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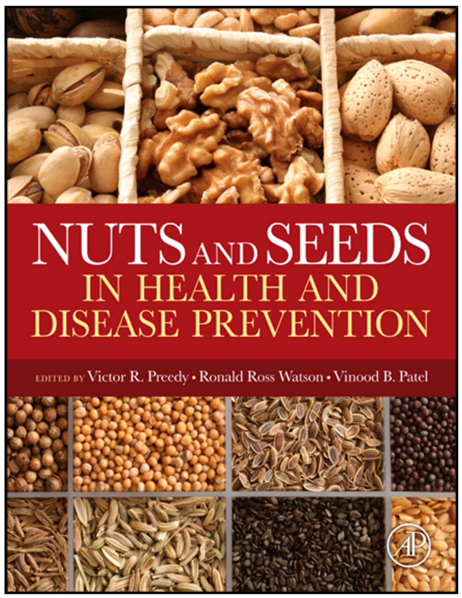

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CHAPTER 110

Pumpkin (*Cucurbita moschata* Duchesne ex Poir.) Seeds as an Anthelmintic Agent?

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CHAPTER OUTLINE

INTRODUCTION

The tropical America native pumpkin *Cucurbita moschata* (Figure 110.1) belongs to the *Cucurbitaceae* family (Fournet, 2002). The fruit is used as a vegetable, and the seed is a high energy source (40–50% lipids and 30–37% proteins in dry embryo material) which is consumed throughout the world with increasing popularity (Table 110.1; Leung *et al.*, 1968; Caili *et al.*, 2006). It is a valuable source of potassium, phosphorus, iron, and β-carotene (TRAMIL, 1999; Caili *et al.*, 2006). The pumpkin seed is used as a vermifuge, galactogogue, and anti-emetic, and to treat various other medical issues, including prostate and bladder problems, in several countries (Caili *et al.*, 2006). It contains a wide range of bioactive compounds, some of which could possess anthelmintic properties, prompting further experimental studies (Figure 110.2).

BOTANICAL DESCRIPTION

Cucurbita moschata is an annual dicotyledonous vegetable, with creeping or climbing stems (growing up to 5 m) bearing tendrils. The stems are strong, cylindrical or pentangular, with petioles measuring 12–30 cm. The stems and leaves are mildly hairy. The leaves are circular, kidney-shaped, heart-shaped, or triangular, often deeply indented at the base, weakly lobed,

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FIGURE 110.1
Longitudinal section of pumpkin *Cucurbita moschata* Duchesne ex Poir. The flesh is orange, and the hollow center contains pulpy loose fibers and flat, oval white seeds.

wavy and toothed, more or less white spotted, up to 20 cm long and 30 cm wide. The flowers are large, yellow, bell-shaped, five-lobed, and up to 12 cm long. The peduncle is strong, with a rounded pentangular base and large apex. Fruits are round, oblate, oval, oblong, or pear-shaped, variously ribbed, 15—60 cm in diameter, and weigh up to 45 kg. Their flesh is deep yellow, orange, pale green, or white, and the hollow center contains pulpy loose fibers and numerous seeds. The seeds (Figure 110.1) are oval, flat, white to brown, thin-shelled, irregularly margined, with a meaty kernel (Morton, 1981; Fournet, 2002).

HISTORICAL CULTIVATION AND USAGE

C. moschata is believed to be native to tropical America (Morton, 1981), where it has been used as a popular vegetable in cooking for several thousands of years. The seeds are edible, and have medicinal applications. In Austria and adjacent countries, pumpkins have been grown for oil production for about three centuries (Caili *et al.*, 2006). *C. moschata* has been used traditionally as a medicine in many countries, including China, Yugoslavia, Argentina, India, Mexico, Brazil, and America (Caili *et al.*, 2006). The anthelmintic activity of the seeds of the

TABLE 110.1 ***Cucurbita moschata*** **Seed: Chemical Composition for 100 g of Husked Seeds**

Constituent	Amount
Water	5.5 g
Energy	2331 kJ (555 kcal)
Proteins	23.4 g
Lipids	46.2 g
Carbohydrates	21.5 g
Fibre	2.2 g
Ca	57 mg
P	900 mg
Fe	2.8 mg
Thiamine	0.15 mg
Niacine	1.4 mg

Data from Leung *et al.* (1968).

