Syzygium cumini (L.) Skeels: Cardiometabolic Properties and Potential Tissue Culture-Based Improvement of Secondary Metabolites Production

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Abstract

Syzygium cumini (L.) Skeels (family Myrtaceae), commonly known as jambolão, jambolan, or jamun, has been suggested as a potential source of bioactive molecules against diabetes and associated cardiometabolic diseases. A wide variety of secondary compounds, mainly, terpenes, and phenolic compounds, such as phenolic acids, flavonoids, and tannins, are present in different parts of this plant species. This chapter describes about the various pharmacological properties of *S. cumini*, including antihyperglycemic, antihyperlipidemic, anti-inflammatory, and antioxidant activities, which make it a very interesting species for multitarget therapeutic purposes. Geographical distribution, botanical description, as well as potential of this plant species for in vitro culture have been discussed. Review of literature shows that despite the recalcitrant nature of this plant species, attempts have been made to standardize the protocol for its micropropagation. Although cells and tissues of *S. cumini* have capacity for in vitro production of bioactive compounds, but basic studies for their mechanisms of production as well as full understanding of biosynthesis pathways are required to be known to exploit the potential of this plant species. Further research towards employing novel elicitors, two-phase culture system, and metabolic engineering may also help in improving the performance of *S. cumini* under in vitro culture system.

Keywords

Antihyperglycemic • Anti-inflammatory • Antioxidant • Bioactive compounds • Jamun • In vitro culture • Medicinal plant • Metabolic syndrome • Micropropagation • Myrtaceae • Pharmacology

1 Introduction

Myrtaceae is a monophyletic family from Myrtales order which comprises about 132 genera and over 5,600 species mainly composed of trees and shrubs [1]. In Brazil, it comprises of about 24 genera and 927 identified species [2]. *Syzygium cumini* (L.) Skeels, formerly classified as *Eugenia jambolana* Lam., is one of the important plant species possessing highest pharmacological and economic potential. This plant species has been proposed as a potential source of bioactive molecules against diabetes and its associated cardiometabolic diseases [3–6]. *S. cumini* is native from Indian subcontinent but widely distributed in tropical and subtropical areas (Fig. 1), where it may be either categorized as an exotic [7] or naturalized [2] species. It is popularly known as jamun or jambul in India, Indian black berry in England, faux pistachier in France, black plum in the United States, jambolan in Spanish-spoken countries, and jambolão in Brazil [8].

Seeds of *S. cumini* have been used for centuries by Indian Ayurvedic medicine practitioners for diverse therapeutic purposes [9]. However, it was only during the nineteenth century that British traders imported it to Europe because of its unique

