3 Literature Review

3.1 Plant Profile of *Amaranthus spinosus*:

![Amaranthus spinosus plant and leaves](image)

**Figure 3.1** *Amaranthus spinosus* plant and leaves

3.1.1 Taxanomical Classification

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae-Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subkingdom</td>
<td>Tracheobionta-Vascular Plants</td>
</tr>
<tr>
<td>Superdivision</td>
<td>Supermatophyta-Seed plants</td>
</tr>
<tr>
<td>Division</td>
<td>Magnoliophyta- Flowering Plants</td>
</tr>
<tr>
<td>Class</td>
<td>Magnoliopsida-Dicotyledons</td>
</tr>
<tr>
<td>Subclass</td>
<td>Caryophyllidae</td>
</tr>
<tr>
<td>Order</td>
<td>Caryophyllales</td>
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<tr>
<td>Family</td>
<td>Amaranthaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Amaranthus L</td>
</tr>
<tr>
<td>Species</td>
<td>A. spinosus L</td>
</tr>
<tr>
<td>Binomial name</td>
<td><em>Amaranthus spinosus L</em></td>
</tr>
</tbody>
</table>

*CRPS, Nanded Pharmacy College, Nanded*
3.1.2 Local Names:

<table>
<thead>
<tr>
<th>Language</th>
<th>Vernacular name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanskrit</td>
<td>Bhandira, Ghanasvana, granthila, Kandakamarisha</td>
</tr>
<tr>
<td>English</td>
<td>Spiny pigweed</td>
</tr>
<tr>
<td>Hindi</td>
<td>Adak-dhatura, Cauleyi, Cholai, Goja</td>
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<tr>
<td>Marathi</td>
<td>Chanalai, Kante bhaji, Kantemat, Tandulja, Thanduliya</td>
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<td>Kannada</td>
<td>Mulla-dantu</td>
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<tr>
<td>Tamil</td>
<td>Cemulli, Cemullikkirai, Varataru</td>
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<td>Telgu</td>
<td>Nalta-doggli</td>
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<tr>
<td>Assamese</td>
<td>Hati khutura</td>
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<tr>
<td>Malayalam</td>
<td>Cerucira, Mullan-chira, Mullancira</td>
</tr>
<tr>
<td>Tibetan</td>
<td>Ta-ndu-la-ka</td>
</tr>
<tr>
<td>Manipuri</td>
<td>Chengkruk</td>
</tr>
</tbody>
</table>

3.1.3 Distribution:

*Amaranthus spinosus* is an annual weed that is widely distributed in the humid zone of the tropics including Kenya.

3.1.4 Plant part used: Leaves

3.1.5 Morphology:

*Amaranthus spinosus* is an plant grows annually with specification as an erect, monoecious herb, tall up to 100-130 cm, greatly branched; terete stem or obtusely angular, slightly pubescent or glabrous, variably or green suffused with purple. The leaves are simple and alternate without stipules; petiole is approximately as long as the leafblade; The blade shape is ovate lanceolate to rhomboid, 3.5-11 cm × 1-4.5 cm acute and often slightly decurrent at base, rounded, obtuse or slightly retuse and often short mucronate at apex, glabrous, entire or slightly pubescent on veins when young. The inflorescence consists of dense clusters, lower ones are axillary, higher often collected in an axillary and terminal spike which is often branched in its lower part; axillary clusters are usually armed with extremely sharp spines up to 2 cm long. Its flowers are unisexual, solitary in the axil of a bract, subtended by 2 bracteoles; bracts and bracteoles scarious, mucronate from a broad base, shorter or as long as the perianth; male flowers are usually arranged in a terminal spike above the base of the inflorescence, green; tepals 5 or in male flowers often 3, free, subequal,
ovate-oblong to oblong-spatulate, up to 2.5 mm long, very convex, membranous, with transparent margins and green or purple median band; male flowers with 5 stamens about as long as tepals; female flowers with superior, oblong ovary, 1-celled, styles 2-3, ultimately recurved. The fruit is ovoid shaped with a short inflated neck below the style base, circumscissile a little below the middle or indehiscent. The seed is about 1 mm in diameter, shiny, compressed, black or brownish-black in colour (Baral M, et al, 2011).

3.1.6 Chemical constituents:
*Amaranthus spinosus* contains 7-p-coumaroyl apigenin 4-O-beta-D-glucopyranoside, a new coumaroyl flavone glycoside called spinoside, xylofuranosyl uracil, beta-D-ribofuranosyl adenine, beta-sitosterol glucoside, hydroxycinnamates, quercetin and kaempferol glycosides, betalains; betaxanthin, betacyanin; amaranthine and isoamaranthine, gomphrenin, betanin, b-sitosterol, stigmasterol, linoleic acid, 0.15% rutin and beta-carotene. (Suryavanshi V, et al, 2007), (Odhava B, et al, 2007), (Barminas T, et al, 1998). The leaves content 1.16 g/100 g of carbohydrate, 27 kcal energy, 91 g moisture, 4 g protein, 0.6 g fat, 2.48 g fiber, 2.76 g ash (Sawangjaroen N, et al, 2005). also contain dry weight (38.4 mg Iron, 968.7 mg calcium, 912.4 mg magnesium, 816.3 mg phosphorus, 6.8 mg manganese, 1.2 mg copper, 6.8 mg zinc) per 100g. (Olumayokun A, et al, 2004).

3.1.7 Uses:
The Chinese as a traditional medicine use *A. spinosus* to treat diabetes. Whereas seeds as a poultice for broken bones, internally given to treat internal bleeding, diarrhoea and excessive menstruation. Roots are identified as an effective diuretic. In South-East Asia a decoction of the root is used to treat gonorrhoea as well as applied as an emmenagogue and antipyretic. Nepalese and some tribes in India apply *A. spinosus* to induce abortion (Azhar-ul-Haq, et al, 2004), (Igoli J, et al, 2005). In Thai traditional medicine, used plant to treat diarrhea (Lin B, et al, 2005) while root is for toothaches (Hilou A, et al, 2006). In many countries, together with those in Africa, the bruised leaves are considered a good emollient and applied externally in cases of ulcerated mouths, eczema, burns, wounds, boils, earache and hemorrhoids also for gastroenteritis, gall bladder inflammation, abscesses, colic menorrhagia, arthritis and for the treatment of snakebites (Ibewuike J, et al, 1997). Plant ash solution was used to wash sores.
Plant saps for eye wash to treat ophthalmia as well as convulsions in children. In Malaysia, plant is used as an expectorant as well as to relieve breathing in acute bronchitis. In mainland South-East Asia, it is also used as a sudorific, febrifuge, an antidote to snake poison, along with as a galactagogue (Mandal M, et al., 2001). The rainy season is a malaria endemic season in which A. spinosus bark decoction is taken in a volume of about one liter three times a day to ward off malaria (Cai Y, et al., 2003).

3.1.8 Other Species:
Amaranthus paniculatus, Amaranthus gangeticus, Amaranthus blitum, Amaranthus viridis

3.1.9 Literature Review of Amaranthus spinosus:
The plant has been mentioned in the ancient text to be a digestive, laxative, diuretic, stomachic, antipyretic, to improve appetite, biliousness, blood diseases, burning sensation, leprosy, bronchitis, rat bite, piles, convulsion and leucorrhea, while the boiled leaves and root are given to children as a laxative, emollient, and poultice for abscesses, boils, and burns (Kirtikar & Basu, 2001). The leaves are also used to treat rheumatic pain, stomach-ache, eczema, gastroenteritis, gallbladder inflammation, boils, abscesses, snakebite, colic menorrhagia, and arthritis. Amaranthus spinosus is in addition used as an anti-inflammatory, antimalarial, antibacterial, antimicrobial, antidiuretic, and antiviral agent and in hepatic disorders (Anonymous, 1956, Olajide, 2004; Stintzing, 2004).

