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REVIEW ARTICLE

Genus Zingiber: A Review on Botanical, Major Bioactivities and Genetic Diversity

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Abstract Zingiberaceae is a perennial plant family that is found across the tropics, particularly in Southeast Asia from low land to hill forests. In Peninsular Malaysia, it is believed that 160 ginger species are widely distributed belonging to 18 genera. Most of the *Zingiber* species in Peninsular Malaysia are less investigated and less understood taxonomically, thus remaining as under-utilized crops. The description of their morphologies in parallel with phytochemicals and molecular information are crucial to provide valuable information for further discovery of potent compounds, identification of potential new sources of genetic variation, as well as to provide insight into the domestication and breeding of ginger. The majority of *Zingiber* species are perennial herbs with a fragrant scent, an upright stem, and a fibrous rhizome. The presence of volatile components such as monoterpenoids, sesquiterpenes, sesquiterpenoids and some non-volatile compounds like gingerols, shagaols, and zingerone have contributed to the strong scent of the ginger oils. Among the dominant components of *Zingiber* are α-zingiberene, geranial, neral, camphene, neral, neric acid, α-curcumene, and zerumbone. The crude extracts and essential oils of *Zingiber* have proven to show some biological activities such as antimicrobial, anti-bacterial, insecticidal, larvicidal, anti-cancer, anti-inflammatory, anti-ulceration, antioxidant, anti-fungal, immunomodulatory, and anti-nociceptive. Most *Zingiber* species are known to have 22 somatic chromosomes (2n=22) which is the lowest among genera in Zingiberaceae. This study underscores the crucial significance of breeding programs and germplasm conservation, specifically emphasizing the potential of common ginger as a prominent contributor.

Keywords: *Zingiber*, ginger, genetic diversity, bioactive compounds.

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Introduction

Over 2000 years ago, ginger was first utilized as a spice and natural flavoring. In addition to being utilized as a flavoring component in many culinary products, spices can now be employed to create innovative traditional remedies to cure disease. These spices regarded as medicinal plants are abundant in secondary metabolites, which serve as substantial sources for novel chemical compounds with important therapeutic effects, such as antioxidant and antibacterial properties. Due to their low toxicities and strong bioactivities, plant-derived antibacterial and antioxidant compounds are famous [1]. For centuries, *Zingiber* has been used therapeutically for its antimicrobial, anti-inflammatory and antioxidant properties

[2]. Alternative medicinal practices such as those from the traditional system have been using gingers to treat the common cold, sore throat, vomiting, nausea, ulcer, stomach disorders, migraine, hypertension, and arthritis [2-4]. There are a lot of beneficial nutrients contained in *Zingiber* which are believed to be very rich in minerals, lipids, proteins, vitamins, and carbohydrates. These nutrients were essentially extracted into essential oils, which have been used worldwide and have been shown to have various pharmacological effects such as insecticidal, antiviral, antibacterial, anti-herbivore, antifungal, antioxidant, and anti-inflammatory effects against viruses, bacteria, protozoa, or fungi [5].

Previous studies have reported the chemical constituents, and antimicrobial activities reside in the essential oil of *Zingiber* spp. Among the primary, significant compositions are α–zingiberene, α-curcumene, zerumbone, and β-sesquiphellandrene [2,6]. Ginger can effectively stave off oxidative stress [7]. Excessive production of free radicals or reactive oxygen species (ROS) during metabolism causes oxidative stress, which is implicated in heart disease, neurological illnesses, cancer, and the ageing process [8]. It has been proposed that eating foods high in antioxidants can prevent or delay the oxidation of important macromolecules within the cell by chelating metals or scavenging free radicals produced by metabolism. As a result, antioxidants are essential to prevent oxidative cell damage, which has been related to a variety of illnesses, as well as to maintain cell components in a reduced form [9].

Synthetic and natural antioxidants are frequently used in the pharmaceutical and food industries to minimise oxidative damage. Nonetheless, research has revealed that synthetic antioxidants typically have negative side effects and are potentially hazardous. As a result, natural antioxidant alternatives made from plants have been advocated [10]. Spices are a fantastic source of antioxidants, and some of them even outperform synthetic antioxidants and are safer for your health [11]. It's worth noting that many Zingiberaceae species, including *Zingiber officinale* Roscoe, *Zingiber officinale* var. *rubrum*, *Zingiber zerumbet*, and *Zingiber cassumunar*, are gaining popularity [7].

Given the benefits of *Zingiber* spp., they must be appropriately identified. *Zingiber* spp. genomes were significantly varied. Thus, molecular techniques may be used to assess genetic diversity within a population or species. Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR), Inter-Simple Sequence Repeats (ISSR), and Amplified Fragment Length Polymorphisms (AFLP) are all frequently utilized in plant ecological, evolutionary, taxonomic, phylogenetic, and genetic diversity studies. PCR is used to amplify a semi-arbitrary marker known as an Inter-Simple sequence repeat (ISSR) in the presence of one primer that matches the microsatellite sequence to be targeted. Microsatellites can be found anywhere in the genome, and they are easy to use. This property serves to be a promising key advantage. ISSRs are likely to uncover gene-rich areas and to be a valuable technique for discriminating between closely related ginger cultivars [12]. This method is crucial because a crop's ability to adapt to changes in climate, farming practices, and pathogenic organisms may be limited if genetic diversity is lacking. Previous studies in morphology, anatomy, physiology, and embryology were also used for identification [13].

However, in the absence of the blooming stage, most *Zingiber* species are phenotypically identical and difficult to differentiate. Flowering takes a long period, making identification a time-consuming task. [14]. Using a molecular marker technique would thus make it simpler to discover novel sources of variation and genetic components that influence inherited quantitative features. ISSR markers, which are highly polymorphic and reproducible even for intraspecific purposes, are used among available genetic markers to determine the genetic variation of ginger species [15].

Origins, History, and Taxonomic Status of Zingiberaceae

The Zingiberaceae family belongs to the Zingiberales order, which is one of the largest in the plant kingdom. They are widespread in the tropics and comprise 53 genera and 1200 species all over the world, with Southeast Asia as the centre of diversity [16-18]. The Malesian region (Indonesia, Malaysia, Singapore, Brunei, the Philippines, and Papua New Guinea) has the highest concentration of genera and species, implying that the family's origin was in the Indo-Malayan region [19]. In Peninsular Malaysia, it is believed that 160 ginger species belonged to 18 genera [20]. Based on morphological traits, Zingiberaceae are classified into four tribes: Alpinieae, Zingiberaceae, Globbeae, and Hedychieae; based on DNA sequences, they are classified into six tribes, which are: Alpinieae, Riedelieae, Zingiberaceae, Globbeae, Tamijieae, and Siphonochilieae [21]. Typically, Zingiberaceae species grow as scattered plants or thickets in wet, shaded lowland environments or on hill slopes [22]. Due to the geographical landmass of Peninsular Malaysia being presumed unaffected by glaciers, drastic climatic changes, and sea floods, the family has been able to constantly evolve. This includes *Zingiber* and many more taxa, which successfully evolved in a stable environment and then expanded to Thailand, China, and Borneo's adjacent regions.