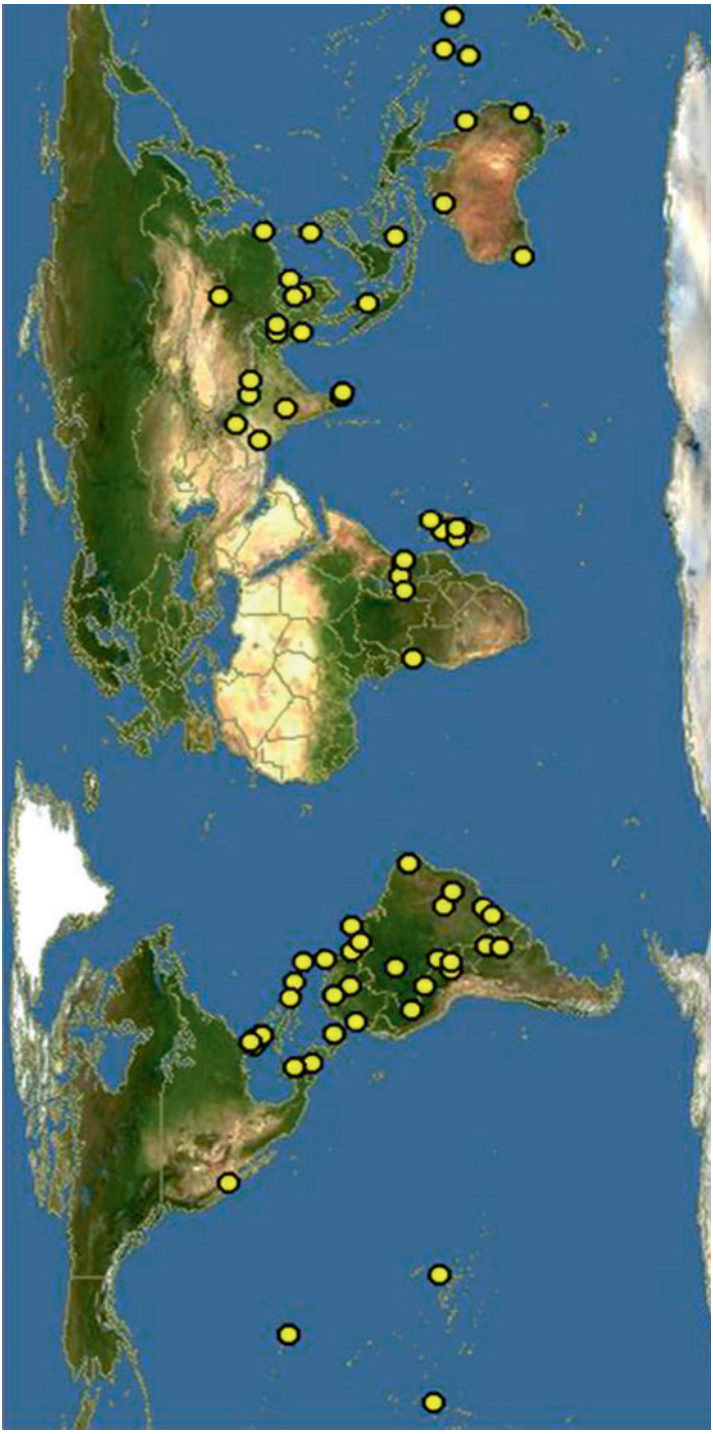

Fig. 1 Phytogeographic domains of *Syzygium cumini*. Illustration showing tropical and subtropical distribution of *S. cumini* (Source: US National Plant Germplasm System: GRIN Taxonomy. Accessed via http://www.gbif.org/species/101390909 on 2016-03-24)

property to decrease urine sugar content in diabetic patients. The first morphological, phytochemical, pharmacological, and clinical reports on the species and its antidiabetic effect were published in the 1880s and readily attracted the attention of scientific community. As a result the use of jambolan seeds had already been included in the therapeutic and phytotherapeutic standard literature by the year 1990 [6]. Ever since, *S. cumini* became a worldwide cultivated medicinal plant. Currently, most of the knowledge on the pharmacological properties of *S. cumini* has come from experimental studies, with very limited data coming from clinical trials. On the other hand, clinical trials demand large amounts of extracts or its isolated and identified secondary metabolites, whose obtention would be considered extremely laborious or ecologically unsustainable.

Biotechnological methods by employing plant cell, tissue, and organ culture techniques offer an important alternative for micropropagation as well as in vitro production of bioactive molecules of interest without harvesting the plants from nature [10–14]. In *S. cumini*, micropropagation technique has been employed for mass multiplication. The other advantage of using in vitro method is to obtain plants throughout the year without any risk of disease. However, there are several problems associated with standardization of protocol for in vitro establishment and culture of tree species like *S. cumini.* This chapter will address the difficulties in raising in vitro cultures in *S. cumini* as well as future challenges. The chapter commences with the short description of plant species, followed by pharmacological properties and tissue culture potential.

2 Botany

S. cumini presents as trees ranging from 10 to 15 m high; evergreen, often twisted trunk, branches (Fig. 2a). Leaves simple, opposite, shortly petiolate, as petiole 16–23 mm length; leaf blade from 5.5 to 13 cm length × 3.5–5.5 cm width, elliptic or elliptic-lanceolate, apex acuminate, base attenuate, margin entire, prominent midrib on the abaxial and furrowed face on the adaxial face, secondary veins 35–45, texture cartácea type, discolor (each side of the leaf exhibits a different shade), venation pattern brochidodromous, numerous oleíferos channels present in the form of translucent glands (Fig. 2c). Inflorescence in panicles or panicles of cyme, 31–59 mm length, terminal, and extra-axillary lateral; bracteoles 0.5–0.6 mm length × 0.3–0.4 mm width, elliptic, caducous before or after anthesis. Sessile flowers; bisexual, actinomorphic, dichlamydeous, sepals with indistinct lobes, persistent calyx tube; petals 4–5, measuring 2.5–3.5 mm length; stamens numerous, free each other, whitish; ovary inferior, two-locular, plain, stiletto 5–5.5 mm length (Fig. 2b). Drupaceous fruit, ellipsoid, 13–17 mm length × 7–8 mm width, plain, varying between red and black (Fig. 2d). Seed ovoid, about 1.5 cm length (Fig. 2e). Description is based on the following examined material: São Luís, Maranhão, Brazil, Campus da UFMA, fl., E.B. Almeida Jr. & I.F. Amorim 1094, 11/IV/2014 (MAR 4574); Campus da UFMA, fl. fr., A. Kely & E.B. Almeida Jr. 01, 10/III/2014 (MAR 4694); Campus da UFMA, próximo ao prédio de anatomia, fl., E.B. Almeida

