

# Karanja (*Milletia pinnata* (L.) Panigrahi): a tropical tree with varied applications

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Abstract Milletia pinnata, popularly known as Karanja, is a perennial tropical tree indigenous to India, South-Eastern Asia, and Australia. This highly favored oilseed plant is used in agroforestry as well as for restoration of wastelands due to its moderate tolerance to drought and salinity. Karanja tree, though widely investigated as biofuel feedstock producer, possess wider commercializing values that can be exploited in agricultural and industrial sectors. The oil, leaves, and bark of the Karanja tree have remarkable medicinal properties. In contrast, oil cakes have broad pesticidal applications due to the presence of various bioactive constituents (e.g., karanjin and pongamol). Due to richness in proteins and fatty acids, oil cakes have also been utilized as feedstock for industrially relevant enzyme production and animal feed. In this paper, we have presented the recent and updated review of varied applications of Karanja biomass classified into medicinal, industrial, and agricultural applications.

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# Introduction

Karanja (Millettia pinnata (L.) Panigrahi) is a promising oilseed crop that belongs to the family Fabaceae (= Leguminosae). This medium-sized tree is also known by the synonyms: Pongamia glabra Vent., Derris indica (Lam.) Bennet, and P. pinnata (L.) Pierre (Bala et al. 2011). It is also named 'Karum Tree' or 'Poonga Oil Tree' in English. Karanja is a glabrous, perennial tree which is probably originated in India and found in South-Eastern Asia and Australia (Sangwan et al. 2010). The tree is known to be drought resistant, nitrogen-fixing with high tolerance to salinity. It has wide adaptability to adversative climatic and soil moisture conditions and often exploited as windbreaks in tea plantations (Divakara et al. 2010). M. pinnata contains a dense network of lateral roots that have been known to prevent soil erosion. It is easily adapted to humid and sub-tropic regions with good drainage and sunny location. These trees can tolerate salinity and alkalinity and are commonly found along the coasts and riverbanks. In India, Karanja trees are commercially cultivated where seed ripens from February to May and are harvested during December and April (Yadav et al.

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2011). *M. pinnata* is a rapidly growing medium size plant which reaches up to a height of 40 feet and spreads a broad canopy for casting shade (Sangwan et al. 2010). The plant has been reported to be grown by several methods, including direct seed sowing or by raising the seedlings in the nursery and planting or by stump cuttings. The most common and successful way is direct seed sowing, which does not require pretreatment, and germination occurs within a period of 1 week to a month. The tree has vast economic significance due to widespread usages of its seeds, seed oils, oil cakes, flowers, fruits, barks, and roots (Fig. 1).

The oil, leaves, and bark of Karanja tree have remarkable medicinal properties while oil cakes have broad pesticidal applications due to the presence of various bioactive antinutritional constituents (e.g., flavonoids, saponins, alkaloids, triterpenes, etc.). Due to rich nutritional values, oil cakes have also been utilized as feedstock for industrially important enzyme production and animal feed (Fig. 2).

This paper aims to provide recent updates on the medicinal, agricultural, and industrial applications of Karanja tree biomass.

# **Medicinal applications**

Karanja is an indigenous medicinal legume tree and one of the non-edible oil yielding trees with high potential for seed yield (20,000 seeds/tree) (Belide et al. 2010). For more than 80 years, all part of *M. pinnata* has been widely used as traditional medicines cross worldwide, and the responsible bioactive compound for such activities is karanjin- a furano flavonoid, which was first isolated in 1925 by Prof. Limaye.

Karanja biomass has been continuously exploited by the pharmacological companies to develop various drugs for the treatment of tumors, ulcers, and brain diseases (Muqarrabun et al. 2013; Pavithra et al. 2010). Table 1 summarizes the studies about the therapeutic properties of different plant parts of *the M. Pinnata* tree.

# Antioxidant property

The physiological process in the human body leads to the production of free radicals and another group of reactive oxygen species. Such free radical leads to the destruction of cells and develop cancer, so to scavenge



Fig. 1 Botanical features of Karanja tree: roots, bark, leaves, flowers, shells, seeds, seed oil and deoiled cake

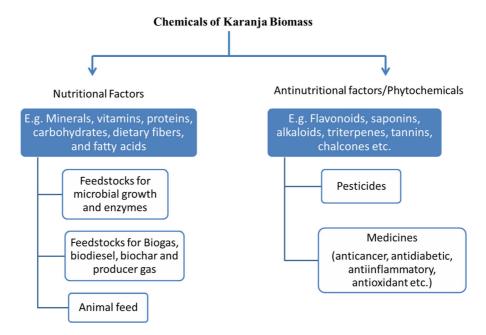


Fig. 2 Potential applications of Karanja biomass on the basis of its chemical constituents

these radicals, antioxidants compounds are required (Phaniendra et al. 2015). It has been reported that the presence of secondary metabolites like phenolics, terpenoids, flavonoids, etc. in various parts of the M. *pinnata* tree are the responsible compound for the most potent antioxidant property (Muqarrabun et al. 2013). Priya et al. (2016), reported that biosynthesis of metal nanoparticles developed from *P. pinnata* leaf extracts are potential natural antioxidants having reliable reducing power and can be essential for health preservation against oxidative stress-related degenerative diseases, such as cancer.