There are between 100 and 150 species of *Zingiber* [23-25], with more than 40 species found in Thailand and Southern China [25]. [24] reported fifteen species of *Zingiber* in Thailand, two of which are cultivated, namely *Zingiber officinale* Roscoe and *Zingiber zerumbet* (L.) Sm. In central and northern Thailand, there are also a few indigenous species. Nineteen species were identified in Peninsular Malaysia [18], sixteen of which are native, while *Z. officinale, Z. zerumbet*, and *Z. montanum* are introduced or cultivated. 37 *Zingiber* taxa were discovered in Myanmar in 2016, four of which are new and belong to the section *Cryptanthium* and *Zingiber* [26].

Ginger flowers are delicate and fragile with a limited life span. In most taxa, they develop from the inflorescence's base. *Curcuma*, *Amomum*, and *Zingiber* are the most often used genera of ginger in commercial production. In certain species (*Zingiber officinale*, *Curcuma domestica*, and *Alpinia galanga*), rhizomes are used as a flavoring ingredient and for therapeutic reasons. More ginger genera have been utilized in recent years for their therapeutic potential as well as for usage as decorations and cut flowers. Ginger is often used in the production of alcoholic beverages including ginger ale, ginger beer, and ginger wine, among other things [15].

Morphological Characteristics

Zingiber is a vast and complicated genus. In recent years, fieldwork in China, Thailand, and Malaysia has revealed the discovery of several undescribed taxa, suggesting an even larger variety of species. On the other hand, the present categorization of this genus is based on the work of [27] and [28], and it is still relevant in revising the genus. In the Flora of the Malay Peninsula, [29] recognised several new taxa for Malayan species, and [19] focused on species in the Malay Peninsula in later revisions. Thelaide's [18] revision closely resembles Holttum's [19]. During an early investigation on *Zingiber* taxonomy, [30] revealed 33 *Zingiber* species, whereas [16] reported 35 *Zingiber* species in Thailand. Hedychieae, Alpinieae, Globbeae, and Zingibereae are the four Zingiberaceae tribes that have been identified [16,31]. *Zingiber* is the sole genus recognised in the Zingibereae tribe, and it is based on both vegetative and floral characteristics, such as numbers of locules and placentation in the ovary, development of staminodia, modification of the fertile anther, and rhizome shoot-leaf orientation. Most of the characteristics used to designate the tribes are inconsistent and changeable, and there are no defining morphological traits that are significant in distinguishing the four tribes.

The *Zingiber* plants are perennial, and the rhizome is the most important component. To thrive, these gingers need to be in a moist, shaded, and damp environment [16,18]. Most of the species in Peninsular Malaysia live on the ground or in mid-mountain forests, with a few located on high mountain ridges [19]. The most notable morphological features are the bract and labellum. During flowering, the bracts are often red or orange in colour, changing to a darker colour as they age. The style is surrounded by a long and curving anther appendage, and the lips are three-lobed with enormous bracts. During pollination, this large bract is particularly important for insect adhesion. In certain species, each flower has a non-tubular bracteole, and the lip is cream or white, but purple mottled with cream is sometimes observed [16,18]. [16] identified four Thai species that are utilised as traditional remedies: *Z. ottensii* Val., *Z. purpureum* Roxb., *Z. zerumbet* (L.) Sm., and *Z. spectabile* Griff. While there are two highly cultivated Malaysian species: *Z. officinale* and *Z. zerumbet*, [32]. These gingers are well-known for their economic significance, as flavour to various dishes, spices, pharmaceuticals, condiment, beverages, and scent goods. As a result, they are considered an important group with substantial economic potential.

Problems in Classification

The majority of *Zingiber* is used in traditional medicines and for household purposes. As a result, plant specimens have become few and exploited, and research on this genus and family is progressing slowly. Flowers are always destroyed because of their delicate structure and limited life span; in many cases, no flowers have been preserved, or the preservation is inadequate; field notes of bloom colour are often lacking [16,19]. Proper preservation techniques, such as storing several flowers or the complete inflorescence in alcohol or FAA and photographing the floral components in colour, are required [24]. Even ancient collections and dried material are typically sterile or destroyed. This makes dealing with the genus *Zingiber* challenging, thus the key has been based on vegetative characteristics and the structure of the inflorescence wherever feasible [18,25]. To complete the family's review, this genus must be investigated further. Ginger has well-known cultivar variation for production and morphological characteristics, with a few basic kinds of outstanding quality. The ginger varieties' popular names, however, are confusing and have led to a geographical bias in ex-situ conservation. Most ginger molecular/biochemical marker studies published reveal low levels of polymorphism in contrast to high levels of phenotypic variability. The main obstacles to further varietal improvement of ginger are the limited genetic diversity, considerable impact of environmental conditions on the amount of essential chemicals, the paucity of seed set, and the ambiguity of the popular names [33].

Zingiber Species in Malaysia

The third-largest genus in the Zingiberaceae family, which consists primarily of plants used for food and medicine, is *Zingiber*. It consists of 141 species, of which 12 species are indigenous to China, particularly southwest China. The majority of the species in this genus are perennial herbs with a fragrant scent, an upright stem, and a fibrous rhizome. In addition to obtaining fragrant oils from the stems, leaves, and roots of *Zingiber* plants, the roots are primarily utilized for food and medicine [34].

Scientific classification of *Zingiber* spp
Kingdom : Plantae
Division : Tracheophyta
Class : Magnoliopsida
Order : Zingiberales
Family : Zingiberaceae
Genus : *Zingiber*
Species : ***Zingiber officinale*** Roscoe, ***Zingiber officinale*** var. ***rubrum***, ***Zingiber zerumbet***, ***Zingiber cassumunar***

Zingiber Officinale Roscoe

Zingiber officinale is also known as edible ginger. There are three types of local ginger: true ginger, or *Halia betul*, *Halia bara* or *Halia padi;* and *Halia udang*. The first has no red color on its rhizome, also known as *Z. officinale* cultivar group *officinale* "ginger" whereas the others have red externally and are highly pungent which known as *Z. officinale* cultivar group "*rubrum*". One of the most widely utilized herbs in Asia, *Zingiber officinale* has been empirically used to treat a variety of diseases. It is a herbaceous perennial that can reach a height of about 100 cm. The branched rhizome is where the leaves grow. Simple, alternating, narrow, oblong-lanceolate leaves with sheathing bases are distichous, 2–3 cm wide and the blade gradually tapers to a point. The inflorescence consists of a single lateral radical pedunculate spike that is rectangular and cylindrical. Rare orchid-like flowers with several overlapping scales on an extended stem are found. Each flower features three yellowish-orange petals and an additional purplish structure that resembles a lip. Rhizomes are fragrant, thick lobed and light yellow. When the plant reaches maturity, bunches of lateral branches from the herb start to dry out [35].
The rhizomes of ginger are most frequently used as a spice and condiment due to their spicy flavor and woody aroma, even though both ginger flowers and bruised stems have pleasant aromas. The *Zingiber officinale* rhizome is highly prized not just for being one of the most widely traded spices but also for its health-improving qualities. The ginger rhizomes were used by locals to treat stomach problems. The use of this plant as traditional medicine has been verified by the rural communities and cross-checked the information at various intervals. The plant has been recognized and certified by experts in plant taxonomy [36].