Hilou A et al. (2006) obtained extracts from two Burkinabe folk medicine plants, spiny amaranth (Amaranthus spinosus L., Amaranthaceae) and erect spiderling (Boerhaavia erecta L., Nyctagynaceae) and screened for antimalarial properties with the aim of testing the validity of their traditional uses. The plant extracts was shown significant antimalarial activities in the 4 days suppressive antimalarial assay with Plasmodium berghei berghei in mice inoculated with red blood cells parasitized. They also obtained values for ED50 of 789 and 564 mg/kg for Amaranthus spinosus and Boerhaavia erecta extracts, respectively. Moreover the tested vegetal material has shown only less toxicity (1450 and 2150 mg/kg as LD50 for Amaranthus spinosus and Boerhaavia erecta respectively).

Ashok Kumar B (2008) estimated rutin and quercetin from Amaranathus spinosus by HPLC. Flavonoids present in the Amaranthus spinosus are rutin and quercetin. It suggested that Rutin and quercetin possess many biochemical
properties like inhibition of enzymes, regulatory role on different hormones and pharmacological activities like antimicrobial, antioxidant, anticancer, antihepatotoxic, protection of cardio vascular system. Author developed HPLC method for estimation of rutin and quercetin from herbal extract of *Amaranthus spinosus*.

Azhar-ul-Haq et al. (2006) isolated Spinoside, new coumaroyl flavone glycoside from the *n*-butanol fraction of the methanol extract of the whole plant of *Amaranthus spinosus* and also assigned the structure 7-*p*-coumaroyl apigenin 4-*O*-β-D-glucopyranoside on the basis of spectroscopic techniques including 1D and 2D NMR spectroscopy. In addition *α*-xylofuranosyl uracil, *β*-D-ribofuranosyl adenine and *β*-sitosterol glucoside have also been isolated for the first time from this species.

Chandana Venkateswara Rao et al. (2009) evaluated for antinociceptive and anti-inflammatory activities with 50% ethanol extract of *Amaranthus spinosus* (whole plant). Analgesic and anti-inflammatory activities were studied by measuring nociception by formalin, acetic acid, hot plate, tail immersion method while inflammation was induced by carrageenan. Study reveals that plant had significant dose dependent percentage protection against acetic acid (0.6% of 10 ml) induced pain and the effects were also compared to aspirin, morphine and naloxone, while formalin induced pain (0.05 ml of 2.5%) was significantly blocked only at higher dose (400 mg/kg) in first phase. Author suggests that plant significantly blocked pain emanating from inflammation at all the doses in second phase. The reaction time in hot plate was increased significantly and dose dependently where as pretreatment with naloxone rigorously reduced the analgesic potentials of plant. Further in tail immersion test the same dose dependent and significant activity was observed by the author. Whereas aspirin had no effect on thermal induced pain i.e. hot plate and tail immersion tests but showed an effect on writhing test. Investigation showed that *Amaranthus spinosus* possess significant and dose dependant anti-inflammatory activity, it has also central and peripheral analgesic activity.

Deenanath Jhade (2011) evaluates the effect of aqueous and ethanol extracts of root of *Amaranthus spinosus* Linn (*Amaranthaceae*) in rats to explore its antifertility activity. Antifertility screenings of water and ethanol extract of plant were done by the reproductive outcome in mice, anti-implantation,
abortificant, estrogenic and anti estrogenic activity in rats. While the water extract of the root of plant showed the decrease in number of implants and number of litters when compared with the ethanol extract as the percentage of implantation failure increased and were found not significant at dose of 500 mg/kg with 41.39% and 12% resorption at 500 mg/kg for ethanol and water extract respectively body weight dose level. The extract shows furthermore, non significant increase in uterine weight in immature ovariectomised rats. Simultaneous administrations of extracts with ethinyl estradiol cause significant antiestrogenic activity. All these observations reveal that aqueous and ethanol extracts of *Amaranthus spinosus* Linn. have weak anti-fertility effect.

Hussain Zeashan et al. (2008) studied on hepatoprotective and antioxidant activity of 50% ethanol extract of whole plant of *Amaranthus spinosus* and evaluated against carbon tetrachloride (CCl₄) induced hepatic damage in rats with dose of 100, 200 and 400 mg/kg were administered orally once daily for fourteen days. Study reveals that substantially elevated serum enzymatic levels of serum glutamate oxaloacetate transaminase (AST), serum glutamate pyruvate transaminase (ALT), serum alkaline phosphatase (SALP) and total bilirubin were restored towards normalization significantly by the plant in a dose dependent manner. While higher dose exhibited significant hepatoprotective activity against carbon tetrachloride induced hepatotoxicity in rats. Biochemical observations were supplemented with histopathological examination of rat liver sections. Meanwhile, *in vivo* antioxidant activities as malondialdehyde (MDA), hydroperoxides, reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) were also screened by authors who were also found significantly positive in a dose dependent manner. The results of this study strongly indicate that whole plants of *A. spinosus* have potent hepatoprotective activity against carbon tetrachloride induced hepatic damage in experimental animals. This study suggests that possible mechanism of this activity may be due to the presence of flavonoids and phenolics compound in the ASE which may be responsible to hepatoprotective activity.

Amin I (2006) determines the antioxidant activity (total antioxidant and free radical-scavenging activities) and total phenolic content of *Amaranthus* sp. The effects of different blanching times (10 and 15 min) on antioxidant activity and phenolic content were also studied. Four types of *Amaranthus* species locally
known as spinach, namely ‘bayam putih’ (*Amaranthus paniculatus*) (BP), ‘bayam merah’ (*Amaranthus gangeticus*) (BM), ‘bayam itik’ (*Amaranthus blitum*) (BI) and ‘bayam panjang’ (*Amaranthus viridis*) (BPG), were selected for this study. Total antioxidant activity of water-soluble components in raw spinach was in the order of $\text{BI} \approx \text{BM} \approx \text{BPG} > \text{BP}$, whereas free radical-scavenging activity was in the order of $\text{BI} > \text{BPG} > \text{BM} > \text{BP}$. The total phenolic contents of BM and BP were significantly higher ($p < 0.05$) than other samples. All the studied spinach species possessed different antioxidant activities and phenolic contents. Antioxidant activities and phenolic contents of all the spinach were in the order of raw $> \text{blanched 10 min} > \text{blanched 15 min}$. Blanching up to 15 min may affect losses of antioxidant activity and phenolic content, depending on the species of spinach.

Dinesh MG (2014) investigate and evaluate the antitumor capacity and chemoprotective effects of *Amaranthus spinosus* leaves. Author tested methanol extracts of *Amaranthus Spinosus* leaves with different doses for different cell lines such as breast, colorectal, liver and normal cell lines. It was observed that the methanol leaf extract at different doses causes a decrease significant level of tumor development and viable cell count. Percentage of leaf methanol extracts HEp G2, MCF-7 and HT-29 cells shows IC$_{50}$ values of less than 30 $\mu$g/ml. *Amaranthus spinosus* leaf extracts also shown significant membrane stability property which shows better anti-inflammatory responses. Furthermore FACS used to analysis the inhibition of cell cycle progressions by using PI Staining for HEp G2 Cells. The results propose that the methanol extracts of *Amaranthus spinosus* leaves reveals significant antitumor effects in cancers of breast, colorectal, liver and normal cell lines. Further studies are essential to elucidate the precise molecular mechanisms and targets for cell growth inhibition.