Vadivel and Biesalski (2011) reported that seed extract exhibited high levels of ferric reducing/ antioxidant power (FRAP, 1179 mmol Fe(II)/mg extract), radical scavenging activity (54.64%), superoxide (54.53%) and also found to have inhibited effects against  $\beta$ -carotene degradation (41.13%). Amongst the plant parts tested, it has been found that extracts of barks contain more antioxidants power as compared to the other parts. A study conducted by Sajid et al. (2012) confirmed that the maximum extraction yield of antioxidant components was obtained from bark (16.31%), as compared to leaves (11.42%) and seeds (21.51%). Also, its extract contained higher levels of total phenolics (6.94 g GAE/ 100 g DW), total flavonoids (3.44 g CE/100 g DW), and DPPH radical scavenging activity having an IC<sub>50</sub> value of 3.21  $\mu$ g/ml. However, their flower extracts also possess remarkable antioxidant activity, and it is reported that aqueous flower extract exhibits high hydroxyl free radical scavenging inhibition capacity (Kumar et al. 2016). In contrast, Hazra et al. (2011a, b) compared the leaf, seed, and flower extracts and proclaimed the best antioxidant activity of leaf extracts.

Such antioxidant activities in plant parts are reported to be associated with the secondary metabolites explored in extracts. Several compounds found in this species are reported to exhibit antioxidant activity, for example, flavones (methylenedeoxy flavones and furano-flavone), a chalcone (pongamol), two triterpenes (cycloart-23-ene-3β-25diol and Lupeol) and an aromatic carboxylic acid (pyperonylic acid), in several studies, demonstrated significant antioxidant activity (Rao et al. 2009; Badole et al. 2011; Muqarrabun et al. 2013). The marker compound of this species, i.e., karanjin (furanoflavone), also found to exhibit strong effects against inhibition of oxidative stress, due to the presence of anti-oxidative enzymes such as catalase, peroxidase, and superoxide dismutase and by the normalization of lipid peroxidation (Vismaya et al. 2011).

Plant part	Bioactive molecule responsible	Activity	Medicinal properties	References
Seed	Secondary metabolites such as phenols, flavonoids, etc.	Antioxidant activity	The extract exhibited high levels of ferric reducing power and radical scavenging activity	Vadivel and Biesalski (2011)
Roots	Flavone and chalcone	Anti- inflammatory effects	Out of the 52 derivatives isolated, tunicatachalone, isocordoin, 3,4,2,4 tetramethoxy chalcone, 2,4,dihydroxy-3,4- dimethoxychalcone exhibited strongest anti- inflammatory activity against nitric acid production in a microglial cell with an IC50 value in the range of 5.3–8.1 µM	Wen et al. (2018)
Seeds	Furano-flavonoid	Anti-ulcer activity	Karanjin inhibited 50 and 74% of ulcers induced by swim stress in rat models at 10 and 20 mg/ kg body weight, respectively	Verma et al. (2011)
Leaves	Not mentioned	Immune modulatory	The aqueous extract induced higher production of nitric oxide in RAW 264.7 cells	Manikannan et al. (2011)
Stem bark	Not mentioned	Neuro- protective activity	Ethanol extract of stem bark (200 and 400 mg/kg) was administered orally to rats, significantly reduced the monosodium glutamate-induced excitotoxicity by decreasing the level of $Ca^{+2}$ and $Na^+$ with a concomitant increase in the level of $K^+$	Swamy et al. (2013)
Seeds	Karanjin	Anti- Alzheimer's activity	Karanjin decreased the transfer latency time in a dose-dependent manner when Swiss albino rats were fed orally with Karanjin (25 and 50 mg/kg bodyweight)	Saini et al. (2017)
Stem bark	Not mentioned	Anti-diabetic	Extract administered at 100 mg/kg significantly decreased blood glucose level, and improved oxidative stress in diabetic rats	Badole et al. (2015)
Seeds	Flavonoid (Karanjin)	Anti-Cancer effects	Inhibitory effects against human cancer cell lines MCF-7 and HeLa	Arulvasu et al. (2012)
Flower	Not mentioned	Anti-microbial activity	Extracts in the form of aqueous, ethanol and fresh juice exhibited effective antibacterial activity against <i>E. coli</i>	Kavitha et al. (2014)
Bark, leaves, and seeds	Phenolic acids and flavonoids	Anti-microbial activity	Bark extracts showed lower $IC_{50}$ values (3.21–10.01 µg/ml) indicating higher radical scavenging activity, as compared to leaves ( $IC_{50}$ values 4.42–16.46 µg/ml) and seeds ( $IC_{50}$ values 15.7–38.0 µg/ml) extracts	Sajid et al. (2012)
Leaf, bark, flower, and root	Secondary metabolites like Saponins, alkaloids, flavonoids, triterpenes, tannins, etc.	Anti-malarial activity	Excellent activity against <i>Plasmodium falciparum</i> (3D7 strain) and <i>P. berghei</i> malaria parasite	Satish and Sunita (2017)

