil conditions which are acidic and alkaline, it favours neutral to marginally acidic (pH 6.3 to 7.0), sandy or loamy soil that can retain moisture.[2] This small deciduous tree begins to yield pods after two years of cultivation and depending on the variety, has a life span of 4 to 40 years.

Interestingly, *M. oleifera* tree has a vast contribution to animal feed, fuel production, cosmetics, water purification, plant growth enhancement, medicine, and functional food.[19-28] Traditionally, every part of the *M. oleifera* tree such as leaves, flowers, pods and roots are consumed as a wholesome food in many countries, specifically in India, Pakistan, Nepal, and some African nations.[29-31] *M. oleifera* has gained robust global demand for the world supply of minerals and vitamins and it has contributed as a critical component of food and nutritional security in some countries such as Ethiopia.[32,33]

Plant parts and application of *Moringa oleifera*

The leaves of *M. oleifera* are bi- pinnate or tri-pinnate, alternatively arranged and usually 25-60 cm long.[34,35] The leaves are extremely popular and accessible vegetable that has its own tasty flavour. *Moringa* leaves have been long reported to be a low cost but rich source of antioxidants, protein, calcium, potassium and vitamins. In addition, the antioxidant compounds available in *Moringa* can prolong the shelf-life of most fat containing foods.[31]

The leaves also contain anti-inflammatory and anthelmintic properties.[36] *Moringa* referred as 'mother's best friend' in Philippines. This is due to the ability of the plant to produce more milk in nursing women. Also, this plant is prescribed to people who suffer from anemia.[37,38] Besides that, it has numerous medicinal uses including anti-cancer, anti-microbial, anti-diabetic agents, anti-atherosclerotic, and neuroprotectant properties.[39] *Moringa* leaves are also used as one of the components for *Moringa* foliage in animal feed. *Moringa* foliage consists of leaves, stems, petioles, and rachis that are fed to ruminants as source of protein.[25,27] *M. oleifera* oil extracted from the leaves and processed for biodiesel synthesis mainly because of the great oxidation performance and stability.[26] Besides that, extract from *Moringa* leaves is also useful for growth enhancement of plant crops.[19,20]

The *Moringa* tree's fruit appears to resemble pea pods. When they reach maturity the fruit colour changes from white to brown.[34] Proteins, lipids, carbs, minerals, fibre, Vitamin A, and bactericidal and fungicidal compounds make the fruit of *M. oleifera* a very nutrient-dense food.[40,41] Pods of *Moringa* is consumed to treat digestive issues such as inflammatory-associated gastrointestinal diseases and to promote healthy gut environment.[42] When the tree is about eight months old creamy white flowers of *M. oleifera* species begin to bloom.[34] Flowers are boiled and consumed as tonic to promote diuresis and to increase the flow of bile.[43]

Moringa seeds appears to be in pale greyish white colour and have unique structure that resembles like wing. The seeds are obtained from the pods and can be consumed fresh, steamed, boiled, roasted or dried for other functions.[34] These seeds have proven to have anti-pyretic and antimicrobial activities in many studies.[36,44,45] Also, the *Moringa* seeds can be hard-pressed to obtain sweet edible oil which is non-desiccant. 'Ben oil' is the name given to the oil extracted from the seed this oil and can be utilised for cosmetic purposes.[24,37] The physical and chemical characteristics of 'Ben oil' and olive oil are alike, hence the 'Ben oil' is suitable to be used as vegetable oil.[46] Other than that, the dried seed cake remains after extracting oil contain polypeptides that can be utilized as coagulants to purify the water.[47,48] The seeds are used as treatment for sexually transmitted infections as well.[37] The seeds and roots are prescribed for remedy of snake bite and scorpion sting.[36]

In addition, the root of *M. oleifera* is bitter and consumed as tonic for the body and the lungs. It is known as remedy for diuretic, stomatitis, urinary discharges, asthma, soreness of mouth and throat.[37] Extract from *Moringa* root is also useful for growth, development and yield of plant crops.[19,20] Other than that, the *Moringa* bark has both tough and smooth texture and it is not fissured. The bark is used to terminate pregnancy in females.[49] The gum that is found in the *Moringa* stem can be used for dental carries.[37]

Genetic variability of *Moringa oleifera*

The crop *M. oleifera* is famous for its efficacy to grow fast, resistance to drought, and is high yielding. Although *M. oleifera* crops have the potential for high yielding, they heavily depend on the season, the weather conditions, and the application of fertilizers. The knowledge of the genetic variability of *M. oleifera* is useful for improving the quality and quantity of the crop. Therefore, it is crucial to understand the differences in the DNA of *M. oleifera* genotypes found in various regions.