Zingiber Officinale Var Rubrum

Zingiber officinale var. *rubrum* is also known as red ginger. As an annual plant, this variety can reach heights of 50–100 cm. The lance-shaped leaves have lengthy sheaths that clasp the stem and are 5–25 cm long and 1.5–2 cm wide. The tip of the leaves is pointy. Instead of being branched, stems grow perpendicularly and flatly. Compound and ovoid in shape, the blooms have a stem length of 10–25 cm, an oval shape, and a purple flower crown that is 2–2.5 cm in diameter. Three tubular, angular petals make up little flowers. The thick, reddish-brown, and red rhizome skin of the fleshy rhizomes is noticeable. With age, the single root becomes larger to produce the rhizomes and branches that will develop into new plants [37].

Red ginger has become popular, particularly in Asia, where it is valued for its therapeutic properties, including its ability to reduce inflammation, improve blood circulation, and clear the body of wind. Several therapeutic medicines have been developed as a result of the positive effects attributed to red ginger's ingredients. Red ginger is also referred to as 'halia bara', 'halia merah', or 'jahe merah' in Southeast Asia. Currently, foods like dried ginger pieces and pickled ginger, as well as red ginger extracts in instant beverages like tea and coffee are among the red ginger goods sold in Southeast Asia. Additionally, red ginger extracts are included in body creams, lotions, ointments, and pills [38].

Zingiber Zerumbet

Zingiber zerumbet is also known as "lempoyang". *Z. zerumbet* is a variegated wild edible ginger with stems that are about 1-2 m long, erect, oblique, spherical, annual, and covered with the smooth sheaths of the leaves. It may be identified by the presence of a pulvinus between the base of the petiole and ligule. The thick, knobby rhizome, or subterranean stem, of pinecone ginger grows just below the soil's surface, from which the plant's leaves and inflorescences emerge. The 25-35 cm long, thin leaves, which are occasionally purplish beneath young shoots, have midribs that are sharply elevated on the underside. The ligule is exceedingly thin, whole, and broad, measuring only 1.5-2.5 cm in length compared to the petiole's around 6 mm length. Along an arching pseudo stem that lengthens to about one to two meters, the leaflets are placed alternately [39].

A distinct pseudo stems from the leaves bearing the inflorescence, which is roughly 6–12 cm long, green when young, and turns red as it ages. The inflorescence includes closely overlapping bracts or bracts that form an open pouch in which flowers are present, one in each bract. It is a spike that ranges in shape from ovoid to ellipsoid. Bracts support the position of each flower, giving the inflorescence a pinecone-like appearance. The mucilaginous substance found in the inflorescence is utilized by the Hawaiians as shampoo and natural hair conditioner, which is why *Z. zerumbet* is also known as "ginger shampoo". When macerated in ethanol, the rhizome can be utilised as a tonic and stimulant. A cure for wounds and bruised skin was also made using the ashes from burnt *Z. zerumbet* leaves, which were mixed with *Schizostachyum glaucifolium, Aleurites moluccana*, and *Z. zerumbet* tuber sap [39].

Terpenes and polyphenols are the primary chemical components of *Z. zerumbet*. The main bioactive component of this species is zerumbone, a sesquiterpene that is being extensively researched for its therapeutic characteristics, including analgesic, antibacterial, anti-inflammatory, antidiabetic, and antioxidant. According to research, sesquiterpenes dominate the complex blend of terpenes produced by bitter ginger. Commercially, Z. *zerumbet* is a medicinal plant with enormous potential for growth and low cultivation expenses. High concentrations of (Z)-nerolidol (22–36%) have been detected in stem, leaf, and flower extracts, but not in rhizomes, while zerumbone was found to predominate in leaves [39]. Nerolidol can be found in ginger, and it possesses biological activity such as antioxidants [40].

Zingiber Cassumunar

Zingiber cassumunar is also known as "bonglai". This plant's root is perennial, tuberous, and equipped with long, white fleshy fibers and jointed like ginger, but much larger. When fresh, it is a deep yellow color with a warm, spicy flavor that is somewhat bitter. It also has a strong camphoraceous odor [72]. Since the rhizome is the primary part of the plant, it is employed medicinally. The phytochemicals and bioactivities of Z. *cassumunar* have been the subject of several previous research to build the scientific foundation for its application in conventional medicine [41].

Z. cassumunar is one of many medicinal plants that have historically been used extensively for skin beautification, prevention of asthma, persistent colds, nausea, poultice, decoction, and therapeutic massage. Additionally, it has cassumunarin, which is particularly high in antioxidant properties [41]. Prior research on cassumunar ginger's essential oils discovered the main terpenic components that contain sabinene and terpinen-4-ol [42].

Bioactive Compounds in Zingiber

The variation, quantity, and quality of essential oil are contributed by a lot of factors which are geographic origin, maturity at harvest, time of harvest, the age of plants, cultivation methods, environmental and climatic conditions, agricultural practices, analytical techniques, drying processes, storage time, methods of extractions, and different plant parts for extractions [43-45]. The physical color of essential oil from *Zingiber* appears to be pale yellow to light-amber, containing both pungent and aromatic compounds [46,47]. In Malaysia, the bioactive compounds of some *Zingiber* species have been investigated in previous studies. Among the studied species are *Z. cassumunar* Roxb, *Z. montanum* (J. Koenig), *Z. officinale* Rosc., *Z. officinale* var. *rubrum* Theilade, *Z. ottensii* Valeton, *Z. puberulum* Ridl, *Z. spectabile* Griff, and *Z. zerumbet* L. (Smith).