 Table 1
 Medicinal uses of Karanja biomass

# Anti-inflammatory property

Inflammation generally occurs due to the damage to the living tissues from bacterial, fungal, and viral infections. However, the inflammatory activity is a series of responses in the body which aims to localize, eliminate, and remove the damaged tissue and to heal the affected organ, tissue or system (Oguntibeju 2018). It has been reported that karanjin possesses antiinflammatory effects against several kinds of ulcers. Karnajin extracted from the seeds could positively inhibit swim stress-induced ulcers in rats by 50 and 74% when administered at 10 and 20 mg/kg body weight. Patel and Trivedi (2017) also concluded that karanjin has the potential to cure colitis. They reported when pure karanjin was administered to colitic mice in two different concentrations 100 and 200 mg/kg for seven consecutive days, it significantly and dosedependently ameliorates the visible damage. It could be due to that karanjin helps in the reduction of the myeloperoxidase activity, which catalyzes the formation of hypochlorous acid, a potent cytotoxic oxidant, and also significantly decreases the nitric oxide production (Patel and Trivedi 2017). It has also been reported that the presence of flavonoids, triterpenes, carotenoids, and saponins in the extracts of Karanja seeds are responsible for anti-ulcerative properties (Patil et al. 2010).

#### Anti-diabetic property

In today's era, diabetes is one of the foremost public health problems, which is a metabolic disorder caused due to impaired insulin secretion and/or insulin action (International Diabetes Federation 2015). Compounds extracted from plants play an essential role in curing diabetes. It has been investigated that chemical constituents like karanjin, pongamin, pongamol, etc., have anti-hyperglycemic activity (Tamrakar et al. 2008). Badole et al. (2015) revealed that stem bark extracts of M. pinnata (100 mg/kg, per oral) treatment significantly reduced the blood glucose levels in streptozotocin-nicotinamide induced diabetes in Sprague-Dawley rats. Extracts reduced the blood glucose levels because of their ability to stimulate the secretion of insulin from the surviving pancreatic  $\beta$  cells. Also, in their study, the preliminary phytochemical analysis of extracts indicated the presence of alkaloids, terpenoids, triterpenes, flavonoids, steroids, and volatile oils. Another study revealed Karanja leaf extracts as a potential drug to control diabetes (Sikarwar and Patil 2010). Ethanolic and aqueous extracts of Karanja leaves when studied in comparison with a known antidiabetic drug glibenclamide in experimental diabetic rats, showed significant (P < 0.001) antidiabetic activity, with a blood glucose levels of  $155.83 \pm 11.211 \text{ mg/dl}$  (ethanolic extracts) and  $132.00 \pm 4.955$  mg/dl (aqueous extracts) in comparison to diabetic control (413.50  $\pm$  4.752 mg/dl) respectively. Similarly, petroleum ether extract of stem bark of *M. pinnata* showed positive antihyperglycaemic activity, administration of 25, 50, 100, 200 and 400 mg/kg to alloxan-induced diabetic mice showed a significant reduction in serum glucose level (Badole and Bodhankar 2009). On the other hand, Punitha and Manoharan (2006) inferred that oral administration of ethanolic extract of *M. pinnata* flowers (300 mg/kg, body weight) to the alloxan-induced diabetic rats enhances anti-hyper-glycemic, anti-lipid peroxidative effects.

# Neuroprotective property

Neuronal cells are sensitive to the oxidative stress caused by free radicals as they lack internal antioxidant defense mechanisms (Masilamani et al. 2017). Thus, in view to combat and prevent neural damage, natural products rich in antioxidants are used as preventive measures. Scientists have also explored the neuroprotective role of M. pinnata extracts. A clinical study revealed that the stem bark extract of Karanja significantly reduced the excitotoxicity in albino rats induced by Monosodium glutamate neurotoxicity. It was observed that oral administration of ethanol extract at 200 and 400 mg/kg reduced the excitotoxicity by decreasing the level of Calcium and sodium ions with a simultaneous increase in the concentration of potassium ions, levels of Serum gamma-aminobutyric acid in rats' models (Swamy et al. 2013). It has been evident that the accumulation of high levels of Ca, Na, and low levels of K intracellularly trigger the mitochondrial dysfunction and free radical generation resulting in neurotoxicity. According to Nobre-Junior et al. (2009), extracts containing furano-flavonoids and chalcones exert neuroprotective activity in various neurodegenerative diseases; this could be one of the reasons for this property in Karanja plant too.