Fig. 2 **Botanical aspects of** *Syzygium cumini* (L.) Skeels: (**a**) tree picture, (**b**) detail of flower and stamens, (**c**) leaves, (**d**) fruit (color varying between *red* and *black*), (**e**) open fruit with detail for seed (Figures adapted: **a**, **b**, Mercadante, 2016; **c**, Upcavage, 2016; **d**, **e**, Santos 2015)

Fig. 3 **Specimen of** *Syzygium cumini* (L.) Skeels: Specimen recorded in Herbarium of Maranhão (*MAR*)

Jr. & A.N. Silva 1093, 11/IV/2014 (MAR 4573); and Campus Bacanga-CCBS, fl., D.N de. Melo 01, 09/V/2014 (MAR 7556) (Fig. 3).

3 Phytochemistry

S. cumini presents a wide variety of secondary metabolites thoroughly distributed on its different parts, from roots to fruits, mainly consisting of terpenes and phenolic compounds, such as phenolic acids, flavonoids, and tannins. Early assessment of its phytochemical profile, performed in late nineteenth century, led authors to propose jambulin, a yellow and unstable glucoside extracted from its seed, as responsible for the already well-known glucose-lowering effects of jambolan. Half a century later, Wastl and coworkers stated that no substance of glycosidic nature could be found in the plant even though jambolan's fame remained [6]. Table 1 summarizes the main compounds already identified in different parts of *S. cumini*. The leaf contains high levels of flavonoids, especially quercetin and myricetin derivatives, besides simple phenols like ellagic acid, ferulic acid, and gallic acid. The leaf presents a terpene-rich

Table 1 Phytochemical compounds identified in different parts of *Syzygium cumini* tree

Part	Class	Compounds	References
Leaf	Flavonoids	Catechin, (*epi*)gallocatechin-(*epi*)gallocatechin-*O*-gallate, kaempferol, myricetin, myricetin deoxyhexoside, acetylated myricetin deoxyhexoside, acetylated methylmyricetin deoxyhexoside, myricetin 3-*O*-β-D-glucuronopyranoside, myricetin-4′-methyl ether 3-*O*-α-rhamnopyranoside, myricetrin 4″-*O*-acetate, myricetrin 4″-*O*-acetyl-2-*O*-gallate, myricitrin, quercetin-3-*O*-α-rhamno_ pyranoside	[17, 18]
	Phenolic acids	Caffeic acid, chlorogenic acid, ellagic acid, ferulic acid, gallic acid	[17, 19, 20]
	Tannins	Nilocitin, HHDP-glucose, pedunculagin I, casuarinin, trigalloylglucose, tetragalloylglucose, pentagalloylglucose	[17, 18]
	Terpenes	α-Pinene, α-cadinol, pinocarvone, pinocarveol, α-terpineol, myrtenol, eucarvone, muurolol, myrtenal, cineole, geranylacetone	[21, 22]
Seed	Flavonoids	Quercetin, rutin, 3,5,7,4′-tetrahydroxy flavanone	[23, 24]
	Phenolic acids	Caffeic acid, ellagic acid, ferulic acid, gallic acid	[23]
	Tannins	Corilagin, 3,6-HHDP-glucose, 4,6-HHDP-glucose, 1-galloylglucose, 3-galloyl glucose	[23]
	Terpenes	α-Terpineol, β-pinene, β-terpinene, betulinic acid, eugenol	[24, 25]
Fruit	Flavonoids	Myricetin, myricetin deoxyhexoside	[26]
	Phenolic acids	Ellagic acid, gallic acid	[26, 27]
	Tannins	HHDP-galloylglucose, trigalloylglucose	[26]
	Terpenes	Citronellol, geraniol, hotrienol, nerol, β-phenylethanol, phenylpropanal	[28]
	Anthocyanins	Cyanidin, delphinidin, petunidin	[29]
Flower	Flavonoids	Kaempferol, myricetin, dihydromyricetin, myricetin-3-L-arabinoside, isoquercetin, quercetin, quercetin-3-D-galactoside	[30]
	Phenolic acids	Ellagic acid	[31]
	Terpenes	Eugenol, oleanolic acid	[16]
Bark	Flavonoids	Myricetin, quercetin, kaempferol	[31]
	Phenolic acids	3,3′-di-*O*-methyl ellagic acid, 3,3′, 4-tri-*O*-methyl ellagic acid, gallic acid	[16, 23]
	Terpenes	β-Sitosterol, friedelin, betulinic acid	[16]