[48] has identified a total of 15 compounds in the essential oil of *Z. cassumunar* of Kuantan, Pahang. The abundant components were 2,6,9,9-tetramethyl-2,6,10-cycloundecatrien-1-one (60.77%) and α-caryophyllene (23.92%) [48]. Meanwhile, [49] reported 3-cyclohexen-1-ol,4-methyl-1-(1-methylethyl), and terpinene-4-ol to be among the most dominant compounds found in the essential oils of *Z. cassumunar* rhizomes of Malim Nawar, Perak [49]. [50] investigated the crude extracts of *Z. montanum* of Gombak, Selangor and reported a number of 82 compounds extracted from the rhizomes of the species. Among the dominant compounds were Dimethyl 4-methylphthalate, carbendazim, 6.7-Dimethoxyquinoxaline, 2,4,5-trichlorophenol, and noscapine.

According to [45], there are around more than 400 different compounds identified in *Zingiber officinale*. The amount of essential oil presented would determine the odor of ginger [45,51]. The presence of volatile components such as monoterpenoids, sesquiterpenes, sesquiterpenoids and some non-volatile compounds like gingerols, shagaols, and zingerone have contributed to the strong scent of the oils [2,45]. [52] reported 82 volatile components of essential oils extracted from rhizomes of *Z. officinale* Rosc. originated from Bentong, Pahang. Among the dominant components are α-zingiberene, geranial, neral, trans-caryophyllene, eucalyptol, β-phellandrene, and camphene. In another study, [53] reported 44 compounds of *Z. officinale* essential oil extracts of the same locality. The major compounds include neral, α -curcumene, borneol, eucalyptol, and neric acid. The same author also reported 43 compounds of *Z. officinale* essential oil extracts of Keningau, Sabah. The major compounds are α -curcumene, geranial, geraniol [53].

Meanwhile, [5] reported a number of 46 compounds of essential oil extracted from the leaves of *Z. officinale* var. *rubrum* of Negeri Sembilan. Sesquiterpenoids and monoterpenoids were dominant with β-caryophyllene, geranial, neral, and caryophyllene oxide were abundantly presented. On the other hand, 54 compounds were identified in the oil from the rhizomes of *Z. officinale* var. *rubrum* [5]. The oil from the rhizomes was predominantly monoterpenoid, with camphene, gernial, and geranyl acetate as the three most abundant constituents [5]. [50] reported 43 components from the rhizome crude extract of *Z. officinale* var. *rubrum* of Selangor. The major extracted compounds were zingerone, benzaldehyde dimethyl thiol acetal, α-curcumene, zingiberene, β-sesquiphellandrene, gingerol, and 3-methoxy-ltyrosine.

A study conducted by [54] reported 28 components from the rhizomes of *Z. ottensii* of Sabah, East Malaysia. The most abundant component of *Z. ottensii* was zerumbone while other major components included terpinene-4-ol, α-humulene, and sabinene. Meanwhile, [55] identified 26 components from the essential oils extracts of *Z. ottensii* of Johor Bharu of which the major component was found to be zerumbone while the minor components have been identified as α-humulene, humulene epoxide II, β-pinene, γ-terpinene, terpinene-4-ol, and sabinene [55]. [56] reported 21 compounds in the extract of *Z. puberulum* of Cameron Highlands, Pahang with fatty acids which are palmitic acid and oleic acid as the major constituents while various terpenic compounds presented in smaller amounts. The sesquiterpene compounds identified in the extract of *Z. puberulum* were α-bisabolol, β-elemene and caryophyllene [56]. [50] identified 50 active compounds from the rhizomes crude extract of *Z. spectabile* of Selangor with 1,1’-ethylenebisdecalin, 1-pentadecyne, γ-sitosterol, and β-sitosterol reported to be dominating. Meanwhile, [57] in their study reported a number of 19 compounds in the oil of *Z. spectabile* of Skudai, Johor. The chemical compositions of the rhizome essential oil of *Zingiber spectabile* were terpinen-4-ol, labda-8, 12-diene-15,16-dial, α-terpineol, and β-pinene [57]. In another study, [5] identified 80 compounds in oils extracted from leaves and rhizomes of *Z. spectabile*. The most abundant components in the leaf oil were β-caryophyllene and β-elemene, whereas the rhizomes yielded an oil rich in zerumbone [5]. It was also reported that the oil extracted from rhizomes was more pungent compared to the leaves [5].

The chemical compositions of *Z. zerumbet* essential oils have been widely studied in Malaysia. Majority of studies reported zerumbone as the main component of the essential oil. However, the zerumbone contents varied in the analyzed samples from the same region or different regions [44]. According to [58], major bioactive compounds found in *Z. zerumbet* were zerumbone, limonene, and humulene. [59] identified 56 active compounds of *Z. zerumbet* essential oil extracts, followed by [60] with 50 compounds, [54] with 18 compounds, and [61] with 17 compounds. The species studied originated from Penang, Selangor, Sabah, and Pahang respectively. The most abundant components of *Z. zerumbet* rhizome oil were zerumbone, α-humulene, camphene, and caryophyllene oxide [54,59,60,61]. In another study by [50] who investigated the crude extracts of *Z. zerumbet* of Selangor, 51 bioactive components were reported with humulene epoxide II and zerumbone as the major constituents of the extracts. Besides *Z. zerumbet*, zerumbone has also been identified as the major constituent in the rhizome oils of other members of the *Zingiber* genus such as *Z. ottensii* and *Z. spectabile* [5]. The major bioactive compounds extracted from *Zingiber* species in Malaysia are summarized in Table 1.