Maiyo ZC et al. (2010) study the phytochemical constituents and antimicrobial activity of hexane, ethyl acetate, dichloromethane and methanol leave extracts of plant species *Amaranthus hybridus*, *Amaranthus spinosus* and *Amaranthus caudatus*. They also assayed for antimicrobial activity with some gram positive (*Staphylococcus aureus* and *Bacillus* spp), gram-negative (*Escherichia coli, Salmonella typhi, Pseudomonas aeruginosae, Proteus mirabilis* and *Klebsiella pneumonia*) and a pathogenic fungus *Candida albicans*. The leaves extracts showed a broad spectrum anti-bacterial activity but resistance
The leaf extracts of the three *Amaranthus* species commonly encountered phytochemical constituents included flavonoids, steroids, terpenoids and cardiac glycosides. The minimum inhibitory concentration (MIC) of *A. spinosus* extracts exhibited against the *Salmonella typhi* was found to be 129 mg/ml. The MIC exhibited by *A. hybridus* extracts against the tested organisms ranged between 200 and 755 mg/ml whereas that of *A. caudatus* was between 162.2 and 665 mg/ml.

Stintzing, FC (2004) isolate Betacyanins and Phenolic Compounds from *Amaranthus spinosus* L. and *Boerhavia erecta* L. Stem bark extracts. *Boerhavia erecta* L. (erect spiderling) and *Amaranthus spinosus* L. (spiny amaranth) are two wild growing weed plants used in traditional African medicine, were characterized with respect to their phenolic profile including the betalains while the main betalains in *A. spinosus* were identified as amaranthine and isoamaranthine, the major betacyanins in *B. erecta* were identified as betanin, isobetanin together with neobetanin. The *A. spinosus* showed higher betalain concentrations amounting to 186 mg/100 g, while the *B. erecta* contained 24 mg betacyanins in 100 g of the ground plant material. *A. spinosus* extracts were found to contain hydroxycinnamates, quercetin and kaempferol glycosides, whereas *B. erecta* contains catechins, procyanidins and quercetin, kaempferol and isorhamnetin glycosides. The amounts of these compounds ranged from 305 mg/100 g for *A. spinosus* to 329 mg/100 g for *B. erecta*.

Baral Manik et al., (2011) studies pharmacognostic character of stem and leaves, macroscopic and microscopic analysis, Isolation of epidermal layers, physicochemical studies, preliminary phytochemical screening of *Amaranthus spinosus* Linn.

Mathur Jaya et. al. (2013) studied on macroscopic, microscopic and preliminary phytochemical investigation of leaves of *Amaranthus spinosus* which includes leaf constants, physiochemical parameters like ash values, extractive values and moisture content. The total ash, acid insoluble, ash, water-soluble ash values and sulfated ash were find to be 6.33%, 3.60%, 2.44% and 0.80% w/w respectively. Alcohol soluble and water-soluble extractive values of the leaves were finding to be 6.40%, 3.30%, respectively. Powdered leaves with different chemicals were also subjected to fluorescence analysis. Phytochemical investigation of methanol and petroleum ether extracts revealed the presence of
flavonoids, phytosterols, glycosides, tannins, phenolic compounds and carbohydrates.

Deenanath Jhade, et. al., (2011) studied traditional, clinical potential, ethanopharmacology studies and safety profile of *Amaranthus spinosus*

Stintzinga FC et. al. (2004) characterized the phenolic profile including the betalains present in the plant while the main betalains in *A. spinosus* were identified as amaranthine and isoamaranthine. Extracts of *A. spinosus* were found to contain hydroxycinnamates, quercetin and kaempferol glycosides.

Azhar-ul-Haq et al. (2004) isolate spinoside, new coumaroyl flavone glycoside from the n-butanol fraction of the methanol extract of the whole plant of *Amaranthus spinosus* and assigned the structure 7-p-coumaroyl apigenin 4-O-a-D-glucopyranoside on the basis of spectroscopic techniques including 1D and 2D NMR spectroscopy. In addition alpha xylofuranosyl uracil, a-D-ribofuranosyl adenine and a-sitosterol glucoside have also been isolated for the first time from this species.

Suryavanshi VL et. al. (2008) isolates the rutin with the camag TLC Scanner from the plant extract by overlaying the spectrum chromatogram of rutin in plant with that of the standard rutin.

Bavarva JH et. al. (2013) studied on ethanol extract of *Amaranthus spinosus* leaves by administered (150, 300 and 450 mg/kg bw) to type-1 and type-2 diabetic rats with standard drugs, glibenclamide and metformin as a positive control for comparison. Also study on changes in carbohydrate and lipid metabolism and antioxidants and compared with control and standard drug treated animals. The study suggest that higher doses of extract significantly decreased plasma glucose levels, hepatic glucose-6-phophatase activity and increased the hepatic glycogen content with a concurrent increase in hexokinase activity in both type 1 and 2 diabetic rats. It also significantly lowered the plasma and hepatic lipids, urea, creatinine levels and lipid peroxidation with an improvement in the antioxidant profiles of both type-1 and type-2 diabetic rats. The study reveals that the *A. spinosus* has potential antidiabetic activity and significantly improves disrupted metabolisms and antioxidant defense in type-1 and type-2 diabetic rats.

Balkrishnan S (2010) studied on anti-hyperglycemic and anti-hyperlipidaemic effects in male Wister albino rats. In this study diabetes was
induced in the albino rats by administration of a single dose of alloxan monohydrate (150 mg/kg, i.p). The methanol extract of *A. spinosus* (MEAS) was administered daily at single doses of 250 and 500 mg/kg, p.o to diabetes-induced rats for a period of 15 days. The effect of methanol extract of *A. spinosus* on blood glucose level was measured in the diabetic rats as well as serum lipid profiles were determined. The activities were also compared to the effect produced by a standard anti diabetic agent like glibenclamide. It is established the pharmacological evidence which support the folklore claim that MEAS is an anti-diabetic agent.

Girija K et. al. (2011) studied the anti-diabetic and anti-cholesterolemic activity of methanol extracts of leaves of *Amaranthus caudatus, Amaranthus spinosus* and *Amaranthus viridis* was evaluated by using normal and streptozotocin (STZ) induced diabetic rats at a dose of 200 mg/kg and 400 mg/kg p.o. daily for 21 days. Blood glucose levels and body weight were monitored at specific intervals, and different biochemical parameters such as serum cholesterol, serum triglyceride; high density lipoprotein, low density lipoprotein and very low density lipoprotein were assessed. Histology of pancreas was performed. It was found that all the three plants showed significant anti-diabetic and anti-cholesterolemic activity.

Amuthan A et. al (2012) evaluated different concentrations of ASAE (200, 500, 1000, 1500mg/kg), thiazide (10 mg/kg) and vehicle were orally administered to rats (n=6 animals per group) and their urine output was collected after 24 h. Volume, pH, Na⁺, K⁺ and Cl⁻ concentrations of urine were estimated. ASAE produced increase in Na⁺, K⁺ and Cl⁻ excretion, caused alkalization of urine, and showed strong saluretic activity and carbonic anhydrase inhibition activity. These effects were observed predominantly at 500 mg/kg dose and suggested that the *A. spinosus* is acting as a thiazide like diuretic.