# Anti-cancer property

As per the data of India Council of Medical Research (ICMR), about 1300 patients die every day in India due to cancer (NCRP Report 2017). Cancer is nothing but a stage where cells start to grow uncontrollably. It has been reported that specific secondary metabolites like flavones possess the anticancerous property and help in the cure of the disease. Nagaprashantha et al. (2011) found that flavones exhibit anti-proliferative, anti-angiogenic, and pro-apoptotic effects in carcinoma of prostate (CaP) cells (PC-3, DU-145, and

LNCaP). According to a study conducted by Arulvasu et al. (2012), M. pinnata seed oil extracts also possesses the anti-cancer property against human cancers. They reported that seed oil extracts of Karanja exhibited high cytotoxic property against the human cell lines MCF-7 (Human breast cancer cell line) and HeLa (Cervix). Both the cells were treated with or without the seed oil extracts at a concentration ranging from 2 to 10 mg/ml to check the cell viability. Results indicated a significant decrease in cell viability. As a confirmatory test, DNA fragmentation was also measured, which again showed high DNA fragmentations in cell lines with Karanja extracts indicating strong inhibitory effects against human cancer. George et al. (2010) also concluded that M. pinnata extracts possess high inhibitory actions against human pancreatic cancer. The methanolic extracts of the *M. pinnata*, when presented as the fraction of Panc-1 cell survival in comparison to controls, the extract exhibited high anti-proliferation activity of 0.1470.07 fraction of Panc-1 cell survival. The presence of various classes of flavonoids in M. pinnata extracts could be one of the primary reasons behind the anti-cancerous property of this medicinal plant.

#### Miscellaneous medicinal properties

The aqueous extract of Karanja leaves showed immuno-modulating activity demonstrated by the enhanced production of nitric oxide activity by the RAW 246.7 cells (Manikannan et al. 2011). The methanolic extract of Karanja biomass (dried leaves, bark, flowers, and roots) exhibited anti-plasmodial properties against Plasmodium falciparum (3D7 strain) and P. berghei. The extract at the concentration of 1000 mg/kg b.wt./day showed 83.90, 87.47, and 94.67% of chemo-suppression during Suppressive, Repository, and Curative tests respectively (Satish and Sunita 2017). The anti-microbial activity of Karanja biomass is also well documented. The aqueous methanolic extract of bark showed maximum antimicrobial activity against a set of common pathogenic microorganisms, which includes Gram-negative bacteria: Pseudomonas aeruginosa, P. stutzeri, Escherichia coli, and fungi: Aspergillus oryzae, A. niger, and Fusarium solani (Sajid et al. 2012). A comparative study revealed extract (aqueous, ethanol and fresh juice) of Karanja flowers more efficient in exhibiting 65.25% more antibacterial activity against *E. coli* than *M. oleifera* (Kavitha et al. 2014).

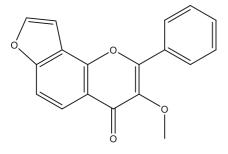
The majority of the phytochemicals that renders above mentioned medicinal properties to Karanja biomass belongs to the class flavonoids (flavones, flavans, and chalcones) while some are terpenes and carboxylic acids. Some of the major metabolites of *M. pinnata* representing an individual chemical class are shown in Fig. 3.

# **Agricultural applications**

The use of oil cakes as organic manures, due to its richness in NPK content, has been in practice in the agriculture field for many decades. From improving rhizosphere health to enhancing plant growth parameters, oil cakes as fertilizers have been reviewed intensively on almost all crops. However, the biomass has also been applied or valorized as biopesticides due to the presence of toxic phytochemicals. Recently, the biomass has also been promoted as a phytoremediator of heavy metals, thereby enhancing the production in agriculture. From a veterinary point of view, the Karanja deoiled cake has been detoxified by various means and served as a protein supplement in animal feed.

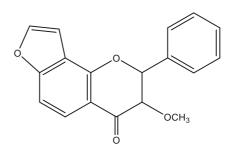
# Pesticidal activities

Phytochemical biopesticides hold great value for sustainable pest management in lieu of significant drawbacks of synthetic pesticides. M. pinnata possesses several toxic constituents (discussed elsewhere) due to which it has gained attention as potential pesticides (Walia et al. 2017; Kalra and Kaushik 2015) (Table 2). The pesticidal effect of Karanja oil on whiteflies (Trialeurodes vaporariorum) on Chrysanthemum plants has been reported (Pavela and Herda 2007b). A bioformulation (PONNEM) of Karanja oil in combination with neem oil showed oviposition deterrent effect against insect pests Helicoverpa armigera and Spodoptera litura (Packiam et al. 2012). Later, the same product was converted into an advanced nanoformulation through cross-linkage with chitosan nanoparticles, and the resultant product showed 88.5% antifeedant activity and 90.2% larvicidal activity against *H. armigera* (Paulraj et al. 2017). A comparative study of silver and gold nanoparticle of