Table 1. Major Bioactive Compounds Extracted from *Zingiber* species in Malaysia

No.	*Zingiber* sp.	Origin	Part used and Method of Extraction	Number of Volatile Components	Product	Major compounds (>5%)	Source
1	*Z. cassumunar* Roxb.	Kuantan, Pahang	Rhizome (Steam distillation)	15	Essential oil	2,6,9,9-tetramethyl-2,6,10-cycloundecatrien-1-one, α -caryophyllene, caryophyllene oxide	[48]
2	*Z. cassumunar* Roxb.	Malim Nawar, Perak	Rhizome (hydro-distillation)	NA	Essential oil	3-cyclohexen-1-ol,4-methyl-1-(1-methylethyl), terpinen-4-ol	[49]
3	*Z. montanum* (J.Koenig)	Gombak, Selangor	Rhizome (Hexane crude extract)	82	Crude extract	Dimethyl 4-methylphthalate, carbendazim, 6.7-Dimethoxyquinoxaline, 2,4,5-trichlorophenol, noscapine	[50]
4	*Z. officinale* Rosc.	Bentong, Pahang	Rhizome (hydro-distillation)	82	Essential oil	α-zingiberene, geranial, neral, *Trans*-caryophyllene, Eucalyptol, β-phellandrene, camphene	[52]
5	*Z. officinale* Rosc.	Keningau, Sabah	Rhizome (hydro-distillation)	43	Essential oil	α -curcumene, geranial, geraniol	[53]
6	*Z. officinale* Rosc.	Bentong, Pahang	Rhizome (hydro-distillation)	44	Essential oil	Neral, α -curcumene, borneol, eucalyptol, neric acid	[53]
7	*Z. officinale* var. rubrum Theilade	Selangor, Malaysia	Rhizome (Hexane crude extract)	43	Crude extract	Zingerone, benzaldehyde dimethyl thiol acetal, α-curcumene, zingiberene, β-sesquiphellandrene, gingerol, 3-Methoxy-Ltyrosine	[50]
8	*Z. officinale* var. rubrum Theilade	Negeri Sembilan	Leaf (hydro-distillation)	46	Essential oil	β-caryophyllene, geranial, neral, caryophyllene oxide	[5]
9	*Z. officinale* var. rubrum Theilade	Negeri Sembilan	Rhizome (hydro-distillation)	54	Essential oil	Camphene, geranial, geranyl acetate, neral, geraniol	[5]
10	*Z. ottensii* Valeton	Johor, Malaysia	Rhizome	26	Essential oil	Sabinene, β-pinene, γ-Terpinene, Terpinen-4-ol, α-Humulene, Zerumbone	[55]
11	*Z. ottensii* Valeton	Sabah, Malaysia	Rhizome	27	Essential oil	α-Humulene, Zerumbone	[54]
12	*Z. ottensii* Valeton	Johor Bharu	Rhizome (hydro-distillation)	26	Essential oil	Zerumbone, sabinene, β-pinene, γ-terpinene, terpinene-4-ol, α-humulene	[55]
13	*Z. puberulum* Ridl.	Cameron Highlands, Pahang	Rhizome (Supercritical fluid extraction)	21	Essential oil	Palmitic acid, 1-heptatriacotanol, oleic acid	[56]
14	*Z. spectabile* Griff.	Selangor, Malaysia	Rhizome (Hexane crude extract)	50	Crude extract	1,1’-ethylenebisdecalin, 1-pentadecyne, γ-sitosterol, β-sitosterol	[50]
15	*Z. spectabile* Griff.	Skudai, Johore	Rhizome (hydro-distillation)	19	Essential oil	Terpinene-4-ol, labda-8(17),12-diene-15,16-dial, α-terpineol, β-pinene	[57]
16	*Z. spectabile* Griff.	Negeri Sembilan	Leaf (hydro-distillation)	54	Essential oil	β-caryophyllene, β-elemene, caryophyllene oxide, α-pinene, β-pinene	[5]
17	*Z. spectabile* Griff.	Negeri Sembilan	Rhizome (hydro-distillation)	56	Essential oil	zerumbone	[5]

No.	*Zingiber* sp.	Origin	Part used and Method of Extraction	Number of Volatile Components	Product	Major compounds (>5%)	Source
18	*Z. zerumbet* L. (Smith)	Selangor, Malaysia	Rhizome (hydro-distillation)	50	Essential oil	Zerumbone, humulene, camphene	[60]
19	*Z. zerumbet* L. (Smith)	Selangor, Malaysia	Rhizome (hexane extraction)	51	Crude extract	Zerumbone, humulene epoxide II	[50]
20	*Z. zerumbet* L. (Smith)	Sabah, Malaysia	Rhizome	14	Essential oil	α-Humulene	[54]
21	*Z. zerumbet* L. (Smith)	Johore	Rhizome (Crude extraction)	*NA*	Pure extract	Zerumbone	[62]
22	*Z. zerumbet* L. (Smith)	Batu Pahat, Johore	Rhizome (hydro-distillation)	*NA*	Essential oil	Zerumbone	[63]
23	*Z. zerumbet* L. (Smith)	Penang, Malaysia	Rhizome (hydro-distillation)	56	Essential oil	Zerumbone, α-humulene	[59]
24	*Z. zerumbet* L. (Smith)	Selangor, Malaysia	Rhizome (Hexane crude extract)	51	Crude extract	Humulene epoxide II, zerumbone,	[50]
25	*Z. zerumbet* L. (Smith)	Kuala Lumpur	Rhizome (Stream-distillation)	50	Essential oil	Zerumbone, humulene	[60]
26	*Z. zerumbet* L. (Smith)	Kuantan, Pahang	Rhizome (hydro-distillation)	17	Essential oil	Zerumbone, camphene, α-humulene	[61]

Major Biological Activities of *Zingiber*

Zingiber has long been used traditionally to treat minor illnesses such as stomachaches, nausea, diarrhea, sores, loss of appetite, relieving rheumatic pain, ear inflammation, and as medicine for worm infestation [43,47,64]. The crude extracts and essential oils of *Zingiber* have proven to show some biological activities such as anti-microbial [39], anti-bacterial [2], insecticidal [65], larvicidal [39,44], anti-cancer [66], anti-inflammatory [67,68], anti-ulceration [69], anti-oxidant [70], anti-fungal [45,71], immunomodulatory [72] and anti-nociceptive [60,73].

The antibacterial properties possessed by *Zingiber officinale* have been tested against the pathogenic Gram-positive and Gram-negative bacterial strains [2]. According to [2], the essential oils of *Z. officinale* were found to have a stronger effect and higher antibacterial activity against Gram-positive bacteria than Gram-negative bacteria. The presence of biologically active compounds such as zingiberene, α-curcumene, and β-sesquiphellandrene contributed to the antimicrobial properties of the essential oil [2]). In another study conducted by [52], the antimicrobial activity of *Z. officinale* essential oils was tested against fungi and bacteria. The phytopathogen was *Xanthomonas oryzae* where a strong correlation between *Z. officinale* essential oils and its bioactivities was reported. It was suggested that *Z. officinale* essential oils could be used as a new antimicrobial agent in terminating the growth of phytopathogens which is suggested as significant, environment-friendly alternatives to non-renewable fungicides and bactericides [52].

The insecticidal properties of *Z. officinale* are shown to be effective against pulse beetle *Callosobruchus maculatus* [74] *Tribolium castaneum* [75], and *Callosobruchus chinensis* [65] which species are all associated with stored grain pests. Besides, the essential oils are also effective against cowpea aphid *Aphis craccivora* Koch [76], larvae of *Spodoptera litura* [77], *Spilosoma obliqua* [78], and *Plutella xylostella* [79]. Zingiberene and α-curcumene are the major volatile constituents that are known for insecticidal and insect feeding-disincentive activities [59]. Insecticidal and larvicidal activities also demonstrated good larvicide effects towards crude extracts of *Z. officinale* var. *rubrum*, *Z. montanum, Z. spectabile*, and *Z. zerumbet* [50]. The extracts are effective against *Aedes albopictus, Aedes aegypti*, and *Culex quinquefasciatus* larvae [50] which are vectors of dengue, chikungunya and filariasis diseases, respectively. Larvicidal assays exhibited the toxicity of the hexane extracts of these four *Zingiber* species against the insects even at low dosages [50].