Zeashan H et. al. (2014) was evaluated hepatoprotective activity of the 50% ethanol extracts of the whole plant of *Amaranthus spinosus* Linn. (Amaranthaceae) against d-galactosamine/lipopolysaccharide (d-GalN/LPS)-induced liver injury in rats. d-GalN/LPS (300 mg/kg body weight/30 μg/kg body weight)-induced hepatic damage was manifested increase in the activities of marker enzymes (aspartate transaminase, alanine transaminase, alkaline phosphatase, lactate dehydrogenase and gamma glutamyl transferase) and
bilirubin level in serum while phospholipids significantly decreased. All other parameters, viz. cholesterol, triglycerides and free fatty acids were increased significantly in both serum and liver compared to the control group. Pretreatment of rats with *A. spinosus* extract (400 mg/kg) reversed these altered parameters to normal compared to the intoxicated group. The biochemical observations were supplemented by histopathological examination of liver sections. Results of this study revealed that *A. spinosus* extract could afford a significant protection against d-GalN/LPS-induced hepatocellular injury.

Harsha VS (2011) was study on ethanol and aqueous extracts of *Amaranthus spinosus* (roots) for their antibacterial activity against ten bacterial strains including Gram-positive and Gram-negative bacteria using the agar-well diffusion method. As compared to other extracts tested, the ethanol extract presented the best results while the aqueous extract showed moderate inhibition of the microbial growth. Each extract is unique against different microorganisms.

Olumayokun AO et. al. (2004) evaluated the methanol extracts of *Amaranthus spinosus* Linn leaves for anti-inflammatory activities in different animal models. The extract (25-100 mg/kg) inhibited the carrageenan-induced rat paw edema and produced significant inhibition of acetic acid-induced increased vascular permeability. Inhibition of the cotton pellet granuloma was also inhibited by 100 mg/kg of the plant extract. Analgesic activity was exhibited with the significant and dose-related reduction in the number of writhings induced with acetic acid, as well reduction in paw licking induced by injection of formalin in mice. These results demonstrate the anti-inflammatory properties of the leaf extract of *A. spinosus*. It is also suggested that the plant extract probably acts by the inhibition of prostaglandin biosynthesis.

Hussain Z et. al. (2009) studied that the ethanol extract (50%) of the whole plant of *Amaranthus spinosus* Linn. (ASE) significantly inhibited travel of a charcoal meal at three different doses of ASE, but when 400 mg/kg of ASE was repeated in the presence of yohimbine, intestinal propulsive inhibition decreased, while morphine reversed the activity. The percentages related to controls for the onset of diarrhoea were 16.58, 83.42, and 116.18% at doses of 100, 200, and 400 mg/kg of ASE, while with morphine this value was 123.93% compared to controls. The percentage purging frequency related to controls was 41.09, 64.38, 71.23, and 86.30% at three different doses of ASE and with morphine,
respectively. The inhibitions in intestinal accumulation were 8.9, 48.16, and 68.06% at doses of 100, 200, and 400 mg/kg of ASE, respectively, compared to control, while inhibition with yohimbine was 50.78%. Antidiarrheal indices of ASE were 23.55, 49.16, and 76.53 at the three different doses of ASE, while morphine had a maximum index of 88.4.

Jhade D et. al (2011) studied on aqueous and ethanolic extracts of root of Amaranthus spinosus Linn for antifertility activity in rats. Antifertility screenings of water and ethanolic extract of A. spinosus were done by the reproductive outcome in mice, anti-implantation, abortifacient, estrogenic and anti estrogenic activity in rats. The water extract of the root of plant showed the decrease in number of implants and number of litters when compared with the ethanolic extract as the percentage of implantation failure increased. The extract shows furthermore, non significant increase in uterine weight in immature ovariectomised rats. Simultaneous administrations of extracts with ethinyl estradiol cause significant antiestrogenic activity. All these observations suggest that aqueous and ethanolic extracts of A. spinosus Linn. have weak antifertility effect.

Ashok BSK et. al (2010) screened methanolic extract of Amaranthus spinosus leaves for antipyretic activities. Antipyretic activity of methanolic extract of A. spinosus was measured by yeast induced pyrexia method at concentration of 200 and 400 mg/kg using paracetamol as standard drug. Methanolic extract of A. spinosus showed significant (P<0.01) antipyretic activity.

Ghosh D et. al. (2013) studied on anti peptic ulcer activity of the leaves of Amaranthus spinosus Linn plant of Eastern Himalaya, in peptic ulcer models rats. Gastric and duodenal ulcers were induced by ethanol and cysteamine respectively. Results were compared with omeprazole, a known drug for peptic ulcer. It was found out that the leaves of A. spinosus Linn. exerted anti peptic ulcer activity against ethanol and cysteamine induced peptic ulcerations but the activity was less than that of omeprazole.

Mitra PK et. al. (2013) studied on anti gastric ulcer activity of root stems and leaves of Amaranthus spinosus Linn against ethanol, hydrochloric acid, indomethacin, stress and pyloric ligation induced gastric ulceration in albino rats. Omeprazole was used as standard anti gastric ulcer drug. Significant anti gastric
ulcer activity was noted in root, stem and leaves of *A. spinosus* Linn. Root of the plant, however, showed highest activity which was comparable to that of omeprazole.

Joshua LS et. al. (2010) studied antitumor potentials of *Amaranthus spinosus* against EAC bearing Swiss albino mice. The ethanol extract of its leaves given orally to mice at the dose of 100 and 200 mg/kg body weight for 16 days. It was observed that decrease in tumor volume and viable cell count, while increase in mean survival time and non viable tumor cell count, when compared to the mice of the EAC control group. Restoration of hematological and biochemical parameters towards normal was also observed. The results suggest that the ethanol extract of *A. spinosus* leaves exhibits significant antitumor effects in EAC bearing mice.

Gul T et. al. (2011) studied the biochemical role of methanolic extract of *Amaranthus spinosus* on the liver of Sprague Dawley rats indicate that significant change in protein and glycogen contents.

Thapar R et. al, (2005) studies Competitive impact of *Amaranthus spinosus* Linn. in *Celosia argentia* and *Corchorus solitorius* production in Southwestern Nigeria. The leaf residue of *Amaranthus spinosus* Linn., effect on the growth and metabolism of *Pertheneum hysterophorus* L., in pot culture. The leaf residue of *Amaranthus spinosus* inhibits the height of the plant, length of the leaves and the number of branches capitula and seeds per plant. The total sugar content was also decreased as well. The accumulation of organic acids revels that respiration was hampered in *A. spinosus*. An increase in the amino acids might be an adaptation of plant in environmental stress.