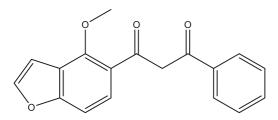


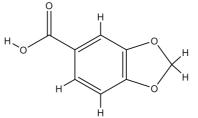
(a) Karanjin

(b) Pongamol

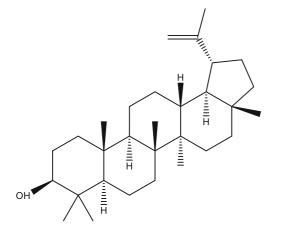


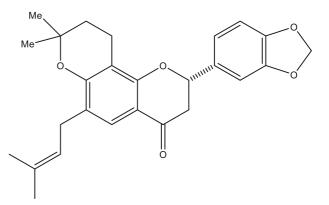
(d) Pongapin





(e) Pyperonylic acid





(c) Lupeol

Fig. 3 Chemical Structure of major secondary metabolites of *M. pinnata*. The majority of the phytochemicals are derivatives of flavonoids i.e. flavones (Karanjin, Pongapin), flavans

(f) Pongamone C

(Pongamone C), chalcones (Pongamol), and miscellaneous like triterpenes (Lupeol) and carboxylic acid (Pyperonylic acid)

Karanja oil revealed the better pesticidal efficacy of the latter, against hairy caterpillar, *Pericalia ricini* (Sahayaraj et al. 2016). Both Pongam oil and karanjin (a furanoflavonol) effectively inhibited the growth of rice weevil, *Sitophilus oryzae* (Biswas and Biswas 2006). Karanjin has been investigated for its deterrent

S. no.	Active ingredient	Pests controlled	Efficacy	References
1.	Karanja Oil (2% v/v)	Trialeurodes vaporariorum	99.33%-Repellency 99.63% deterrence	Pavela and Herda (2007a, b)
2.	Karanaj oil in combination with neem oil (20 µl/l)	Helicoverpa armigera Spodoptera litura	68.12% deterrence 77.48% deterrence	Packiam et al. (2012)
3.	Karanja oil, neem oil with chitosan nanoparticles (0.3% v/v)	Helicoverpa armigera	88.5% antifeedant activity 90.2% larvicidal activity	Paulraj et al. (2017)
4.	Karanja oil (0.03% v/v)	Pericalia ricini	60% mortality	Sahayaraj et al. (2016)
5.	Karanjin (0.25–0.5 g/ml)	Odontotermes obesus	100% mortality	Verma et al. (2011)
6.	Karanjin (0.25% w/v)	Lipaphis erysimi	100% mortality	Mondal et al. (2010)
7.	Leaf extract with ZnO nanoparticles (25 $\mu g/ml$ )	Callosobruchus maculatus	100% mortality	Malaikozhundan and Vinodhini (2018)
8.	Seed kernel (50 ml/l)	Amrasca devastans Aphis gossypii	34.66–35.72% reduction in aphid population	Vinodhini and Malaikozhundan (2011)
9.	Seed extracts (1% w/v)	Earias vittella	> 65% and 75% first instar larval mortality	Reena and Singh (2007)
10.	Karanja + Jatropha de-oiled cake (1:1)	Aedes aegypti	96% mortality	Pant et al. (2016)
		Tribolium castaneum	88–100% mortality	Pant et al. (2014)

 Table 2
 Pesticidal properties of Karanja biomass

activity against termites, *Odontotermes obesus* (Verma et al. 2011). The insecticidal property of karanjin against mustard aphid, *Lipaphis erysimi*, was enhanced by altering the chemical structure into karanj ketone oxime esters with  $LC_{50}$  of 0.048% (Mondal et al. 2010).

Besides the oil, the pesticidal efficacy of other parts of Karanja biomass has also been investigated. Nanopesticidal formulation of Karanja leaf extract developed with ZnO nanoparticles has been effective against pulse beetle, Callosobruchus maculatus (Malaikozhundan, and Vinodhini 2018). The pesticide significantly reduced the  $\alpha$ -amylase and cysteine protease activity of C. maculatus. Aqueous extract of Karanja seed kernel (5% w/w) showed antifeedant activity against leafhopper, Amrasca devastans, and aphid, Aphis gossypii (Vinodhini and Malaikozhundan 2011). Hexane fraction of both mature and immature methanolic seed extracts caused disruptive behavioral and physiological activities in Earias vittella (Reena and Singh 2007). The pesticidal activity of deoiled cake of M. pinnata against Aedes aegypti larvae (Pant et al. 2016), and Tribolium castaneum (Pant et al.