Besides, according to [5] the most abundant component of *Z. officinale* var. *rubrum* which is β-caryophyllene, is known for its anti-inflammatory and anesthetic activities. However, the leaf and the rhizome oils of *Z. officinale* var. *rubrum* were moderately anti-microbial active against the Gram-positive bacteria which were *Bacillus licheniformis*, *Bacillus spizizenii* and *Staphylococcus aureus*, and the Gram-negative bacteria *Eschericia coli, Klebsiella pneumoniae*, and *Pseudomonas stutzeri* [5]. *Z. zerumbet*, a wild edible ginger, has been widely studied for its diverse biological activity [39]. The rhizomes of *Z. zerumbet* are traditionally used as a puree for stomachache relief, an anesthetic for toothache, minor treatment of bruises, swellings, and strains [54,59], in treating sores, loss of appetite [63], and treating enterobiasis [67].

Crude extracts and essential oils from *Z. zerumbet* have been utilised for their antifungal and anti-mycotoxin efficacy against some pathogenic microbes such as *Aspergillus flavus* and *Aspergillus ochraceus* [44,80]. In another study conducted by [45], the antifungal activity has been shown by the essential oil of *Z. zerumbet* tested against *Aspergillus terrus, Aspergillus niger, Aspergillus flavus, Trichothecium roseum, Fusarium graminearum, Fusarium oxysporum* and *Fusarium monoliforme* [45]. The extracts and essential oils of *Z. zerumbet* have also been demonstrated effective and used for mosquito vector control [81]. The larvicidal and pupicidal activities of *Z. zerumbet* essential oil had been reported and recommended to be used as mosquito larvicide [81]. In Vietnam, the essential oil extracted from the rhizome of *Z. zerumbet* exhibited larvicidal activity and was effective in the control of tested mosquitoes; *Culex quinquefasciatus* and *Aedes albopictus* as well as the microbe, *Aspergillus niger* [44].

Apart from that, *Z. zerumbet al*so showed properties involved in cancer chemoprevention and chemotherapy [62]. Zerumbone compound in *Z. zerumbet* essential oil was known to exhibit medicinal properties such as anti-inflammatory activity and anti-tumor activity [82]. According to [62], *Z. zerumbet* extracts have a strong antiproliferative effect on human breast carcinoma (MCF-7) cell lines. Previous studies also demonstrated the antitumor activities of zerumbone and its cytotoxic effects on hepatoma (HTC) cell lines [62], which also inhibited the proliferation of human colonic adenocarcinoma cell lines [83]. It was further reported that zerumbone has the potential as a chemopreventive and chemotherapeutic strategy against cancer [62]. The crude extract as well as the active compounds extracted from the rhizome and leaves of *Z. zerumbet* have been reported to possess various pharmacological properties including anti-inflammatory [84], antitumoral [85], antioxidant [86], antibacterial [87], antiviral [88], analgesic [89], anti-allergic [90] and useful for treating stomach problems [58,91]. Anti-allergic effects also could be found in *Z. zerumbet* extracts and their properties are terpene compounds such as zerumbone, limonene, and humulene [58].

Meanwhile, the compounds 3-cyclohexen-1-ol,4-methyl-1-(1-methylethyl), and terpinene-4-ol presented in *Z. cassumunar* played an important role in the antimicrobial activities [49,92]. [93] in their studies stated the essential oil extracted from *Z. cassumunar* was found to exhibit complete fungitoxic activity. According to [94], the rhizome oil of *Z. cassumunar* was found to exhibit high antifungal activity against yeast. However, *Z. cassumunar* had very low or weak activity against bacteria tested which were *Bacillus cereus, Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa* and two fungi *Candida albicans* and *Cyptococcus neoformans* [48]. According to [56], *Z. puberulum* consists of β-elemene, a sesquiterpene hydrocarbon that was found abundantly. The compound was a novel anticancer drug, which has also been found in the *Z. officinalis* species [56].

One of the most significant compounds in *Zingiber* species is zerumbone which is a sesquiterpene phytochemical and a potential compound with anticancer, anti-inflammatory, anti-HIV properties [59] and chemopreventive [59,95-97]. The anticancer property has been shown in a study conducted by [98] who reported the compound to inhibit the multiplication of human leukemic HL-60 cells. [83,97] also suggested similar properties by testing zerumbone on human colonic adenocarcinoma cell lines. Meanwhile, the HIV-inhibitory property is shown by [99].

Genetic Diversity of *Zingiber*

Most *Zingiber* species are known to have 2n=22 chromosome number which is the lowest among genera in Zingiberaceae. The common ginger *Z. officinale* was reported to have 22 somatic chromosomes as well as six investigated species, *Z. zerumbet, Z. spectabile, Z. cylindricum Z. roseum, Z. wightianum, Z. macrostachyum* [100-104]. In rare cases, the abnormal spindle function occurred in metaphase during clonal multiplication of some cultivars of ginger resulting in the 2n=24 chromosome number [105]. [106] suggested that the somatic chromosome number 55 of *Z. mioga* indicates pentaploidy with a basic number of 11. A recent cytological study by [107] reported the tetraploid chromosome species, *Z. kangleipakense* with 2n = 44.

The majority of ginger cultivars are named for a region or a specific attribute of the cultivar such as *Zingiber* is the generic name for Ginger which has been derived from the Greek word 'zingiberis' having origin in the Sanskrit word 'singabera' meaning spice attributed to its pungent spicy rhizome [108]. This absence of obvious morphological traits among accessions, combined with the lack of cultivar identification, has caused significant difficulty in gene pool conservation and exploitation. In comparison to morphological markers, the use of molecular markers for plant genotype identification appears to be more effective because it gives direct access to the genetic material [15].

Improper flowering and seed development are potential barriers to ginger breeding. The majority of this species' plant breeding programs focus on evaluating and selecting naturally occurring clonal variants. Until samples are taken from a variety of agro-ecological environments, the level of genetic diversity in such species is limited. As a result, the ginger enhancement program places a premium on diversity analysis and the discovery of genetically dissimilar genotypes or clones [7].

Many scholars have investigated ginger genetic variation in geographical accessibility. Because they indicate changes in DNA nucleotide sequences, molecular markers are widely regarded as significant and adaptable techniques in plant breeding, development, genetic modification, and classification. Evidence suggests that molecular markers can be useful in defining and analyzing the level of genetic diversity within both species and habitats. Considering variability may improve its characterization, as well as the creation of adaptation strategies in preparation for genetic improvement [15].