Genotypic variation refers to the difference between individuals of the same species or between species brought on by genetic mutation, an unusual meiotic event, or gene flow. The genetic variability measures the potential of the genotype or trait to vary within a population. Studies of genetic variation in plants are conducted in order to determine variety that possess superior

characteristics and to develop or enhance crops with desired features that the farmer or breeder prefers. The variability information in genotypes help farmers and breeders to select and breed plant genotypes that possess high genetic variation for better adaptation and survival in a harsh condition. Understanding the genetic variability of agronomically important characteristics help improve, enhance, and increase the yield of the crop.

However, due to the polygenic nature of the traits involved, the environment can affect the agronomically important characteristics.[50,51] Hence, the molecular marker studies are necessary for the detection of polymorphisms among the different genotypes in DNA level. A specific DNA segment that represents the differences at the genome analysis is known as a molecular marker.[52] Markers can be used to separate or identify between the groups being studied and they accelerate genotype development.[53] Molecular markers are primers employed for DNA amplification in genetic studies where they function as a nucleic acid chain. They are particularly used as an initial point for DNA synthesis. In order to obtain high polymorphism level that is desired for primer screening, primers can be chosen from a variety of plant species that belong to the same family as *M. oleifera*.

Molecular markers for *Moringa oleifera* diversity

Molecular markers are broadly used for genetic variation findings owing to their accuracy and more importantly, they are not influenced by the environment.[54] They can be either dominant or codominant markers. The codominant markers can indicate homozygosity and heterozygosity for a trait. These type of DNA markers have a high degree of reproducibility, are equally dispersed throughout the genome, and may detect larger levels of polymorphism.[55] In contrast, dominant markers do not specify homozygote and heterozygote individuals. Instead, they show presence of bands for homodominants and heterozygotes while absence of bands for recessive individuals. Dominant markers are generally non-transferable and has problems with reproducibility.[56]

Random Amplified Polymorphic DNAs (RAPDs), microsatellites or Simple Sequence Repeats (SSRs), Amplified Fragment Length Polymorphisms (AFLPs), Single Nucleotide Polymorphism (SNP) and Inter Simple Sequence Repeats (ISSRs) are among the extensively utilised PCR-based molecular marker techniques.[57-61] Among these markers, ISSR, AFLP and RAPD markers are used to identify a single dominant allele. On the other hand, SSR and SNP markers are used to distinguish multiple different codominant alleles. There are certain criteria that an ideal marker technique should meet to assess genetic diversity. The criteria include replicability, resolution of genetic differences, polymorphism level, easy automation, ability to distinguish genome of an organism that has no prior information, use of extremely small samples, time efficiency, cost efficiency, and able to screen multiple independent markers.[62]

Based on the variance detected in the areas between microsatellites, Inter Simple Sequence Repeat (ISSR) marker is commonly used dominant marker technique for genetic variability study. ISSR-PCR has been recognised as a quick and affordable finger-printing technology.[63] ISSR primers which are typically 16 to 25 base pairs length nucleotide.[60] The nucleotides can be di-, tri-, tetra- or penta-nucleotide repeats. During PCR, ISSR marker amplifies DNA segments that are situated at a distance that permits amplification between two microsatellite repeat regions that are identical but opposite in orientation.[64] It targets multiple genomic loci.[65] However, ISSRs fall below than other molecular markers because they are relatively less reproducible.[66,67] Yet, the high polymorphism level and the possibility for improved marker resolution are the reasons for ISSR's broad application in genetic variability studies.[68] Moreover, ISSR markers are easy to conduct compared to RAPD marker and they require no prior genomic knowledge.[69,70]

Furthermore, Random Amplified Polymorphic DNA (RAPD) marker is a dominant marker and this marker technology uses single short oligonucleotides that are 8 to 10 base pairs long for genetic variability studies. RAPD markers are PCR based fingerprinting method. Amplification in RAPD-PCR occurs when two hybridization sites are similar to one another and face the opposite way.[71,72] Although RAPD has a disadvantage of low reproducibility and generate fewer loci simultaneously compared to AFLP, SNP and microsatellites, they offer many advantages as well. This marker also has a high marker index compared to other dominant markers.[73] RAPDs are able to screen multiple loci from a single primer.[74] Moreover, RAPD markers able to distinguish a large number of polymorphic bands without prior DNA sequence information.[75] This system is simple to be automated, inexpensive and it is time efficient.[76] In addition, this technique also requires less quantity of DNA.[75]