Ashok BSK (2011) evaluates the alpha amylase and the antioxidant potential of methanol extract of *A. spinosus* (MEAS). The aim of this study was to investigate in vitro alpha amylase enzyme inhibition by CNPG3 (2-chloro-4-nitrophenol a-D-maltotrioside) and in vivo antioxidant potential of malondialdehyde (MDA), glutathione (GSH), catalase (CAT) and total thiols (TT) in alloxan-induced diabetic rats of a methanolic extract of *A. spinosus*. This study provides evidence that the methanolic extract of *A. spinosus* has potent alpha amylase, anti-diabetic and antioxidant activities.
3.2 Plant Profile of *Annona squamosa*:

![Annona squamosa Fruit and Pulp](image)

*Figure 3.2 Annona squamosa Fruit and Pulp*

### 3.2.1 Taxonomical Classification:

- **Kingdom**: Plantae - Plants
- **Subkingdom**: Tracheobionta - Vascular Plants
- **Superdivision**: Supermatophyta - Seed plants
- **Division**: Magnoliophyta - Flowering Plants
- **Class**: Magnoliopsida - Dicotyledons
- **Subclass**: Magnoliopsida
- **Order**: Magnoliopsida
- **Family**: Annonaceae - Custard-apple family
- **Genus**: Annona L. - annona
- **Species**: Annona squamosa L. - sugar apple
- **Binomial name**: Annona squamosa L

### 3.2.2 Local Name:

- **English**: Custard apple, sugarapple, sweetsop
- **Hindi**: Sitafal
- **Bengali**: Ata
- **Malayalam**: Aathappazham, seethe pazham
- **Telugu**: Seetha phalam
- **Chamorro**: Ates, atis
- **Dutch**: Kaneelappel

### 3.2.3 Plant part used: Pulps
3.2.4 General Information:
The sugar apple tree ranges from 10 to 20 ft (3-6 m) in height with open crown of irregular branches, and some-what zigzag twigs. Deciduous leaves, alternately arranged on short, hairy petioles, are lanceolate or oblong, blunt tipped, 2 to 6 in (5-15 cm) long and 3/4 to 2 in (2-5 cm) wide; dull-green on the upperside, pale, with a bloom, below; slightly hairy when young; aromatic when crushed. Along the branch tips, opposite the leaves, the fragrant flowers are borne singly or in groups of 2 to 4. They are oblong, 1 to 1 1/2 in (2.5-3.8 cm) long, never fully open; with 1 in (2.5 cm) long, drooping stalks, and 3 fleshy outer petals, yellow-green on the outside and pale-yellow inside with a purple or dark-red spot at the base. The 3 inner petals are merely tiny scales. The compound fruit is nearly round, ovoid, or conical; 2 1/3 to 4 in (6-10 cm) long; its thick rind composed of knobby segments, pale-green, gray-green, bluish-green, or, in one form, dull, deep-pink externally (nearly always with a bloom); separating when the fruit is ripe and revealing the mass of conically segmented, creamy-white, glistening, delightfully fragrant, juicy, sweet, delicious flesh. Many of the segments enclose a single oblong-cylindric, black or dark-brown seed about 1/2 in (1.25 cm) long. There may be a total of 20 to 38, or perhaps more, seeds in the average fruit. Some trees, however, bear seedless fruits.

3.2.5 Cultivation:
It is the most widely cultivated of all the species of *Annona*, being grown widely throughout the tropics and warmer subtropics, such as Indonesia, Thailand, and Taiwan; hit was introduced to southern Asia before 1590. Like most species of *Annona*, it requires a tropical or subtropical climate with summer temperatures from 25 °C (77 °F) to 41 °C (106 °F), and means winter temperatures above 15 °C (59 °F). It is sensitive to cold and frost, being defoliated below 10 °C and killed by temperatures of a couple of degrees below freezing. It is only moderately drought-tolerant, requiring rainfall above 700 mm, and will not produce fruit well during droughts.

3.2.6 Phytochemistry:
Numerous acetogenins were isolated from the seeds of *Annona* squamosa. For the most part, they were found to be a mono- or adjacent bis-THF-ring bearing compounds. *Annonaceous acetogenins* were a group of compounds that were
isolated so far only from the Annonaceae family, but were recently reported to be present in the family of Vitaceae. These compounds were characterized by the presence of terminal g-methyl-glaactone and by the presence of a long aliphatic chain bearing tetrahydrofuranic THF and tetrahydropyranic rings, and the epoxy rings and (or) the double bonds. They were reported to inhibit the first complex of mitochondrial respiratory chain (NADH-ubiquinone oxido-reductase), and also exhibits parasiticide, insecticide and other cytotoxic activities, and were also represented as the anti-tumoral candidates. Regained attention occurred with the recent works which shows their probable implication in the treatment of typical Parkinsonism in the tropical areas, through the consumption of the Annonaceous edible species. A new category of natural compounds, called as the *Annonaceous acetogenins*, was recently reported to inhibit the ATP production at a similar site of action and at the higher levels of potency as a rotenone, i.e., at the NADH-ubiquinone oxido-reductase, complex I in the mitochondrial electron-transport chain. Caryophyllene oxide was also the active agent isolated from an unsaponified petroleum ether extract of the bark of *Annona squamosa*. The prolonged continuous work on the biologically active-directed fractionation of the bark of *Annona squamosa* was resulted in the discovery of new *Annonaceous acetogenins*, called as (2, 4-cis and trans)-squamolinone, (2, 4-cis and trans)-9-oxo-asimicinone, and bullacin B9.

The Chromatographic purification of the seeds of n-BuOH soluble fraction was resulted in the isolation of seven cyclic peptides which were named as cyclosquamosins A-G. Cyclic peptides were the molecules possessing a wide range of biological activities. Hence, the Conformational determination of such cyclic peptides plays an important role, because of their biological activities that were known to be closely related with their conformational states. Recently, there was a report on the conformations of a list of cyclic heptapeptides, such as hymenamide, pseudostellarin D, and yunnanin A, and segetalins D and E10. Two bis-tetrahydrofuran acetogenins, squamocin-O₁ and squamocin-O₂, were the compounds isolated from a MeOH extract of the seeds of *Annona squamosa* L.

The discovery of a compound uvaricin in 1982 was the first report on the *Annonaceous acetogenins*, found to act as an *in vivo* active anti-leukemia (P-388) agent that has invigorated a wide interest in the family of Annonaceae. The fractionation work as directed by the brine shrimp lethality test (BST) has led to
the isolation of three new bioactive acetogenins, namely 4-deoxy annoreticuin, *cis*- 4-deoxyannoreticuin, and \((2,4\text{-cis and trans)- squamoxinone.}\) Two more new *Annonaceous acetogenins* called as the squamostanin-C and squamostanin-D were isolated from the 95% EtOH extract of the seeds of *Annona squamosa*. Rollicosin was the compound isolated from Rollinia mucosa and Squamostolide from the *Annona squamosa*. These compounds contain a partial skeleton of an ordinary *Annonaceous acetogenins* with two c-lactone moieties on both the sides of an aliphatic chain. Rollicosin can be generated from the oxidative degradation of the ordinary acetogenins such as murisolin and/or from the cis-murisolin and also squamostolide from solamin and/or from the cis-solamin. Moreover, these compounds were found to be helpful to investigate the role of the terminal hydroxylated lactone moiety instead of the hydroxylated THF moiety with long aliphatic chain that could be seen in the ordinary acetogenins for its bioactivity.