Molecular Markers Available for Diversity Study

Numerous methods are used to assess genetic diversity within and between plant populations, such as morphological, biochemical (allozyme), and DNA (or molecular) marker analysis [109]. Markers can have the same modes of inheritance as other characteristics, such as dominant/recessive or codominant. A marker is considered to be codominant if the genetic pattern of homozygotes can be separated from that of heterozygotes. Codominant markers are generally more informative than dominant markers [110]. These markers can detect chromosomal variations caused by deletion, duplication, inversion, and/or insertion. Because such markers are only found near or connected to genes that govern the characteristics of interest, they do not affect the phenotype of the traits of interest [109].

DNA markers classified according to their inheritance mode include dominant where only two alleles are produced by dominant markers, which display homozygote dominants paired with heterozygotes as one composite present band and recessives as absent of band, or the other way around, respectively [111]. DNA markers may shorten the breeding cycle and genetically modify the selected crops. Plant breeders may increase the efficacy of selection with the use of genetic markers, and new molecular techniques can improve gene bank libraries and agrobiodiversity management. The ideal markers for studying genetic polymorphism (both nuclear and morphological) would be those that are polymorphic, informative, repeatable, transferable, dominant or codominant in inheritance, non-selective in abundance (neutral), and would exhibit widespread distribution across the plant genome. DNA-based molecular markers are primarily classified into two categories: those that are "based" (like RFLPs) and those that are "based" (eg. RAPDs, SSRs, ISSRs etc.). Some markers are of a hybrid kind, combining restriction and amplification of target DNA for instance, restriction fragment length polymorphism RFLPs and cleaved amplified polymorphic (CAPS) markers [112].

DNA markers are different in terms of polymorphism, developmental and running cost. The first class of markers with moderate polymorphism is often utilised for single-locus and di-allelic analysis of conserved coding regions, with methods like restriction fragment length polymorphism (RFLP) and simple sequence length polymorphism (SSLP) being particularly useful. The second class is markers which include microsatellites, may be created quickly and readily, and provide more detailed information on polymorphisms than traditional RFLPs [113]. For example, the maize genome's SSR distributions were not random; their density was highest in untranslated regions (UTR), and it gradually decreased in the promotor, intron and coding sequence regions, in that order (Vieira *et al*., 2016). In terms of DNA fingerprinting, estimating genetic diversity, evaluating seed genetic purity, helping with breeding programme selection, genetic mapping, and isolating genes. The capacity to identify polymorphisms, cost, simplicity of use, and consistency of results are all areas in which these markers vary are shown in Table 2.

Molecular approaches for identifying plant kinds or genotypes are more widely used than visual markers because they provide a direct connection to genetic material and the ability to explore plant relationships [15]. Several species of plants use molecular markers that are PCR-based for identification and analysis purposes, population genetics, phylogenetic analysis, and genetic linkage mapping. ISSR markers have a high level of repeatability [15].

Table 2. The different types of DNA markers based on technical differences used in polymorphism evaluation with important advantages and disadvantages

Molecular Marker	Acronym	Inheritance Pattern	Principle	Advantages	Disadvantage	Source
Inter simple sequence repeat	ISSR	Dominant	Polymorphisms in the inter-microsatellite DNA regions are analyzed using a PCR-based method. ISSR sequences range 15-30bp.	High polymorphic, minimal development and operating costs. 10–50 ng of excellent quality DNA is sufficient for ISSR assay. ISSR marker applications overcome many of the constraints of SSR, AFLP, and RAPD analyses.	Technical problem regarding faint bands formation and bands overlapping. ISSR sequence could be conserved or non-conserved region. Therefore, this method is best used for phylogeographical studies or to potentially regions that have access species rather than for identifying specific individuals. Less effective in combining genetic and physical characterization.	[111,113, 114]
Simple sequence repeat	SSR	Co-dominant	It is a PCR based technique. sequences range from 2 to 9 nucleotides (microsatellites marker) that distribute through coding and non-coding regions of genes.	Results in high locus specific; Can be done quickly and easily; Reveals a high degree of polymorphism. Stable and easy to replicate. Mono-locus, highly informative, simple, and automated. Useful to calculate gene flow. SSR loci reflect DNA quantitative traits thus it useful in gene mapping. To define DNA fingerprints of cultivar. Aid in link genetic, physical, and sequence-based mapping. An important tool of breeders to link phenotypic and genotypic variation.	The costs both of designing and running the operation are quite expensive. Repeats are unstable because of the high rate of mutation in these regions. Development of a primer is a difficult and time-consuming process that often necessitates the use of polyacrylamide gel electrophoresis.	[114-116]

Molecular Marker	Acronym	Inheritance Pattern	Principle	Advantages	Disadvantage	Source
Simple sequence tandem repeats	SSTR	Codominant	PCR-based methods (Microsatellites marker) concentrates on sections of DNA that contain repeat units between 2 - 6 bp in length. Distribute through both the noncoding and coding regions of a of genes. Mostly in noncoding regions.it is longer than SSR markers and shorter than VNTR.	High polymorphism Reduced stutter product formation compared to dinucleotide repeats that benefit the interpretation of sample mixtures. High discriminating power, usually >0.9.	Development costs for them are quite costly.	[113,114]
Variable number tandem repeats	VNTR	Codominant marker	PCR based technique (mini-satellites marker) 10-60 nucleotides. Generally, distribute through non-coding regions of the genome.	It has high polymorphism and is considered a valuable resource for generating RFLP genetic markers for use in genome mapping.	The disadvantage in amplifying VNTR extracted with restriction enzymes by PCR and determining their size by gel electrophoresis.	[113,114, 117]
Simple sequence length polymorphis ms	SSLP	Predominate -ly coding markers	Dinucleotide repeat sequences in intergenic DNA and inside genes are analyzed using SSLP markers for length variation.	Highly polymorphic SSLPs allow for rapid and inexpensive monitoring of inbred strains.	The majority of SSLP are single locus or di-allelic, making them unsuitable for linkage studies. Incorrect findings might be drawn from studies that use cell lines that have been misidentified or that have been contaminated.	[113,114, 118]
Sequence tagged microsatellite site	STMS	Codominant	It is a PCR based technique. Usually located in the genome's non-coding sequences. STMS flanking primers must be consistently conserved.	Highly polymorphism. It's beneficial because allele sizes can be computed with great precision and band profiles may be understood in terms of loci.	Significantly expensive to develop	[113, 114,117]