Another PCR- based dominant marker is Amplified Fragment Length Polymorphism (AFLP) which in general have 50 to 500 bp length of nucleotide. The AFLP is the combined idea of RFLP and RAPD approach. The amplification of genomic DNA fragments by restriction enzymes using PCR is the foundation of AFLP. The "genome representation" capability of AFLP, which allows for simultaneous screening of representative DNA sections randomly scattered throughout the genome, is its unique characteristic. An AFLP primer (17–21 nucleotides long) is made up of an artificial adaptor sequence, the restriction endonuclease recognition sequence, and an arbitrary, non-degenerate sequence (1-3 nucleotides). Before PCR amplification, gDNA is fragmented using REs and ligated using adaptor sequences. The fragments that were cut by the frequent cutter and rare cutter will be amplified during the PCR amplification.[74] The AFLP-PCR is able to screen many loci in the genome simultaneously due to

the key feature of this marker as a multilocus marker.[77] AFLP marker is reproducible and the amplified AFLP products has high locus specificity.[78] In contrast to RAPDs and microsatellites, AFLP markers can evaluate a high number of polymorphic loci concurrently with a single primer combination on a single gel, making it an information-rich method.[74] Also, this marker system requires no DNA sequence information.[79] Nevertheless, the requirement for high amount of pure DNA is a drawback of this marker system. The restriction enzyme needs template DNA devoid of inhibitors to function properly. Another impediment is that AFLP marker system is laborious and expensive to be conducted due to the complicated methodology. AFLP marker has relatively lower marker index as well.[80]

Meanwhile, the utilization of microsatellites or Simple Sequence Repeat (SSR), a codominant marker is required to analyze the genetic diversity because it is able to distinguish both the homozygous and heterozygous traits in an individual. Microsatellite loci have short tandem repeat sequence that involves one to six nucleotide bases.[81,82] Mono-, di-, tri-, tetra-, penta- and hexa-nucleotide are all possible types of microsatellites.[83,84] Building a library of SSR motifs is the initial step in creating SSR markers, which is followed by the identification of specific SSR motifs. After that, in order to carry out PCR, it is necessary to identify favourable genomic sections and design primers. Then SSR PCR fragments are separated using electrophoresis on agarose or polyacrylamide gels.[85] Microsatellite sequences possess a high level of polymorphism due to variation in their repetition count.[56] SSRs are highly reproducible and they require less amount of DNA.[86] They are easy to operate and transferable between populations.[87] The reason behind SSR marker system is widely utilized in genetic variability studies is because they are robust and reliable. Moreover, they are multiallelic by nature and possess good genome coverage.[88] However, they are expensive to be developed and they show high levels of null alleles. The occurrence of homoplasy and high amount of time taken for primer production is a setback of SSR marker system as well.[89-91]

Single Nucleotide Polymorphisms (SNPs) are the most prevalent type of DNA sequence polymorphisms in the genome of an individual.[92] It is a single base pair shift such as substitution, insertion or deletion that occur in the genome sequence of an organism. SNPs are abundant in both plants and animals, with plants having one SNP every 100–300 bp or more frequently.[93] SNPs serve as simplest form of codominant markers that uses polymerase chain reaction technique. They offer many advantages that includes highly reproducible, ubiquitous, requires only a few nanogram of DNA and no need for prior genomic sequence information for the design of primers.[71] SNPs are abundantly distributed in the genome, therefore the markers are available in a large quantity. Also, this system yields high frequency of polymorphism and they offer several applications specifically for genetic diversity studies.[94] However, SNPs are relatively less polymorphic than SSR markers because of their biallelic nature. The major constraint of SNP is that its high developmental cost.[74]

Geographically dissimilar populations may vary in terms of genetic diversity or in the way that diversity is distributed in space. Studies have been recorded using dominant and codominant marker to detect genetic variability in *Moringa*. A study reported, 12 RAPD markers were used to characterize genetic variability of *M. oleifera* populations from Tanzania.[95] Furthermore, 17 RAPD markers were used for the genetic diversity study of 16 *M. oleifera* accessions obtained from the Germplasm Bank of Embrapa.[96] Similarly, Rufai reported a genetic diversity study of 20 MO genotypes from various geographical regions using 20 RAPD markers.[97] A study using 10 ISSR markers and 10 SCoT markers to analyze the genetic variability of 10 MO accessions from Egypt was reported.[63] Rajalakshmi evaluated the genetic diversity of 97 MO accessions from India utilizing 24 ISSR and 39 SRAP markers.[98]

M. oleifera studies on genotype level are scarce owing to the truly little genetic variability present in the genotype level when compared to the species level. However, the smallest of information has a major contribution towards plant breeding. The information in genotype level is necessary for crop enhancement strategy planning. Thus far, *M. oleifera* genotype studies are available within a particular country. The genetic diversity study on 300 genotypes belonging to 12 Indian drumstick populations was evaluated by.[99] Another genetic variability study of eight *M. oleifera* cultivars collected from various states of India.[100]

However, there are limited comparison studies on ISSR molecular markers use on *M. oleifera* varieties from Asia countries. In Saudi Arabia, genetic variation in genotypes of two *Moringa* species was characterized by employing ten ISSR primers.[101] Meanwhile, the genetic variation study done on *M. oleifera* genotypes from Indonesia was conducted using Random Amplified Polymorphic DNA (RAPD) markers.[102] Besides that, genetic variations of *M. oleifera* from Malaysia and six other countries were characterized using Random Amplified Polymorphic DNA (RAPD) markers as well.[97] Those studies on genetic variability of *Moringa oleifera* across different region and their findings are summarized in Table 1.