2014) have been reported. The use of non-edible oil cakes, including Karanja as an organic amendment to control phytoparasitic nematodes, has extensively been reviewed (Akhtar and Malik 2000; Oka 2010). Barring plant-parasitic nematodes where pesticidal activity has been accredited to the release of simpler molecules like ammonia and organic acids during decomposition of deoiled cake (Oka 2010), the significant impact of Karanja biomass as phytopesticide is primarily because of karanjin (Walia et al. 2017). The toxic molecule acts as potent feeding and oviposition deterrent against several field insects and mites. The antifeedant activity of karanjin has been linked to its ability to suppress ecdysteroids (Gonzalez-Coloma et al. 2013) and inhibit protease activity (Malaikozhundan and Vinodhini 2018). Though a potent pesticide, karanjin has been found to have minimal cytotoxic side effects on humans while the mild inhibitory impact on non-target microflora of soil (Raghav et al. 2019). Thus, the use of Karanja based phytopesticide is not only effective and competitive but also safe and sustainable.

#### Phytoremediation

The utility value of *M. pinnata* biomass as a phytoremediation tool for toxic contaminants has been investigated. Karanja tree is indigenous to the Indian environment and offers more tolerance to heavy metals in soil compared to other tree species. Further, linking to the bioenergy potential, phytoremediation using the Karanja tree becomes all the more holistic and sustainable (Pandey et al. 2016). Some believe that phytoremediation through energy crops would not only avoid the food/fodder-fuel conflict but also provide sustenance to a bio-based economy in developing nations (Tripathi et al. 2016). Reclamation of polluted and abandoned land is an added advantage of the 'phytoremediation by energy crop' approach (Abhilash et al. 2013). Experiments on Karanja seedlings revealed the ability of the biomass to tolerate Arsenic (As) up to 0.2-1.0 mM without showing an adverse effect on its growth and chlorophyll content (Kumar et al. 2017a, b). In another study, thermally activated Karanja deoiled cake removed 94.12% of As (III) in the countercurrent fluidized bed column (De et al. 2019). Karanja plants inoculated with plant growth-promoting isolate, Bradyrhizobium liaoningense remediated mine tailings polluted with iron-vanadium-titanium oxide (Yu et al. 2017). The symbiotic association enhanced the remediation potential as the plant accumulated 1753.2 and 3111.0 mg/kg in the roots and shoots of M. pinnata, respectively. An experiment with biosorbent prepared from deoiled seeds of Pongamia demonstrated successful remediation of water contaminant, chromium in the form of Cr(VI) ions in an aqueous medium (Rangabhashiyam et al. 2019).

# Animal feed

The presence of anti-nutritional factors (phytates, tannins, and protease inhibitors, glabrin and karanjin) limits the use of Karanja oil cake as feed for cattle. However, after proper processing, a higher concentration of Karanja cake can be included in the diet of livestock (Soren and Sastry 2009). Treatment of Karanja cake with dilute acid (2% HCl) for one hour at room temperature removes tannins, phytates, and trypsin inhibitors up to 54, 72.5, and 74%, respectively (Vinay and Kanya 2008). Conversely, Habib et al. (2016) observe no improvement in the performance of

broilers when fed with Karanja seed cake detoxified after treatment with dilute alkali or acid. Detoxified Karanja cake obtained through water washing proved to be a promising protein supplement for male lambs as the diet containing 50% N replaced by detoxified Karanja cake did not affect the gross and histopathological architecture of vital organs (Soren et al. 2017). Karanja seeds treated with aqueous methanol mixture removed the toxic constituents and became suitable to partially substituted soybean meal (9% in a concentrated mixture) as livestock feed for lambs (Rao and Kumar 2015). Incorporation of detoxified Karanja seed cake at 5.85% of total mixed ratio (maize grain, deoiled soybean meal, urea, molasses, mineral mixture, and salt) improved the milk yield of crossbred dairy cows. However, comparatively, authors observed the performance of Karanja inferior to deoiled Neem seed cake (Raj et al. 2016). Alkali processed Karanja seed cake (1.5% NaOH) supplemented with 0.2% methionine replaced 12.5% nitrogen of Soybean meal in broiler diets and showed no adverse effect on carcass characteristics and sensory attributes (Panda et al. 2007).

# **Industrial applications**

Any industrial process is deemed sustainable if the nutrient (substrate) input is a low cost yet widely available. Both oil and oil cakes of the Karanja tree are a cheap and abundant source of fats, proteins, and carbohydrates and therefore, can serve as a potential tool for an economically viable bioprocess industry. The general characterization of Karanja oil and deoiled cake is given in Tables 3 and 4, respectively. The ability of the Karanja biomass as a renewable feedstock was investigated for industrial applications to keep the economics of bioprocess in check.