Molecular Marker	Acronym	Inheritance Pattern	Principle	Advantages	Disadvantage	Source
Restriction fragment length polymorphism	RFLP	Codominant	RFLP technique does not rely on polymerase chain reaction (PCR), but rather on restriction digestion. RFLP is useful to gather predictive data on the frequency of mutations in restriction sites.	Levels of polymorphism are moderate.	High running and development cost. They are predominantly di-allelic and single locus, rendering them useless for linkage analyses. Methodology that is too complex	[113,114, 119]
Amplified fragment length polymorphism	AFLP	Dominant	PCR-based technique The DNA is fragmented into segments using restriction enzymes, and then adaptors are grafted onto the ends. After polymerase chain reaction (PCR) amplification, fragments of different lengths may be seen in gel or capillary-based platforms.	AFLP has a high polymorphism rate.	Significant amounts of high-quality DNA are essential, time-consuming.	[114,119-121]
Single nucleotide polymorphisms	SNP	Co dominant marker.	PCR-based. Direct sequencing methodology detects a single nucleotide mutation. SNPs are found in coding and noncoding areas. novel polymorphisms to identify polymorphism alleles in target sequences.	High SNP Polymorphisms are present in coding, non-coding, and intergenic regions of genes with varying rates.	Polymorphism can be identified using a costly platform and DNA sequencing. High-quality nucleic acid is required.	[113,114, 122,123]
Sequence-tagged sites	STSs	Codominant	PCR based technique, STS is a single occurrence in the organism's DNA. It's easy to detect STS in a sequenced genome using microsatellites (SSRs, STMS, or SSRPs), SCARs, CAPs, and ISSRs.	High polymorphism and helpful for gene mapping and microdeletion detection.	Expensive STS creation requires high quality DNA and sequencing.	[113,114, 124]
Cleaved amplified polymorphic DNA	CAPS	Co-dominant	CAPS is a PCR-RFLP hybrid. It went like this: 1. Sequence the RFLP probe. 2. Design primers to amplify 800–2,000-bp DNA fragments. 3. Cloning and sequencing PCR product.	It has high polymorphism. A relatively tiny quantity of DNA is needed for PCR analysis to reveal polymorphisms. It is also locus specific.	High developmental cost.	[113,114, 125]

Molecular Marker	Acronym	Inheritance Pattern	Principle	Advantages	Disadvantage	Source
Random amplified polymorphic DNA	RAPD	Co-dominant	Technology based on PCR. This method uses a single primer (10-mer) to perform random amplification in accordance with predetermined PCR parameters. Distribution and abundance of annealing sites throughout the genome determine the total amount of amplified fragments.	A highly polymorphic marker Even DNA of low quality will do. Cheap to develop and running.	The dominant expression of alleles is the most limiting feature of RAPD markers, since it complicates the understanding of multi-locus patterns.	[113,114, 119]
Sequence-characterized amplified regions	*SCAR*	Co-dominant and mono-locus markers	SCAR markers are made by purifying PCR fragments and creating SCAR primer. Polymorphic bands are discovered using agarose gel, and then the DNA's nucleotide sequence is examined. The sequence of this polymorphic DNA is analyzed using the NCBI's database. Then, this polymorphic DNA sequence is used to create SCAR primers.	SCAR markers are straightforward, reliable, and repeatable to analyze. SCARs are reproducible, unlike RAPDs. Converting RAPDs to SCARs may provide a co-dominant marker. AFLP, ISSR, SSR, and RFLP less reliable for herbal drug verification. SCAR markers may be able to overcome the constraints of ITS sequences.	High developmental cost.	[113,114, 122,126, 127]

The method of evaluating the degree of genetic variation among individuals, groups, and communities is known as "genetic diversity studies." The study contains quantitative data as well as a variety of variables. Pedigree, biochemical, morphological, and identification data have all been used to assess genetic diversity in crops. DNA-based marker information, on the other hand, has been utilized to acquire more accurate genotype variation. A molecular-based approach, including DNA analysis and isozyme, can be used to obtain the extent of diversity uniquely. To understand the molecular foundation of various biological functions, there is a need to study and analyze genetic variation. The molecular characterization of cultivars is a vital factor in ginger genetic resource conservation and exploitation. In terms of actual considerations, the ongoing production of novel cultivars necessitates the establishment of innovative procedures for determining genetic purity. According to interest in commercializing various ginger cultivars as fresh spice crops, it is now required to define the genetic diversity found in cultivars, natural populations, and advanced selections [128].

Several authors investigated inter-specific DNA-based evolutionary relatedness in the *Zingiber* genus. *Zingiber officinale* is the most common *Zingiber* species to which molecular markers have been applied. The rbcL sequences were utilized to investigate the genetic similarity of 9 *Zingiber* cultivars from South India. On an industrial level, RAPD analysis can be used to examine the genetic integrity of micropropagated *Zingiber officinale* plants produced in vitro being a portion of a crop development effort [107].

There are few publications on the genetic diversity of *Z. zerumbet* that used RAPD markers to examine the genetic diversity of this species obtained from Thailand. This plant's genetic diversity was also revealed using the AFLP marker. Neither one of those publications goes into detail regarding the genetic diversity within this species based on geographical germplasm. However, the ISSR marker to be an essential approach for assessing genetic variety [129]. Furthermore, RAPD primers are the least reliable, more sensitive to reaction circumstances, and least consistent. To overcome these limits, the diversity of ISSR-markers has been explored in numerous plants. It is a significant tool for studying genetic diversity and has a cheap analytical cost. As a result, the study was carried out to examine the chemical diversity in the essential oil content of *Z. zerumbet* from various locations in Eastern India using GC/MS research, as well as the genetic variation amongst them via ISSR markers [130].

ISSR primers are typically 16–25 base pairs (bp) in length and are made up primarily or entirely of repeating DNA motifs about 2 to 4 bp each, designed to be complementary to microsatellite areas in the genome. ISSR primers are classified into three types based on their usage: unanchored (primer consists only of a repeated motif, that is, 5'–(AC)8–3'), 5'-anchored (primer consists of a repeated motif with one or several non-motif nucleotides at the 5'-end, for example, 5'–GA(AC)8–3'), and 3'- anchored (primer consists of a repeated motif with one or several non-motif nucleotides at the 3'-end, e.g., 5'–(AC)8–3') [131]. The effects of employing these various primers for the production of ISSR bands are thoroughly examined. As a result, researchers recommend using either the 3'-or 5'-anchored ISSR primers for research aimed at evaluating genetic diversity. Unanchored ISSR primers may slide across the microsatellite area under PCR, resulting in uneven amplification in each cycle and impacting repeatability [132].

Conclusions

Ginger has been the topic of intense scientific inquiry over the last few decades due to its abundance, low cost, and safety in intake. This review concludes that the genus *Zingiber* encapsulates botanical diversity, potent bioactivities, and genetic richness, epitomized by the widespread use of medicinal ginger. Its efficacy in mitigating motion sickness and supporting cancer treatment underscores its therapeutic relevance. Moreover, ginger's affordability and safety have spurred intense scientific exploration. This study emphasizes the pivotal role of genetic diversity in breeding programs and germplasm conservation, highlighting the common ginger's potential as a key player.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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