Available commercial *Moringa oleifera* varieties

Many varieties of *Moringa oleifera* are developed and commercialized by public and private sectors globally. Particularly, most of the research and development of *Moringa* varieties has taken place in India. The commercialized *Moringa oleifera* varieties include PKM 1, PKM 2, ODC 3, AMAR 32, Jaffna, MOL'E, MS series, MX3, Rohit 1, Sarpan SD2, Andipatty, Bhagya KDM 01, Moolanur, Multiplex, PKM 1 dwarf, PKM 2 dwarf, Shobhanjana, STX-1, STX-2, Malawi and Mbololo.[121] Among the MO varieties, most commonly available varieties in the market are PKM 1, PKM 2 and ODC 3. The *Moringa oleifera*

Table 1: Genetic variability of *Moringa oleifera* genotypes using molecular markers conducted in various regions.

Sl. No.	Molecular markers	Primers	Region	References
1.	AFLP	P12, P14, P15, P17, M51	India, Malawi, Kenya	[103]
2.	RAPD	KFP-1, KFP-3, KFP-4, KFP-5, KFP-6, KFP-7, KFP-8, KFP-9, KFP-10, KFP-11, KFP-13, KFP-21	Tanzania	[95]
		A3, A4, A8, A12, A15, A16, A18, IDT02, IDT3, IDT15, S01, S18, W02, W13, W19, B02, B18	Brazil	[96]
		OPA17, OPA19, OPB17, OPBC10, OPBD18, OPBD19, OPF20, OPH19, OPO3, OPM6, OPM8, OPQ2	Malaysia, USA, Thailand, India, Tanzania and Taiwan	[97]
		OPAD-09, OPAE-04, OPAE-05, OPAE-09, OPAF-07, OPAF-08, OPAF-09, OPAF-11 OPAF-12	Nigeria	[104]
		RAPD-1, RAPD-2, RAPD-3, RAPD-4, RAPD-5, RAPD-6, RAPD-7, RAPD-8, RAPD-9, RAPD-10, RAPD-11, RAPD-12, RAPD-13, RAPD-14, RAPD-15, RAPD-16, RAPD-17	India	[100]
		OPA-19 OPB-17 OPB-20 OPBC-10 OPF-02 OPO-03 OPJ-13 OPC-15 OPA-09 OPA-04 OPA-11 OPC-06 OPD-08 OPC-10 OPD-16 OPJ-01 OPJ-03 RAPD-2 RAPD-3 RAPD-4 RAPD-5 RAPD-6 RAPD-8 KFP-1 KFP-3 KFP-4 KFP-5 KFP-6 KFP-7 KFP-8 KFP-9 KFP-10 KFP-11 KFP-13 KFP-21	India	[105]
		AP-1, AP-2, AP-3, AP-5, AP-6, AP-8, AP-9, AP-10, AP-11, AP-12, AP-13, AP-14, AP-15	India	[106]
		OPA-7, OPA-8, OPA-10, OPA-13, OPA-17, OPA-18, OPA-19, OPA-20, OPB-17, OPB-20, OPF-20, OPH-19, OPM-6, OPM-8, OPO-2, OPO-3, OPO-13, OPQ-2, OPBB-7, OPBC-2, OPBC-10, OPBD-18, OPBD-19, OPU-17	Northern Nigeria	[107]
		OPB-3, OPB-6, OPH-2, OPH-5, OPH-6, OPH-8, OPT-2, OPT-3, OPT-4, OPT-5	Western Nigeria	[108]
		D01, A17, U23	Iran	[109]
		OPA 1, OPA 2, OPA 3, OPA 4, OPA 7, OPA 8, OPA 9, OPA 10, OPA 11, OPA 12, OPA 13, OPA 14, OPA 15, OPA 16, OPA 17, OPA 18, OPA 19, OPA 20	Indonesia	[102]
3.	RAPD-SCAR	OPB-17, OPB-20, OPO-03, OPJ-13, OPC-15, OPA-04, OPA-11, OPC-06, OPC-10, OPD-16, RAPD-2, RAPD-3, RAPD-4, RAPD-5, RAPD-6, RAPD-8, KFP-3, KFP-4, KFP-5, KFP-6, KFP-7, KFO-8, KFP-9, KFP-10, KFP-11, KFP-13, KFP-21	India	[112]
4.	ISSR	ISSR4, ISSR6, ISSR7, ISSR8, ISSR11, ISSR12,	India	[100]
		ISSR-2, ISSR-3, ISSR-4, ISSR-8, ISSR-10, ISSR-12, ISSR-14, ISSR-18, ISSR-28, ISSR-29	Egypt	[63]
		24	India	[98]
		H876, H808, H834, H844	Iran	[109]
		UBC807, UBC810, UBC811, UBC822, UBC823, UBC824, UBC825, UBC826, UBC827, UBC864	Saudi Arabia	[101]
	R-ISSR	H808 & U23, H834 & U23, H844 & D01, H876 & A17	Iran	[109]