essential oil, which contains α-cadinol, pinocarvone, and pinocarveol. A number of hydrolyzable tannins have also been identified in leaves, mainly consisting of galloyl monosaccharides, which resulted from esterification of glucose by three to five gallic acid units. Interestingly, it has been shown that the number of galloyl units determine

antioxidant capacity of galloylglucose isomers [15]. The flower and seed show similar phytochemical profile, whereas there is some scarcity of flowers' studies. The seed particularly contains hydrolyzable tannins, terpenes, and phenolic acids. The fruit is rich in anthocyanins such as cyanidin, delphinidin, and petunidin, which give it a typical bright purple color. The stem and barks essentially contain the same phenolic profile found in the leaves [3, 9, 16]. From all phytochemical classes mentioned above, (poly)phenolic compounds are considered as the most prominent compounds responsible for the pharmacological properties described in the next sections.

4 Pharmacological Properties

Traditional practitioners of Indian Ayurveda medicine have applied *S. cumini*-based remedies for countless illnesses over the centuries [9]. Its particular capacity to decrease urine sugar content in diabetic patients has consistently impelled scientific researchers to investigate its antidiabetic and metabolism-related properties since the late nineteenth century, even before the discovery of insulin [6]. Ever since, a number of in vitro, in vivo, and even clinical studies have validated multiple pharmacological properties for jambolan, as described in the following sections.

4.1 Antihyperglycemic Activity

Blood glucose-lowering activity of *S. cumini* has been assessed with extracts prepared from its different parts, although the spotlight has been mainly put on the seed. Administration of ethanolic extract of seed kernel (100 mg/kg/day) for 30 days to streptozotocin-induced diabetic rats decreased both glycemia and glycosuria [32]. Similarly, flavonoid-rich extract of seed further restored peripheral glucose tolerance in the same animal model, an effect ascribed to adipocytic activation of peroxisome proliferator-activated receptors (PPAR) alpha and gamma [33, 34], whose downstream pathways control fatty acid oxidation, adipocyte differentiation, and insulin sensitivity (Fig. 4). Quercetin, a flavonoid importantly found in jambolan seeds, has also been shown to improve adipocyte glucose uptake via PPAR gamma upregulation [35]. On the other hand, glucose-lowering effect of seed and bark extracts has also been attributed to direct stimulus of insulin release from pancreatic beta cells of rats, a property further extended to leaf extracts [3, 36] (Fig. 4). Among flavonoids found in *S. cumini*, quercetin and quercetin-3-rutinoside are able to improve insulin release through stimulation of calcium influx [37, 38]. More recently, Sanches and coworkers [18] demonstrated that a polyphenol-rich extract from jambolan leaves improved metabolic status of monosodium L-glutamate-induced obese rats by dual effect on peripheral insulin sensitivity and insulin release modulation (Fig. 4). These effects were mainly ascribed to myricetin and its derivatives, which have been shown to improve insulin signaling pathways in skeletal