### 3.2.7 Traditional uses:

The plant is attributed with the medicinal properties that include anti-fertility and antitumour activities which were observed in mice and rats. The young leaves of *Annona squamosa* were used extensively due to its anti-diabetic activity by the tribal men villagers used to consume a mixture of 4-5 newly grown young leaves along with black pepper (*Piper nigrum*) of about five grains, earlier in the morning in the treatment of diabetes, with the continued therapy that ensures up to 80% of the positive results. The treatment was particularly common and was popular in the Lodha community as the plant was considered to be a sacred fruit. The formula was successfully used and being used by some Unani and Allopathic physicians that is in existence till date. The aqueous leaf extract of *Annona squamosa* was also reported to ameliorate hyperthyroidism, which is the major causative factor for diabetes mellitus. Though there was no such scientific evidence to prove the anti-diabetic effect of *Annona squamosa*, tribal men continued to use the plant in order to manage the diabetes. Its leaves were used as the insecticidal and antispasmodic agents that were used in the treatment of rheumatism and painful spleen. The plant was reported traditionally to possess analgesic, antiinflammatory, anti-pyretic, anti-ulcer, and antiseptic and abortifacient activities. Its utilization as an insecticidal agent was investigated by several workers and other various phytochemical, pharmacological, anti-bacterial and anti-ovulatory studies was carried out with the extracts obtained from the
seeds. Post-cortical anti-fertility activity of *A. squamosa* was reported from studies with the seed extract. Seeds, fruits and leaves were found to be effective as an insecticide, fish poison, and as a powerful irritant of the conjunctiva. The roots were found to be effective as a drastic purgative and in the acute dysentery. The hot aqueous extract of *Annona squamosa* leaves was investigated to possess a significant hypoglycemic and anti-diabetic activity and its fruit has much higher nutritional value with the biological activity of lowering blood glucose level which was tested to be positive in the experimental animals. *Annona squamosa* Vell was astringent and was found to be useful for the treatment of chronic diarrhoea and estomatic disease and also useful as an insecticide.

**3.2.8 Other species of *Annona squamosa*:**

*A. cherimola, A. muricata A. reticulate, A. senegalensis, A. asiatica, A. chrysophylla*

**3.2.9 Literature review of *Annona squamosa*:**

Smita Sharma et. al. (2010) conducted anti-inflammatory and antidepressant activity of leaves and rhizome extract of *Annona squamosa* and *Curcuma longa*. The polyherbal formulation showed the significant anti-inflammatory activity comparable to the standard drug Indomethacin against carrageenan induced rat paw edema method. The polyherbal formulation reduced the inflammation induced by carrageenan by 49.3% and 61.73% on oral administration at 100 mg/ kg and 200 mg/kg respectively as compared to the control treated group an analgesic activity comparable to the standard drug tramadol HCl. Antidepressant activity comparable to the standard drug Fluoxetine HCl.

Sharma MC et. al. (2010) studied *In vitro* using some medicinal herbal leaves against antidepressant, analgesic activity, and anti-inflammatory activity. The polyherbal formulation showed the significant anti-inflammatory activity comparable to the standard drug Indomethacin against carrageenan induced rat paw edema method. The polyherbal formulation reduced the inflammation induced by carrageenan by 53.0% and 47.0% on oral administration at 100 mg/ kg and 200 mg/kg respectively as compared to the control treated group an analgesic activity comparable to the standard drug tramadol HCl.

Mujeeb Mohd et. al. (2009) evaluated antidiabetic activity of the aqueous extract of *Annona squamosa* in streptozotocin induced-hyperglycemic rats. The
aqueous extract of roots of *Annona squamosa* L. at a dose of 250 mg/kg and 500 mg/kg body weight respectively was tested for antidiabetic activity in Streptozotocin (STZ) - induced hyperglycaemic rats. The blood glucose levels were measured at 0, 2h, 4h and 6h after the treatment. The aqueous extract reduced the blood glucose in STZ- induced diabetic rats from 285.52 to 208.81 mg/dl, 6h after oral administration of extract (P < 0.01). The antidiabetic activity of aq. extract of *Annona squamosa* was compared with glibenclamide, an oral hypoglycaemic agent (3mg/kg).

Chandrashekar C et. al. (2011) explored isolation, characterizations and free radical scavenging activity of *Annona squamosa* leaf. The chemical compound isolated was analyzed by IR, LC-MS and the compound was confirmed flavones type compound on the basis of spectral data. The in vitro antioxidant activity of isolated compound (AS-1) was evaluated by free radical scavenging activity of different concentrations (10μg, 50 μg, and 100 μg) using 1, 1-diphenyl-2 picryl hydrazil method (DPPH). The results of assay were then compared with synthetic antioxidant Butylated hydroxyl anisole (BHA).The isolated compound exhibit (9.62, 24.28, and 45.62%) significant free radical scavenging activity.

Mukhlesur Rahman M et. al. (2005) studied anti-bacterial and anti-fungal activities of the plant compounds such as Petroleum ether extract, CHCl₃ extract, EtOH extract, annotemoyin-1, annotemoyin-2, squamocin and cholesteryl glucopyranoside showed maximum inhibition against the gram positive and the gram negative organisms. The cytotoxicity of the plant extracts was studied by the brine shrimp lethality bioassay and the LC50 values of the petroleum ether and chloroform extracts were calculated.

Singh Sanjiv et. al. (2010) studied the effect of Polyherbal formulation of *Annona squamosa* on blood glucose, plasma insulin, tissue lipid profile, and lipidperoxidation in streptozotocin induced diabetic rats.

Mishar A, et. al. (1979) investigated seed extract of *A. squamosa* Linn for post coitus antifertility activity.

Yang Y.L. et. al. (2002) isolated ent-kaurane diterpenoids, from stem of *A. squamosa* Linn and investigated for anti-platelet activity.

Panda S. et. al. (2007) studied on methanolic extract of seeds of *A. squamosa* Linn, which shows ameliorative effect in the regulation of hyperthyroidism in mouse model.

Ahir A. et. al. (1999) tested antiplasmodial activity of methanolic extract of plant *A. squamosa* Linn on chloroquine sensitive strain 3D7 and chloroquine resistant strain Dd2 of *P. falciparum*. The methanolic extract of *A. squamosa* leaves showed high antiplasmodial activity with IC₅₀ values of 2 and 30 μg/ml on 3D7 and Dd2, respectively.
3.3 Plant Profile of *Brassica nigra*:

![Figure 3.3 Brassica nigra Linn plant and seeds](image)

3.3.1 Taxonomical Classification:

- **Kingdom**: Plantae - Plants
- **Subkingdom**: Tracheobionta - Vascular Plants
- **Superdivision**: Supermatophyta - Seed plants
- **Division**: Magnoliophyta - Flowering Plants
- **Class**: Magnoliopsida - Dicotyledons
- **Subclass**: Dilleniidae
- **Order**: Capparales
- **Family**: Brassicaceae - Mustard family
- **Genus**: Brassica Linn. - Mustard
- **Species**: Brassica nigra Linn. - Black mustard

3.3.2 Local Names:

- **Synonym**: Indian mustard
- **English**: Black mustard
- **Hindi**: Sarson
- **Marathi**: Mohari
- **Telugu**: Avalu
- **Gujarati**: Rai
- **Punjabi**: Rai
- **Tamil**: Kadugu
3.3.3 Plant part used: Seeds