Sl. No.	Molecular markers	Primers	Region	References
5.	CytP450	CYP1A1, CYP2B6, CYP2C19, Cyt01, Cyt02, Cyt03, Cyt04, Cyt05, Cyt06, Cyt07	India	[111]
		CYP1A1, CYP2C19, Cyt02, Cyt03, Cyt06, Cyt07, Cyt08	India	[100]
		CYP1A1, CYP2C19, Cty02, Cyt03, Cyt06, Cyt07, Cyt08	India	[110]
6.	SCoT	SCoT-1, SCoT-2, SCoT-5, SCoT-6, SCoT-7, SCoT-8, SCoT-10, SCoT-11, SCoT-12, SCoT-14	Egypt	[63]
7.	SSR	MO10, MO12, MO15, MO41, MO44, MO58, MO61, MO62, MO64	Saudi Arabia	[101]
		MO1, MO6, MO8, MO10, MO12, MO13, MO15, MO18, MO41, MO44, MO45, MO46, MO48, MO55, MO56, MO58, MO61, MO62, MO64, MO68	India, Myanmar	[115]
		MO1, MO6, MO8, MO10, MO12, MO13, MO15, MO18, MO41, MO44, MO45, MO46, MO48, MO55, MO56, MO58, MO61, MO62, MO68	Pakistan, ECHO	[116]
		MO1, MO6, MO8, MO10, MO12, MO13, MO15, MO18, MO41, MO44, MO45, MO46, MO48, MO55, MO56, MO58, MO61, MO62, MO64	Northern and Southern India	[99]
		MO1, MO6, MO10, MO15, MO41	Nigeria	[117]
		MO1, MO6, MO8, MO10, MO12, MO13, MO15, MO18, MO41, MO44, MO45, MO46, MO48, MO55, MO56, MO58, MO61, MO62, MO64, MO68	Southwest China	[120]
		MO8, MO10, MO15, MO41, MO45, MO48, MO58, MO68	China	[114]
		MO6, MO8, MO12, MO13, MO15, MO18, MO46, MO48, MO58, MO61	Nigeria	[113]
8.	RAM	CGA CT CCA GT TG CA	Colombia	[118]
9.	EST-SSR	EMS-1, EMS-2, EMS-3…… EMS-35	Rajasthan, India	[119]

varieties available in the market are developed specifically for leaf, pod and seed production.

The most popular varieties for commercial purposes are PKM 1 *Moringa* that has been developed by the Horticulture Research Station of Tamil Nadu Agricultural University (TNAU) and released in the year 1989. It is the most commercially distributed variety. PKM 1 variety is an improved pureline selected annual *Moringa* developed through continuous selfing for six generations. The features of these varieties such as rapid growth with the height of 4-6m and abundance of green nutritive leaves, early mature period thus produce fruit early. They produce long pods with 70% flesh that contribute to high yield between 50-54 tons per hectare as reported by previous literature.[46,121] These superior phenotypic characteristics will make this plant more applicable for breeding programme purposes.

The PKM2 variety has been developed from PKM1 with more improved characteristics such as more lateral branching, desirable for production of more leaves at a lower height for ease of harvesting with pod containing more flesh than seeds. The production of fruit per tree is 240 with an average yield at 98 tonnes/ha. Furthermore, the pod can be harvested between 7 to 8 months from planting. The pods are as long as about 125-130 cm with a girth of 8.40 cm and an average weight of about 280 g. They are low in fibre, 70% fleshy, and excellent for cooking. They have an average pod yield of 98 tons per hectare. PKM2 can be grown in a variety of cropping systems, can be maintained as a ratoon crop for three years and grows in most soil types with good drainage as well as containing lower fiber and good cooking quality. The ideal planting densities for maximizing production vary for these two types, according to TNAU. With two plants per hill, the PKM1 spacing is 1.5 X 1.0 m; the PKM2 spacing is closest at 1.2 x 1.2 m. PKM2 is appropriate for intercropping as an intermediate crop with coconut and tropical fruit plantations since it demands more water than PKM1 does.[121]

ODC 3 variety from Oddanchatram, an area in the Dindigul district in the southwest of the Indian state of Tamil Nadu is favored because of its reduced water and fertilizer demand, high pod yield, taste, shelf life and consumer preference.[122] It can be planted with the normal spacing of 3 m row to row distance and 2.4 plant to plant distance, or in 2 m row to row spacing and 1.5

Table 2: Parts of *Moringa oleifera* and their therapeutic properties.