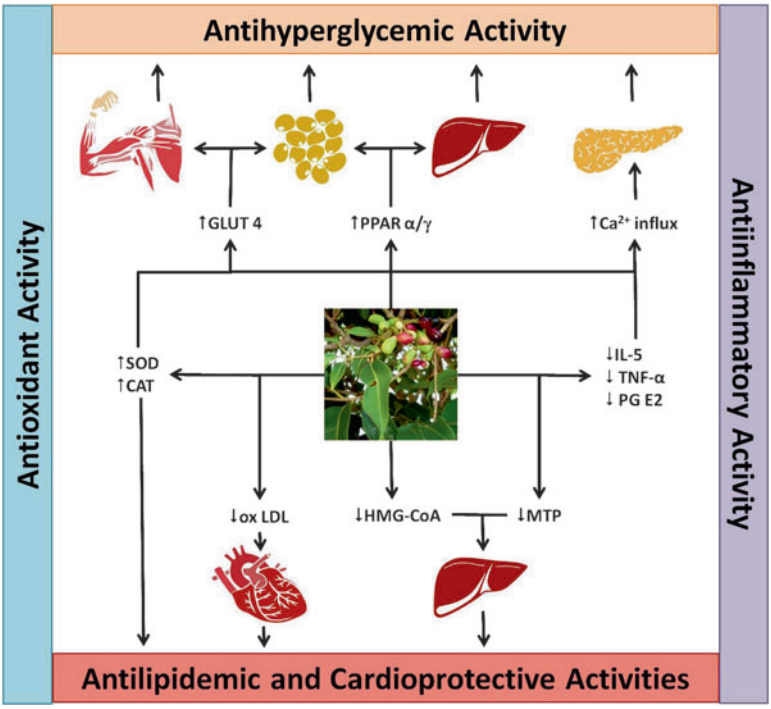

Fig. 4 Main pharmacological activities of *Syzygium cumini*. Extracts prepared from different parts of *S. cumini* have been shown to contain compounds with antihyperglycemic, antihyperlipidemic, anti-inflammatory, and antioxidant activities. Abbreviations: *SOD* superoxide dismutase, *CAT* catalase, *ox LDL* oxidized LDL, *HMG-CoA reductase* 3-hydroxy-3-methyl-glutaryl-CoA reductase, *MTP* microsomal triglyceride transfer protein, *GLUT4* glucose transporter 4, *IL-5* interleukin-5, *TNF-α* tumor necrosis factor-alpha, *PGE2* prostaglandin E2, *PPAR* peroxisome proliferator-activated receptors

muscles [39] and adipocytes [40], as well as to protect pancreatic beta cells from cytokine-induced cell death [41].

Besides the aforementioned mechanisms, other insulin-dependent effects have been shown for jambolan extracts. Methanol extract of leaf was seen to elevate mRNA expression of glucose transporter 4 and phosphatidylinositol-3 kinase, important mediators of insulin actions on adipocytes and skeletal muscle [42]. Moreover, there are studies demonstrating that *S. cumini* increases hepatic and muscular glycogen stores, as well as inhibits pancreatic alpha-amylase activity. As shown in Table 1, *S. cumini* leaf contains (*epi*)gallocatechins, important proanthocyanidins with inhibitory activity against alpha-amylase, a particularly interesting property for diabetes treatment [43]. Both properties directly impact carbohydrate metabolism and further support the use of this plant species for diabetes control.

4.2 Antihyperlipidemic Activity

Although less extensive than antidiabetic, studies on antilipidemic properties of *S. cumini* have demonstrated that flavonoid-rich extract of its seeds reduces blood levels of total cholesterol, LDL cholesterol, and triglycerides while increasing HDL cholesterol levels [33]. Flavonoids have acknowledged hypolipemiant activity mainly achieved by inhibition of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase, a key enzyme on cholesterol biosynthesis [44] (Fig. 4). Such ability to decrease serum lipoprotein levels also gives jambolan a cardioprotective-associated activity. In fact, α-hydroxy succinamic acid, a compound isolated from *S. cumini* fruit pulp aqueous extract, was able to improve endothelial dysfunction markers, like oxidized LDL, as a consequence of lowering serum lipoproteins and triglyceride levels associated to anti-inflammatory effects [45]. Moreover, pancreatic lipase inhibition has been considered the mechanism by which *S. cumini* extracts decrease gut absorption of triglycerides [46]. Our research group has been interested on the effects of polyphenol-rich extract of jambolan's leaves on triglyceride metabolism. Unpublished data shows that this extract restores normal blood triglyceride levels by inhibiting both expression and activity of the hepatic isoform of microsomal triglyceride transfer protein (MTP), which is responsible for very low density lipoprotein (VLDL) particle assembly and exportation into the bloodstream (Fig. 4).

4.3 Anti-inflammatory Activity

Assessment of anti-inflammatory properties of *S. cumini* has demonstrated antiedematogenic effects promoted by its different parts. Methanol and ethyl acetate extracts of leaf, as well as ethanolic extract of bark, reduced edema volume in both acute [47, 48] and chronic [49] inflammatory animal models. These effects have been attributed to inhibition of leukocyte migration consequent to lower production of secondary mediators like prostaglandin E2, serotonin, and histamine. Furthermore, *S. cumini* has also proven to be effective against allergies [50] and lethal sepsis [51] through modulation of inflammatory versus anti-inflammatory cytokine production, such as interleukin-5 and tumor necrosis factor-alpha (Fig. 4).