3.3.4 Plant History
The earliest mention of mustard seeds in the history of India is in a story of Gautama Buddha, where he is seen advising a crying mother to gather a handful of mustard seeds from a house which has never seen the death of a family member. The mother realizes that she is not alone who has experienced the death of her son, there are many like her who have gone through the same pain. The ancient Greek physicians held this plant in such esteem for the medicinal use of its seeds that they attributed its discovery to Esculapius. When it was first employed as a condiment is unknown, but it was most likely used in England by the Saxons. Probably the Romans, who were great eaters of mustard, pounded and steeped in new wine, brought the condiment with them to Britain. Mustard gets its name from mustum (the must), or newly-fermented grape juice, and ardens (burning). It was originally eaten whole, or slightly crushed. Gerard in 1623 says that “the seede of Mustard pounded with vinegar is an excellent sauce, good to be eaten with any grosse meates, either fish or flesh, because it doth help digestion, warmeth the stomache and provoketh appetite”. Tusser mentions its garden cultivation and domestic use in the sixteenth century, and Shakespeare alludes more than once to it: Tewkesbury mustard is referred to in Henry IV. The herbalist Coles, writing in 1657, says: “In Glostershire about Teuxbury they grind Mustard seed and make it up into balls which are brought to London and other remote places as being the best that the world affords”. It was sold in balls till Mrs. Clements, of Durham, at the close of the eighteenth century, invented the method of preparing mustard flour, which long went under the name of Durham Mustard. John Evelyn recommends for mustard-making 'best Tewkesbury' or the 'soundest and weightiest Yorkshire seeds,' and tells us that the Italians in making mustard as a condiment mix orange and lemon peel with the black seed. At Dijon, where the best Continental mustard is made, the condiment is seasoned with various spices and savouries, such as Anchovies, Capers, Tarragon and Catsup of Walnuts or Mushrooms. The Black Mustard is said to have been employed by the Romans as a green vegetable. The young leaves may be eaten as salad in place of those of the White variety, but are more pungent (Duke’s Handbook of Medicinal Plants of the Bible).
3.3.5 Description of *Brassica nigra* plant

3.3.5.1 General Information

Black mustard seeds, the tiny round structures derived from a mustard plant, have been used from time immemorial as a condiment in food at regions all over the world. Mustard seeds are very popular for the aroma that they lend to world cuisines. The strong and spicy taste of mustard seeds varies according to their types, which are brown Indian mustard, white mustard, and black mustard. The black colored mustard seeds are more acrid to taste compared to the white colored versions. They are loaded with several health promoting properties, giving a myriad of reasons to blend them in daily food. (Ahmed S, et al, 2013)

**Stem:** The stems are usually glabrous and glaucous, sometimes they have scattered stiff hairs toward the base. The root system consists of a taproot. This plant spreads by reseeding itself.

**Leaves:** The alternate leaves are up to 10" long and 3" across, becoming smaller as they ascend the stems, stalked, basal leaves blade pinnately lobed, terminal lobe large, elliptic. Stem leaves blade elliptic-lanceolate, with toothed-entire margin, glabrous, bluish green. The lower leaves are dentate (toothed), pinnatifid (deeply lobed) or lyrate (deeply lobed, but with an enlarged terminal lobe and smaller lateral lobes), and are often hairy, at least on the underside. The terminal lobe is much larger than the lateral lobes. The upper surface of a lower leaf is often bristly with scattered hairs that are stiff, short, and white. The lower surface is usually glabrous, except for a few hairs along the central vein. The upper leaves are often lanceolate, broadly elliptic, or some other odd shape; they have 1-2 lobes or none.

**Flowers:** The yellow, four-parted and cross-shaped flowers, occur in many racemes (spike-like cluster) and produce 4-sided siliques-capsular fruit that dehisces (splits open) when mature-that may be up to 2.5 cm (1 in) long. The upper stems terminate in narrow racemes of yellow flowers; these racemes are ½-2' long when fully mature. Each flower is up to 1/3" across, consisting of 4 yellow petals, 4 sepals, several stamens, and a pistil. The sepals are 4 initially green, but become yellow while the flower blooms. Stamens 6, of which 4 long and 2 short. Gynoecium fused, a single carpel. Inflorescence an elongating raceme in fruiting stage. The petals are petals 4, dark-veined, 7-9 mm (0.28-0.36 in.), long, well-rounded toward their tips. Corolla regular (actinomorphic),
yellow, approx. 1 cm (0.4 in.) across. The blooming period occurs primarily during the summer and lasts about 1-2 months. A few plants may bloom during the fall.

**Silique:** Each siliqua contains 2 to 12 or more reddish brown to black round seeds. A single plant may produce thousands of seeds, which must be harvested by hand or mechanically before they fully ripen, because the siliques spontaneously split and disperse the seeds when they are mature. Each flower is replaced by a slender siliqua that becomes appressed against the stalk of the raceme as it matures. This siliqua is about 2/3" long, tapering to a conical beak. The petiole of the siliqua (or flower) is about 1/3" long.

**Seeds:** The seeds within this siliqua are dark brown or black. Both the seeds and the foliage have a pungent taste. Seeds are usually about 1 or 2 mm in diameter. Mustard seeds may be colored from yellowish white to black. They are tinny round structure, netlike or honeycombed surface. It has a non aromatic odor & pungent taste.

**Fruit:** Many-seeded, opens lengthwise, 4-edged, 1-2 cm (0.4-0.8 in.) long siliqua, parallel to stem, terminated by a 2-3 mm (0.08-0.12 in.) long, seedless beak. Stalk 2-5 mm (0.08-0.2 in.) (Plant guide, USDA & NRCS)

### 3.3.6 Range and Habitat:

Black Mustard is a common plant in central and northern Illinois, but it is less common or absent in southern Illinois. Habitats include weedy meadows, thickets, areas along railroads and roadsides, fallow fields, vacant lots, loading areas, sometimes gardens and miscellaneous waste places. Black Mustard doesn't invade high quality natural areas to any significant degree. It is native to Eurasia. The Black Mustard grows throughout Europe, except in the north-eastern parts, also in South Siberia, Asia Minor and Northern Africa, and is naturalized in North and South America (Duke. J. Handbook of Energy Crops).

### 3.3.7 Cultivation:

This plant often occurs in full or partial sun, fertile soil, and mesic conditions. It is largely cultivated in England, Holland, Italy, Germany and elsewhere for the sake of the seed, used partly as a condiment, and partly for its oil. Mustard is sown in spring, either broadcast or in drills, a foot or more apart, and ripens towards the end of summer, when, after it has stood in sheaves to dry, the seed is threshed out and dried on trays by gentle artificial heat. In Durham, the
cultivation of Mustard of an excellent quality has been pursued on a considerable scale for the last two hundred years. Before grinding, the husk is usually removed; the seeds are then passed between rollers and afterwards reduced to powder in a mortar. This is the system invented by Mrs. Clements, of Durham (Duke. J. Handbook of Energy Crops)

**Flowering time:** June-September.

### 3.3.8 Production:

Today mustard seeds are one of the most widely traded spices, with the major production coming from Hungary, Great Britain, India, Canada and the United States.

### 3.3.9 Constituents:

The virtues of Black Mustard depend on an acrid, volatile oil contained in the seeds, combined with an active principle containing much sulphur. The acridity of the oil is modified in the seeds by being combined with another fixed oil of a bland nature, which can be separated. The epidermal cells of the seed-coat contain much less mucilage than those of white mustard seeds, but the cotyledons of black mustard seeds contain from 31-33 % of a fixed oil, which consists of the glycerides of Oleic, Stearic and Erucic or Brasic and Behenic acids. The seeds also contain the crystalline glucoside Sinigrin and the enzyme Myrosin. These substances are stored in separate cells. When brought together in water, the volatile oil of mustard is formed. It is distilled from the seeds that have been deprived of most of the fixed oil and macerated in water for several hours, and contains from 90-99 % of the active principle, Allyl isothiocyanate, which is used as a counter irritant. Neither White nor Black Mustard seeds contain starch when ripe.