Sl. No.	Plant parts	Major compound based on chemical structure classification	Pharmacological activity	References
	Leaves	Flavonoids (rutin, quercetin, isoquercetin, astragalin, isorhamnetin, kaempferol, apigenin, luteolin, genistein, daidzein, myricetin, epicatechin, vicenin-2, Kaempferide) Sulphur containing compounds (Glucosinolate and Isothiocyanate). Phenolic acid (gallic acid, salicylic acid, gentisic acid, syringic acid, elagic acid, ferulic acid, caffeic acid, o-coumaric acid, p- coumaric acid, sinapic acid, chlorogenic acid, cryptochlorogenic acid) Alkaloids (marumoside A, marumoside B, niazimicin, niaziminin) Sterol (β- sitosterol).	Hyperthyroidism, Anti-diabetic, Hypo-lipidemic, Anti-helmenthic, Anti-oxidant, Hypo-cholesterolemic, Hepatoprotective, Antifungal, Antibacterial, Antimalarial Anti-oxidant, Nutritional supplement, Anti-ulcer, Antiatherosclerotic, Diarrheal, dysentery, colitis, sores, skin infection, anemia, sign of aging, cardiac stimulants, arthritis, hypertension, parasitic diseases, contraceptive remedy, urinary ailments, boost immune system and elici lactation.	[4,35, 126-129]
	Flowers	Alkaloids, flavonoid (quercetin and kaempferat) and flavonoid pigments (alkaloids, kaempherol, rhamnetin, isoquercitrin, and kaempferitrin).	Anti-arthritic, muscle diseases, tumor, inflammation, hysteria, enlargement of spleen, aphrodisiac substances.	[4,31,127,130,131]
	Barks	Alkaloids (moringine and moringinine).	Antiurolithiatic, Aids digestion, stomach pain, poor vision, ulcer, hypertension, joint pain, anemia, diabetes.	[126-127]
	Pods	Thiocarbamate, isothiocyanate glycosides and cytokinins.	Hypotensive.	[132,133]
	Roots	Isothiocyanate.	Analgesic and anti-convulsive, Antinociceptive, Anti- inflammatory, Anti-cancer, Anti-inflammatory, Antiurolithiatic, toothache, anthelmintic and Anti-paralytic.	[4,126,128]
	Seeds	O-ethyl-4-(α-L-rhamnosyloxy) benzyl carbamate, 4(αL-rhamnosyloxy)-benzyl isothiocyanate, niazimicin, 3-O-(6′ -O-oleoyl-β-D-glucopyranosyl)-β-sitosterol, β-sitosterol-3-O-β-D-glucopyranoside, niazirin, β-sitosterol and glycerol-1-(9-octadecanoate).	Analgesic, Anti-spasmodic, Diuretic, Anti-allergic, Antibacterial, Larvicidal, Anti-Viral and warts.	[134]

m plant to plant spacing for intensive cultivation with 1200-1500 plants per acre. The trees grow rapidly and three months after planting they begin profuse branching. Pods reach edible size 65 days after flowering. The anticipated yield per plant is 200-300 pods and the total annual yield per acre is estimated to be about 25-30 tons. Ratooned trees can be kept up for ten to fifteen years. The trees should be pruned annually by 1 m from the ground.[122]

Another *M. oleifera* variety that is commercially available is AMAR 32, developed by a private sector, Amar Seeds, Maharashtra. This variety is known for its high production of leaves, rapid maturing and they are ready to harvest within 5-6 months of planting. Next, is Jaffna where this variety was developed in Thangachimadam, India but the cuttings were originally brought from Jaffna, Sri Lanka, hence the name. A 40-year-old Jaffna variety tree

Table 3: Parts of *Moringa oleifera* tested with various solvents.