4.4 Antimicrobial Activity

Hydroethanolic extract of *S. cumini* leaf has been shown to be active against *Candida krusei* and multiresistant bacteria like *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. Besides leaves, aqueous and methanolic extracts of seed have also been shown to be effective against Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative (*Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli*) bacteria, as well as fungi, which include *Candida albicans*, *Aspergillus flavus*, *Aspergillus*

fumigatus, and *Aspergillus niger* [52]. Such antimicrobial activity has been ascribed to tannins and other phenolic compounds present in the extracts [53].

4.5 Antioxidant and Antigenotoxic Activities

Antioxidant capacity of *S. cumini* has been vastly assessed in both in vitro and in vivo studies, which have given it a role of prominent source of cardiometabolic bioactive compounds [3]. Considering in vitro studies, methanol extract of *S. cumini* leaf and branch has been shown to scavenge $OH^{\bullet}$ and $DPPH^{\bullet}$·radicals and decrease Fe^{3+} reduction in the FRAP assay in straight correlation with the high content of polyphenols and flavonoids present in the extract [54]. Similarly, antioxidant activity of ethyl acetate fraction prepared from methanolic extract of *S. cumini* leaf was also correlated with its polyphenolic composition, especially to ferulic acid and catechins present in the extract [19]. Finally, ethanolic extracts of fruit and seed, at different concentrations, showed strong antioxidant activity assessed by mean of distinct assays, such as $ABTS^{\bullet+}$, $DPPH^{\bullet}$, FRAP, and ORAC [55]. Noteworthy, $ABTS^{\bullet+}$ method has been shown to correlate with other antioxidant assays that simulate physiological radical reactions, reinforcing the potential activity of polyphenols and flavonoids contained in *S. cumini* [56].

In vivo studies have further reinforced the abovementioned findings. Pretreatment of jambolan extracts was found to increase the activity of antioxidant enzymes such as superoxide dismutase and catalase in animals challenged with oxidative stressors like urethane, 7,12-dimethylbenzanthracene [57], or cyclophosphamide [58] (Fig. 4). Additionally, these treatments also reduced oxidative chromosomal damage, which enable *S. cumini* extract for antigenotoxic purposes, an activity further supported by findings that seed extract was showed to be effective against implantation and proliferation of benzopyrene-induced gastric carcinomas [59]. Noteworthy, the plethoric polyphenolic composition of *S. cumini* is thought to underlie not only its antioxidant capacity but virtually all the pharmacological properties herein discussed, which makes it a very interesting species for multitarget therapeutic purposes.

5 Tissue Culture

The importance of tissue culture approach for the propagation and tree improvement in woody plant species has already been known and described well in literature [60, 61]. It is generally not easy to culture the explants derived from mature trees due to number of factors including recalcitrant nature, microbial contamination, phenolic exudation, limitation of episodic growth patterns, vitrification, and difficulty in root induction [62, 63]. There are not many reports of in vitro studies in *S. cumini* probably because of abovementioned problems in this woody plant species. However, attempts have been made by several researchers to propagate *S. cumini* under in vitro conditions for mass multiplication and to obtain plants at any time of the year

without the risk of disease. In 1990, Yadav and his coworkers described the procedure for in vitro propagation from seedling-derived explants in *S. cumini* [64]. Due to difficulty in surface sterilization of explants from field-grown trees, in vitro raised seedlings were used for the micropropagation and regeneration studies [64–66]. Different explants, viz., epicotyl, nodal segments, shoot tips, zygotic embryos, leaves, and roots, have been reported to be used for in vitro studies of this plant species (Table 2) [65, 67]. In vitro propagation depends on the physiological state of explants as nodal explants were found to be more responsive than shoot tips [64]. Jain and Babber [65] reported the recurrent production (an average of 8.6 shoots/explant) of *S. cumini* from epicotyl segments bearing scaly leaves (nodes) cultured on Murashige and Skoog (MS) [68] medium supplemented with 6-benzylaminopurine (BAP; 4.4 μM). To see the development of strong root system, three different auxins, viz., α-naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA), and indole-3-butyric acid (IBA), at 0.2–1 mg/l concentration were added to Knop's medium with 2 % sucrose. The best rhizogenic response was seen on medium fortified with 1 mg/l IAA [67]. The rooted plantlets have been reported to transfer to soil and then to fields after an acclimatization of 7–8 months. The plants were found to thrive well for more than 3 years without any apparent phenotypic aberrations. The authors reported the development of an efficient protocol to raise plants of *S. cumini* at any time of the year.