It was formerly supposed that black mustard was deficient in the enzyme myrosin, and white mustard was added to correct this and to secure the maximum pungency. It has been proved, however, that black mustard contains sufficient of the enzyme, and that no increase in the yield of the volatile oil is affected by adding white mustard.

### 3.3.10 Medicinal importance and uses:

Components in the black mustard seed include glycoside (sinigrin) and an enzyme (myrosin); on contact with water, these two components interact and create mustard seed oil. The seeds also contain up to 37 percent fixed oils as well
as proteins and mucilage. Black mustard is recommended as an herbal remedy to:-

**Encourage perspiration:** Black mustard can be used in the same way as ginger or cayenne pepper to stimulate the skin and promote emesis (sweating). Black mustard seeds ground up and steeped into a tea can be added to bath water to help dilate blood vessels in the skin, which may reduce fever.

**Improve digestion:** Black mustard is a warming food that actually improves the digestion of foods with which it is served, in addition to providing a spicy flavor.

**Open the lungs and sinuses:** Inhaling mustard eases breathing and helps open the lungs, because black mustard is antibacterial and antifungal, it is especially good for lung infections such as pneumonia and fungal infections. Black mustard mixes may be inhaled to clear sinuses.

**Treat rheumatoid arthritis:** Black mustard may help because of its ability to improve circulation and reduce inflammation.

**Heal the skin:** Black mustard seed oil is a counter-irritant. When it is applied externally to inflamed skin, it dilates the blood vessels and improves circulation. Black mustard may help speed the healing of an inflamed area by allowing the body to carry off toxins more effectively. It has been recommended as an herbal remedy for chilblains, skin irritation caused by excessive cold temperatures.

**Drain excess fluids:** Black mustard seeds are a diuretic.

**Homeopathy:** Black mustard is sometimes used in homeopathic treatments for upper respiratory and gastro-intestinal conditions.

**Indian medicine:** In India, black mustard tea is served to improve bronchitis, fever, or cold misery, as well as muscular and skeletal pain. Black mustard is also used in India to treat hemorrhoids. Powdered black mustard seeds are mixed with goat milk or fresh yogurt and applied to the affected area.

**Black Mustard as a Poultice:** Black mustard seeds can be crushed and used to create a poultice that is applied to the chest (a mustard plaster) or to the feet.

**To create a mustard plaster:** Mix together four ounces of freshly ground mustard seeds and a half cup of warm water; beat into a thick paste. Add wheat flour if needed to make the mix thick. Spread on dampened gauze or flannel and apply to the chest, back, or feet for one minute. Remove the mustard plaster and check for reddened skin. Continue to check every minute for the first five
minutes. CAUTION: Don’t leave the mustard plaster in place if skin begins to redden; remove after no more than twenty minutes, and only if patient is conscious and comfortable. After removal, smooth olive oil on the skin to reduce irritation.

To make a black mustard foot bath: Add one full tablespoon of crushed black mustard seeds to two pints of boiling water and let steep and cool before adding to the foot basin (Alternativz, nutritets and nutracueticals, 2013).

3.3.11 Other species:


3.3.12 Literature review of Brassica nigra:

Uppala P et al. (2013) investigated methanolic extract of Brassica nigra Linn seeds on Maximal electroshock induced seizures (MES), Pentylenetetrazol (PTZ), Picrotoxin (PIC) induced seizures and Biccuculine induced seizures in mice. It was found that the extract (200 & 400 mg/kg, p.o), significantly prolonged the onset of tonic seizures and reduced the duration of incidence of seizures in PTZ, PIC and Biccuculine induced seizure models where as in MES model, the extract showed significant effect in abolishing tonic hind limb extensions by inhibiting voltage dependant Na+ channels or by blocking glutaminergic excitation mediated by the N-methyl- D-aspartate (NMDA) receptor.

Upwar N et al. (2011) studied alcoholic extract from the seeds of Brassica nigra Linn for their anthelmentic activity against Pheretima posthuma and Ascardia galli. Various concentrations (10-100 mg/ml) of extract were tested in bioassay, which involved determination of time of paralysis and time of death of the worms. Alcoholic extracts exhibited significant activity at highest concentration of 100mg/ml. Piperazine citrate (10mg/ml) was included as standard reference and distilled as control.

Vinyas M et al. (2012) investigated ethanolic extract of Brassica Nigra seed has anti-arthritic activity. Arthritis is induced in the albino rats by inducing the freunds complete adjuvant. The seeds of freunds adjuvant were coarsely
powdered and extracted with ethanol (95%) and water using soxhlet. The effect of these plant extracts on arthritic rats were assessed by the various blood parameters and also taking the changes in paw volume. The BN suppressed the anti–arthritic changes induced in rats and results were statically significant.

Kamel E et al. (2013) evaluated cytotoxic activity of the ethanolic, ethyl acetate and hexane extracts of aerial parts of Brassica nigra Linn against five cancer cell lines and it was found to exhibit significant growth inhibitory activities against HepG2, HeLa, HCT, MCF-7 and HEp2 tumor cells. Brassica nigra Linn extracts showed a potent cell growth inhibition activity on all tested cancer cell lines, in a dose dependent manner.

Kamel E et al. (2013) evaluated antibacterial activity using the agar diffusion and microwell dilution assays against five strains of bacteria. The ethanolic extract of Brassica nigra exhibited the best antibacterial activity against all tested bacteria. The ethanolic extract from this plant has a broad spectrum activity since it is effective against both Gram positive and Gram negative bacteria. The extract was found to be active against Streptococcus pyogenes which is the cause of many important human diseases, ranging from mild superficial skin infections to life-threatening systemic diseases.

Jasim A et al. (2012) evaluated antibacterial activity of oils extracts of Brassica nigra seeds on some bacteria isolated from plaque and healthy teeth in children (1-5) years which include pathogenic and non pathogenic staphylococci, streptococcus spp. Escherichia coli and proteus spp.

Anand P et al. (2007) evaluated aqueous extract of Brassica nigra seeds for its antihyperglycemic activity in streptozotocin induced diabetic rats. The effective dose was found to be 200 mg/kg body weight in GTT. Administration of aqueous extract to diabetic animals daily once for one month brought down fasting serum glucose level.

Alam M et al. (2011) evaluated In vivo and in vitro anti-inflammatory activity of the crude extract using carrageenan induced rat paw edema and protease enzyme inhibition assay, respectively. In vivo anti-inflammatory test of the ethanolic extract of Brassica nigra (500 mg/kg) gave 17.9% inhibition whereas standard Phenylbutazone (100 mg/kg) gave 39.38%. In vitro anti-inflammatory test of B. nigra by protease inhibition method also gave 42.57% inhibition of trypsin at dose 250 μg/ml. Alam also studied on ethanolic leaf
extract of *Brassica nigra* Linn to detect anti-oxidant activity. The different antioxidants assays including total antioxidant activity, DPPH, nitric oxide (NO) scavenging, reducing power were performed; Total antioxidant capacity of the extract was found to be 97.08 mg/g of ascorbic acid. *Brassica nigra* showed IC$_{50}$ value of 63.09 µg/ml whereas the standard antioxidant showed IC$_{50}$ value 14.45 µg/ml in DPPH method.