Plant parts	Solvents used	Phytochemical compound	Biochemical assay	References
Leaves	Ethanol	Crypto-chlorogenic acid, Isoquercetin, Quercetin, Kaempferol, Caffeic acid, Rutin, phytol.	Antioxidant activity.	[136-141]
		Flavonoids, Tannins, Steroid, Alkaloid, Saponins.	Antifungal activity.	[142,143]
		polyphenols, tannins, anthocyanin, glycosides, thiocarbamates.	Antistress.	[138]
		kaempferol, myricetin, quercetin, biochanin A, o-coumaric acid, naringin, naringenin, catechin, resveratrol, hydroxybenzoic acids, 4 (ά– L – rhamnosyloxy) benzyl-isothiocyanate, gallic acid, tannin.	Antimicrobial activity.	[144-146]
			hepatoprotective activity.	[139]
	Ethyl acetate		Antioxidant activity.	[147]
		kaempferol, myricetin, quercetin, biochanin A, o-coumaric acid, naringin, naringenin, catechin, resveratrol,	Antimicrobial activity.	[146]
		myricetin, quercetin, kaempferol derivatives, gallic, dihydromyricetin 3-O-rhamnoside, quercetin 3-O-rhamnoside, glyco-sidic forms of kaempferol, gallic acid 4-O-glucoside and isomeric forms of caffeoylquinic acid and chlorogenic acid.	*In vitro* bioactivity.	[148]
Flowers	Ethanol	β-sitosterol.	Total Phenolic content, Total Flavonoids content, Antioxidant activity, Carotenoid content, Lycopene content, Ascorbic acid content, Anthocyanin content,	[141]
Barks	Ethanol	Epiglobulol.	Total Phenolic content, Total Flavonoids content, Antioxidant activity, Carotenoid content, Lycopene content, Ascorbic acid content, Anthocyanin content,	[141]
Pods	Ethanol	polyphenols, tannins, anthocyanin, glycosides, thiocarbamates.	Antioxidant, Anti-stress, Scavenging potential.	[138]
	Aqueous	polyphenols, tannins, anthocyanin, glycosides, thiocarbamates.	Antioxidant, Anti-stress, Scavenging potential.	
Roots	Alkali Aqueous	Pterygospermin, Isothiocyanate, 4-α-L-rhamnosyloxybenzyl isothiocyanate.	Anti-bacterial activity.	[130,149]
Seeds	Ethanol Aqueous	4(α-l-rhamnosyloxy)-benzyl isothiocyanate, niazimicin, 3-O-(6′ -O-oleoyl-β-d-glucopyranosyl)-β -sitosterol, and β -sitosterol-3-O- β -$_D$-glucopyranoside.	Anti-tumor activity Anti-bacterial activity.	[134,149]
	Chloroform	4 (ά– L – rhamnosyloxy) benzyl-isothiocyanate.	Anti-microbial activity.	[144]

produces 1500 pods and it is typically not affected by pests and diseases.[123] MOL'E is also a variety developed by a private sector named Advanced Biofuel Center (ABC), India. This variety is a non-GMO cultivar that is specifically cultivated for *Moringa* leaf production. The number of plants produced per hectare is 30,000 – 167,000. Besides, the MS series *M. oleifera* variety is developed by Ancient Greenfields PVT LTD (AGF), India. The MS series consist of MS01 and MS02 are suitable for large scale planting. These varieties have high productivity and also germination rates. The MX3 variety on the other hand is intended for seed oil extraction. The seeds of MX3 variety have a range of 35-40 percentage of oil content. Next, Rohit 1variety was developed by a farmer, Balasaheb Marale at Nashik, Maharashtra on the year 2004. A five-year-old tree of this variety produces 585 to 650 drumstick pods and the yield per acre is 7 to 12 tons. Moreover, Sarpan SD2 is a variety by Dr. Nijagunadev Gaddagimath of Dharwad's Sarpan Agri Horticultural Research Center and Sarpan Hybrid Seeds. This variety is a hybrid breed line. It can yield between 300-500 pods per plant.[124] Andipatty variety has a tree height of 25 feet. They are unaffected to pest and diseases and grow fast even in poor soil.

Another commercially available variety is Bhagya KDM 01. This variety is derived from PKM 3 selections developed by Drs. Madalgeri and Mulge at the Arabhavi-based Kittur Rani Chennamma College of Horticulture in the Belagavi District. This perennial variety was developed in the year 2011. The variety grows to a height of 2 to 4 meters by pruning. It is suitable for high density planting and can be maintained in production for 15 years. The plant is fast growing and the fruit length is of a desirable medium length of 45 to 75 cm. The trees produce 350 to 400 pods in the first year and 800 to 1000 in the second year, and a total yield of 17-20 tons per acre. Borer infestations and poor management practices may reduce the yield and productive life of the trees of this variety.[124] Next, Moolanur that is a perennial semi-dwarf dwarf ecotype variety cultivated by farmers in Tamil Nadu. They have a height of 15 feet and per tree yields about 500-600 pods per year. Without pruning, the trees can be maintained for up to 15 years.[121] Also, Multiplex is a variety that is a breed line of PKM1 and PKM2 hybrid dwarf. They have a maximum height of 4-6 meters. They produce pods twice a year with a yield of 200-350 pods per tree. The trees have wide adaptability to their surroundings.