Later in 2003, Jain and Babbar [67] tried to develop a protocol for in vitro regeneration of plants from explants derived from mature trees of *S. cumini* with the aim that for micropropagation, it is preferable to use explants from mature plants where superior characteristics are evident. Nodal explants from mature trees of *S. cumini* cultured on BAP (1 mg/l)-supplemented medium induced only the greening and opening of incipient shoot buds, but for further elongation and normal development of these buds, an additional supply of reduced nitrogen was required which was not required in case of explants taken from in vitro grown seedlings [67]. Therefore, MS medium supplemented with BAP (1 mg/l), casein hydrolysate (1.5 g/l), or glutamine (200 mg/l) was devised for shoot initiation and multiplication from ex vitro plants of *S. cumini*. For rooting, these in vitro shoots were transferred to Knop's medium containing NAA (2 mg/l).

Different cytokines, viz., 6-benzylaminopurine, kinetin, thidiazuron (TDZ), and 2-iP, have been tried either alone or in combination with an auxin NAA for optimum shoot multiplication of this plant species. BAP (7.5 shoots per node after 6 weeks of culture) was reported to be the most suitable cytokine followed by Kn and 2-iP for the shoot multiplication from axillary buds in this plant species [69]. The best rooting was reported on half-strength MS medium with NAA (0.5 μM) [69].

There are reports on indirect shoot regeneration and somatic embryos from callus in *S. cumini* [70, 71] (Iyer and Gopinath 2000; Yadav et al. 2014). Callus obtained from seedling explants produced shoot buds on MS medium with BAP (0.5–2.0 mg/l) singly or in combination with NAA (0.05–0.2 mg/l). About 85 % of shoot buds were reported to form shoots and subsequently roots on medium containing BAP (1 mg/l) + NAA (0.05 mg/l) and IBA (1 mg/l) or NAA (1 mg/l) [70].

Table 2 Use of basal medium, plant growth regulators, additives, and response of different explants in *S. cumini* under in vitro conditions

Explant used	Culture medium[a] + sucrose (%)	Plant growth regulators[b]	Additives	Response	Reference (s)
Shoot tips, nodal segments from in vitro raised plants	MS +3	BAP (4.5 μM) or BAP (1.12 μM) + NAA (0.25 μM)	–	Shoot proliferation	[64]
In vitro shoots	MS +3	IBA (2.5, 5.0 μM) or NAA (2.7, 1.14 μM)	–	Root initiation	
In vitro shoots	MS +3	–	–	Root elongation	
Epicotyl segments from in vitro grown shoots	MS +4	BAP (1 mg/l)	–	Multiple shoot formation	[65]
In vitro grown shoots	Knop's +2	IAA (1 mg/l)	–	rooting	[65, 67]
Nodal or microcuttings from mature trees	MS +4	BAP (1 mg/l)	Casein hydrolysate (1.5 g/l), glutamine (200 mg/l)	Shoot bud elongation	
Seeds	Knop's + 1	–	–	Seed germination	[71]
Zygotic embryos	MS +3	–	Activated charcoal (0.25 %)	Plantlet formation	
In vitro shoots	MS +7	IBA (4 mg/l)	–	Root initiation	
In vitro shoots	MS +10	IBA (4 mg/l)	–	Root elongation	
In vitro shoots	MS +3	2,4-D (5 mg/l) + NAA (1 mg/l)	Coconut milk (15 %)	Callus formation	
Callus	MS +10	IBA (4 mg/l)	–	Somatic embryos	
Nodal segments from in vitro raised plants	1/2MS +3	BAP (4.4 μM)	–	Multiple shoots	[69]
In vitro shoots	1/2MS + 3	NAA (0.5 μM)	–	Root formation	

(*continued*)

Table 2 (continued)

Explant used	Culture medium[a] + sucrose (%)	Plant growth regulators[b]	Additives	Response	Reference (s)
In vitro seedlings	MS + 3	2,4-D (0.05–1.0 mg/l) + BAP (0.5–2.0 mg/l)	–	Callus formation	[70]
In vitro seedling-derived callus	MS + 3	BAP (1.0 mg/l) + NAA (0.05 mg/l)		Shoot regeneration	
In vitro seedling-derived callus	MS + 3	IBA (1.0 mg/l) or NAA (1.0 mg/l)		Root initiation	
In vitro seedling-derived callus	MS + 3	–		Root elongation	