#### Bioenergy

Depleting fossil fuels and rising alarms over climate change and greenhouse gas emissions have persuaded the energy sectors across the globe to look for alternate clean and green energy concepts. Under this approach, various plant and algal biomass have been exploited as feedstocks for the production of renewable energy, often termed as bioenergy. India backs non-edible oilseed tree-based biofuel programs as a long-term

S. no.	Properties	Karanja oil
1.	Kinematic viscosity (cSt) at 40 °C	41.71
2.	Density (kg/m <sup>3</sup> )	911
3.	Flashpoint (°C)	241
4.	Fire point (°C)	260
5.	Pour point (°C)	5
6.	Cloud point (°C)	15
7.	Calorific value (MJ/kg)	41.83
8.	Saponification value	190
9.	Iodine value	87
10.	Carbon residue (%)	0.71
11.	Fatty acid (%)	
	(a) Total saturated acid	20.5
	(b) Total unsaturated acid	79.5

Table 3 Characteristics of Karanja oil (Kumar et al. 2018; Dwivedi and Sharma 2014)

investment that has the potential to serve as sustainable feedstocks (Rasool and Hemalatha 2016). The seed oil of *P. pinnata* is non-edible due to the presence of toxic compounds such as karanjin and pongamol (Marriboina et al. 2017). Together with the fact that the oil content is very high ( $\sim$  35%) but mostly underutilized (Dwivedi and Sharma 2016), Karanja tree becomes very promising and potential biomass for bioenergy in context to India and other South-Asian countries.

# Feedstock for biodiesel production

Karanja seeds contain 27-40% (w/w) oil, which is yellowish orange with an unpleasant aroma. Although, various factors are responsible for the quality of biodiesel produced from Karanja oil viz., viscosity, flash point, calorific value, saponification value, specific gravity, and acid value the detrimental factor is free fatty acids (FFA) content (Dwivedi and Sharma 2014). Since FFA content is very high in Karanja oil, biodiesel is preferably produced through a two-step transesterification process wherein Karanja oil undergoes acid esterification before alkali transesterification (Joshi et al. 2016). Under supercritical conditions, both steps can be carried out simultaneously to minimize the duration of the process (Takase et al. 2015) while some suggested use of microwave and CaO as a heterogeneous catalyst to enhance the transesterification process (Joshi et al. 2015). Karanja oil, like any other vegetable oils, is highly viscous and, therefore, unsuitable for direct use in a diesel engine. The transesterification process addresses this issue and produces quality Karanja oil methyl ester (biodiesel) cost-effectively. Pyrolysis, microemulsion, and blending are other methods for the production of biodiesel from Karanja oil (Patel and Sankhavara 2017). In recent years, efforts are being made to market Karanja oil-based biodiesel as a commercial option over mineral fuels. However, the critics are still apprehensive about its production cost and oxidation stability. Though the use of various anti-oxidants (like pyrogallol) in biodiesel is being suggested to improve the oxidation stability, cost-analysis of biodiesel production is still warranted at different research levels (Mofizur et al. 2016).

# Feedstock for bioethanol and biogas generation

The exploitation of Karanja oil as medicine and feedstock for biodiesel leads to the generation of 120,000–130,000 tons/annum de-oiled seed residue (Muktham et al. 2016a). The residual deoiled cake of *M. pinnata* being a cellulosic rich biomass with round the year availability at a cheaper rate, makes it a promising feedstock for bioethanol and biogas production. Like biodiesel, both bioethanol and biogas are being proclaimed as green, non-toxic, biodegradable biofuel options. Bioethanol from Karanja deoiled cakes considered to be a second-generation biofuel as

 Table 4
 Characteristics
 of
 Karanja
 deoiled
 cake
 (Sharma et al. 2013a, b)

S. no.	Parameters	Karanja deoiled cake
1.	Crude protein (%)	30.43
2.	Crude fibre (%)	6.5
3.	Ash (%)	5.2
4.	Carbon (%)	42.26
5.	Nitrogen (%)	4.87
6.	Phosphorus (%)	0.89
7.	Potassium (%)	1.3
8.	C:N ratio	8.68:1
9.	pH	5.5

the feedstock is non-food material and quell the "food for fuel" issues often related to first-generation biofuel (Robak and Balcerek 2018). Acid hydrolysis is a widely spread pretreatment method that converts cellulose and hemicellulose of deoiled cake into soluble sugars using dilute acids (Doshi and Srivastava 2013; Sharmada et al. 2016). In one such study, Karanja deoiled cake was pretreated with different acids (H<sub>2</sub>SO<sub>4</sub>, HCl, and H<sub>3</sub>PO<sub>4</sub>) at varying concentrations to obtain a high yield of glucose (Radhakumari et al. 2017). Treating the Karanja deoiled cake with various in-house lignocellulosic enzymes isolated from microbial isolates grown over the same substrate has also been reported (Radhakumari et al. 2017).

There is a broad scope of using Karanja deoiled cake as co-substrate during anaerobic digestion for the production of biogas. Karanja deoiled cake when mixed with various proportions of cow dung, shown to produce 73% methane in biogas with a heating value of 408 27.5 MJ/kg and approximately 6.5 kW/m<sup>3</sup> of the energy content (Barik and Murugan 2015). On the contrary, Chandra et al. (2012) preferred Karanja deoiled cake as sole substrate and reported 15-20% more methane in the biogas compared to biogas from cattle dung. The authors reasoned the presence of higher volatile solids content (85.3%) in Karanja deoiled cake for the better performance as feedstock for biomethanation process. From an Indian perspective, valorization of deoiled cake holds the key in improving the economics of biodiesel production and providing a sustainable solution for waste disposal problems through bioethanol and biogas production.