Next, STX-1 and STX-2 are landraces *M. oleifera* cultivar. The STX-1 variety is an indigenous landrace non-GMO strain cultivar by Toni Ramirez of Lorado, Texas. It concentrates most of its energy into leaf production, and less on flowering and pod production (low and short pods). They are fast-growing plant and can be grown in areas with colder winters, resists cold, heat, drought, excess soil moisture, and wind better than PKM-1 and STX-2. The STX-2 variety however, is an improved non-GMO landrace cultivar by Toni Ramirez as well. It is a pure line selection from the PKM-1 variety. They are strong producer of leaves and a heavy producer of very large pods of 24-30 inches in length, extremely fast-growing and highly productive. Other MO variety that is commercialized is Malawi. The focus of this variety is the seed oil production.[46] The Kenya Forestry Research Institute (KFRI), located in Nairobi, Kenya, created the Mbololo cultivar. Due to their high oxidation resistance and high ratio of monounsaturated to saturated fatty acids, they are excellent for seed oil, which may be an acceptable substitute for olive oil in the diet.[125]

Therpeutic properties of *Moringa oleifera*

Pytochemical components and their influence are one of the most studied components in *M. oleifera* owing to their therapeutic properties. This plant is a medicinal plant and has high prospect in pharmaceuticals and therefore all parts of *Moringa* are extensively explored for its nutraceutical properties. The studies on bioactivity of plant parts revealed remarkable therapeutic activities. The Table 2 summarizes the studies performed on leaves, flowers, barks, pods, roots and seeds and their evident pharmacological activity.

Studies related to pharmacological activity of *M. oleifera* often involves solvent extraction. Depending on the polarity of the target solute, solvents play a key role in the process of extracting biomolecules from plants. In a solvent that has the same polarity as the solute, the solute will dissolve efficiently.[135] Therefore, solvent has direct influence over the phytochemical compounds obtained. The Table 3 shows the previously reported studies of biochemical assay revealed that different solvents showed notable difference in the phytochemical composition.

Progress and future potentials

There are numerous uses for *M. oleifera* in both food and medicine. High sources of protein, magnesium, potassium, calcium, vitamin A, vitamin B1, vitamin B3, vitamin B6 and vitamin C are present in them. In opposition to various pathogenic bacteria, fungi, viruses, and parasites, they show antibacterial activity. The phytopathogenic fungi that cause disease and interfere with the growth of profitable crops can be better controlled in agriculture with the help of *Moringa oleifera*. Additionally, to obtain MO plantlets that are genotypically, and phenotypically stronger breeders can use the parental *M. oleifera* for intraspecific hybridization. *M. oleifera* also has a bright future in the management of infectious illnesses in marine aquaculture. It is crucial to promote the separation and identification of specific bioactive compounds from *M. oleifera* that may have antibacterial, antifungal, antioxidant, antitumor, antistress and scavenging potential to maximize their use in the future.

CONCLUSION

This review emphasizes the potentials of *M. oleifera* as a definite source for diverse nutritional and therapeutic materials. The countless importance of M. oleifera available as a readily available plant has robust global demand in diverse economics. However, this plant requires improvement in terms of their phytochemicals quality and fruit yield. Efforts also should be geared toward extensive research to encourage Moringa cultivation through mass propropagation employing tissue culture technique to produce Moringa plants in shorter time. This information will find informative for Moringa plant breeders to obtain plantlets that are genotypicaly and phenotypically resilient .

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DNA: Deoxyribonucleic acid; **RAPD:** Random amplified polymorphic DNA; **SSR:** Simple sequence repeat; **AFLP:** Amplified fragment length polymorphism; **SNP:** Single nucleotide polymorphism; **ISSR:** Inter simple sequence repeats; **PCR:** Polymerase chain reaction; **RFLP:** Restriction fragment length polymorphism; **gDNA:** genomic DNA; **RE:** Restriction enzyme; **MO:** Moringa oleifera; **ScOT:** Start codon targeted; **SRAP:** Sequence - related amplified polymorphism; **PKM:** Periyakulam; **ODC:** Oddanchathiran; **SCAR:** Sequence characterized amplified regions; **R-ISSR:** Reverse inter simple sequence repeat; **CytP450:** Cytochrome P450; **EST-SSR:** Expressed sequence tag-derived simple sequence repeat markers; **TNAU:** Tamil Nadu Agricultural University; **GMO:** Genetically modified organism.

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