[a]*MS* Murashige and Skoog [68], *Knop* Knop [73]

[b]*BAP* 6-benzylamino purine, *IAA* indole-3-acetic acid, *IBA* indole-3-butyric acid, *NAA* α-naphthaleneacetic acid, *2,4-D* 2,4-dichlorophenoxyacetic acid

In another report by Iyer and Gopinath [71], immature zygotic embryos were cultured on MS medium containing 3 % sucrose and activated charcoal (0.25 %) to obtain plantlets. Activated charcoal has been reported to use in in vitro studies of many plant species due to absorption of phenolic exudation and soil-like properties, which help in growth enhancement. Shoots when placed vertically on medium with enhanced concentration of sucrose (7 %) and IBA (4 mg/l) were reported to form roots. On the other hand, callus was formed from shoots when placed horizontally on medium with 3 % sucrose, 2,4-D (5 mgl), NAA (1 mg/l), and coconut milk (15 %). Indirect somatic embryogenesis was obtained upon transfer of callus to medium with high concentration of sucrose (10 %) and replacing the auxins 2,4-D and NAA with IBA (4 mg/l) [71].

The agar is the most frequently used gelling agent in tissue culture media due to its inertness, stability, and nontoxicity. However, the use of isabgol (the husk derived from the seeds of *Plantago ovata*) as a gelling agent has also been described for seed germination as well as shoot and root formation [72]. The efficiency and response of *S. cumini* cultures were compared with media gelled with agar or isabgol. The seeds cultured on agar or isabgol media showed the initiation of germination after 3 weeks of inoculation and growth, morphology, and the percentage of shoot as well as root formation were found to be almost similar without any significant difference in both the media [72].

6 Genetic Transformation Studies in *S. cumini* and Related Species of the Family Myrtaceae

Genetic transformation is an important advancement in biotechnology for introducing a gene of interest especially to tree and woody plant species without disturbing its genetic global organization. In family Myrtaceae, there are examples of genetic transformation studies in economically important plant species including *Eucalyptus tereticornis* Sm., *Eucalyptus camaldulensis* Dehnh, *Psidium guajava* L., and *Verticordia grandis* J.L. Drumm [74–77]. *Eucalyptus tereticornis* Sm. is one among the economically important plant species, mainly used for raising plantations due to its use as fuel wood and raw material for paper pulp. Precultured cotyledons and hypocotyls of this plant species were co-cultured with *A. tumefaciens* strain LBA 4404 harboring the binary vector pBI121 (containing *uid* A and *npt* II genes) to obtain transformed plants upon direct regeneration. Transgenic nature of plants was confirmed by polymerase chain reaction and southern hybridization [74]. In another report by Aggarwal et al. [75], a procedure for *A. tumefaciens* mediated T-DNA delivery into the tissues taken from selected *E. tereticornis* elite clones, and subsequent regeneration of transformed shoots has been developed, with the aim to introduce the useful properties in this commercially important tree species. Similarly agrobalistic method has been used for genetic transformation in *E. camaldulensis* Dehnh [78]. Stummer et al. [77] described a system for transformation and regeneration of transgenic *V. grandis* J.L. Drumm by using *A. rhizogenes*. In *S. cumini*, however, there are no reports on genetic transformation studies.

7 Conclusions

Literature studies show that *S. cumini* is an important multipurpose medicinal plant species possessing various pharmacological properties. These properties are attributed to the presence of various bioactive molecules. Despite therapeutic potential for a number of diseases, there is lack of research in understanding the mechanisms involved, clinical trials. There are scanty reports on establishment of micropropagation protocols due to recalcitrant nature of this tree species. However, in vitro studies depict that this plant species has potential for in vitro production of bioactive compounds of interest, but lack of fundamental research and poor understanding of biosynthesis pathways involved impede the genetic manipulation strategies. Further studies are required to be focused on proper understanding of pathways involved in bioactive compounds of interest. An increase in production of compounds of interest using cell culture technique would be helpful in obtaining compounds of interest in an eco-friendly way. The strategies like elicitation, two-phase culture system, immobilization, and metabolic engineering may help in bringing this medicinal plant species as one of the promising sources of herbal product to prevent several diseases.

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