# Feedstock for biochar and producer gas (syngas)

Thermal decomposition of Karanja deoiled cake (pyrolysis) in the absence of oxygen yields carbonrich biochar and bio-oil, also known as pyrolytic oil (Zhu et al. 2018). Besides being utilized as a solid ecofriendly biofuel, biochar is also used as an organic amendment to improve soil fertility (Song et al. 2014) and adsorbent for pollutant removal (Manyà 2012). Thermogravimetric analysis revealed that the slow pyrolysis of Karanja deoiled cake at 450-500 °C produces the biochar with enhanced carbonization (Muktham et al. 2016b). Chutia et al. (2014) conducted a pyrolysis experiment of KDOC in lab-scale fixed-bed pyrolyzer and obtained highly basic biochar at 500 °C with a heating rate of 40 °C/min. Kumar and Pant (2015) exploited modified hydrothermal treatment to convert KDOC into biochar with high calorific values (24.7-26.3 MJ/kg). Pyrolysis of small-sized (125-425 µm) Karanja deoiled cake at 500 °C using zeolite Y as catalyst gave the 99% hydrocarbon yield (Soongprasit et al. 2017).

Gasification of biomass converts lignocellulosic matter into producer gas that can be directly used for the production of electricity and liquid fuels (Chutia et al. 2014). The process is simple compared to pyrolysis and is considered as low carbon emission technology. An investigation during thermochemical gasification of Karanja shell (covering of seeds) revealed the correlation between pelletization and gasification efficiency (Prasad et al. 2015). Pellet size of 17 mm diameter failed to achieve complete gasification (73%), while 11.5 mm pellet of Karanja shells produced 95% gasification efficiency. Producer gas produced from an 11.5 mm pellet had a calorific value of 4.66 MJ/Nm<sup>3</sup>. Deoiled cake of Karanja did not prove to be the suitable feedstock for gasification as both the pellet size (17 and 11.5 mm) failed to achieve complete gasification (Prasad et al. 2014). During oxygen-steam gasification of Karanja press seed cake, heating the feedstock at 1000 °C produced the syngas with 95% cold gas efficiency and ~ 12 MJ/Nm<sup>3</sup> lower heating value (Dhanavath et al. 2018). Recently, biochar obtained from Karanja deoiled cake has been used as feedstock for the synthesis of production gas

using the air gasification method in a fluidized bed reactor (Marshal et al. 2019).

# *Feedstock for the production of enzymes and metabolites*

Industrial production of enzymes takes place through fermentation (solid or submerged) of substrate employing bacteria or fungi (Sivaramakrishnan and Gangadharan 2009). Deoiled cake of Karanja is rich in proteins, and different studies have investigated their viability as a substrate for various enzyme production. Sharma et al. (2014a) demonstrated the usefulness of Karanja deoiled cake as feedstock for protease production using entomopathogenic fungus Paecilomyces lilacinus 6029. In another study, the deoiled cake was used as the substrate to produce protease (9840 U/g DM) and lipase (1974 U/g DM) using B. pumilus SG2 (Sangeetha et al. 2011). The authors did not supplement the cake with any carbon or nitrogen source as the cake itself was found sufficient to produce an optimum quantity of targeted enzymes. Endophytic fungus Colletotrichum gloeosporioides showed lipase activity (983 U/g DM) using Karanja oil cake as a substrate (Balaji and Ebenezer 2008). The exploitation of deoiled cake as a growth substrate for the mass multiplication of biocontrol agents like Paecilomyces lilacinus, P. variottii, and Trichoderma harzianum has been investigated (Sharma et al. 2013a, b, 2014b; Arora et al. 2017; Singh et al. 2015). Research work indicates that KDOC is also a potential substrate for the production of secondary metabolites viz., fatty acids from P. lilacinus 6029 (Sharma et al. 2014a), dipicolinic acid from P. variottii (Arora et al. 2017), glycolipid (biosurfactant) from Pseudomonas aeruginosa AB4 (Hazra et al. 2011a, b), and iturin A from B. amyloliquefaciens RHNK22 (Kumar et al. 2017a, b).

# **Conclusion and future perspectives**

Through the review, authors have presented the broader perspective of the Karanja tree besides serving as feedstock for biodiesel production. The multiple potential utilities of this legume have been credited to its rich nutritional and bioactive constituents. Further, the ability of the plant to tolerate abiotic stress and adapt to varied edaphic and ecological regions establishes the candidature of the Karanja tree to be promoted for afforestation programs. Henceforth, the focus shall now be on crop improvement through genetics. Research should be conducted to develop the genetically stable disease-free clones of this critical legume plant. High throughput sequencing and transcriptome analysis should be performed to elucidate the mechanisms behind the synthesis of fatty acids and other prominent phytochemicals and ways to enhance their expressions in the plant.

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