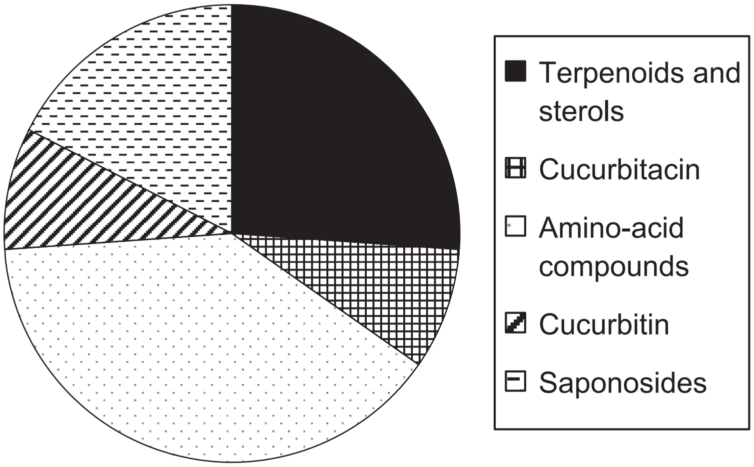

FIGURE 110.2
Qualitative phytochemical composition of *Cucurbita moschata* seed, suspected to have anthelmintic activity. The phytochemical determination was performed by thin layer chromatography methods. Qualitative composition and quantitative evaluation were assessed according to the intensity of the obtained spots. *Data from Marie-Magdeleine (2009), PhD thesis.*

Cucurbita species has long been known (Fang *et al.*, 1961). The Surinam and Cherokee Amerindians used pumpkin seed as an anthelmintic, and also as a pediatric urinary aid to treat bed-wetting (Vogel, 1990).

PRESENT-DAY CULTIVATION AND USAGE

Nowadays, *C. moschata* represents an economically important vegetable species, commonly cultivated worldwide (Taylor & Brant, 2002). The cultivation characteristics are summarized in Table 110.2.

C. moschata seeds are eaten in several countries as snacks, following salting and roasting (Koike *et al.*, 2005). They are commonly used for treating diabetes, prostate gland disorders, and parasites. Data from the ethno-pharmacological literature indicate that *C. moschata* seeds are a potential vermifuge. Eaten fresh or roasted, they help relieve abdominal cramps and distension due to intestinal worms (Caili *et al.*, 2006). Pumpkin seeds are also an important traditional Chinese medicine in the treatment of cestodiasis, ascariasis, and schistosomiasis (Koike *et al.*, 2005). Seeds are used in the Middle East as an effective taenifuge (Karamanukian, 1961). In Middle America, a vermifugal preparation containing shelled and powdered pumpkin seeds is used to expel intestinal worms, including tapeworms (Morton, 1981).

TABLE 110.2 ***Cucurbita moschata*** **Cultivation Characteristics**

Plant Needs	*C. moschata* Constraints
Location	Tropical countries, up to 1800 m altitude
Plant density	2–3 kg of seed/ha, 2–4 seeds per hill; planting distance 2 m × 2 m
Temperature	Temperature above 20°C (day) and 14°C (night).
Period of growth	Photoperiod-insensitive, year-round crop, but better growth during rainy season, if without irrigation.
Soil	Not very demanding with respect to soil conditions; can be cultivated on fertile and well-drained soil, pH 5.5–6.8.
Sensitivity	Drought-tolerant, but sensitive to frost and water-logging; excessive humidity stimulates fungal and bacterial development

Data from Grubben & Denton (2004).

APPLICATIONS TO HEALTH PROMOTION AND DISEASE PREVENTION

Over the years, scientists have studied the many pharmacological actions and potential uses of pumpkin and its extracts. Clearly, there is a lot to learn about the health effects of this plant. Popular medicinal uses of pumpkin seeds have motivated experimental studies on their anthelmintic properties. Such studies have been conducted on different helminth models, and have produced conclusions that call for further research.

Veen and Collier (1949) showed the efficacy of an aqueous extract of the seeds of *C. moschata* as an anthelmintic in humans. Methylene chloride and methanolic extracts of *C. moschata* seed were evaluated on *Caenorhabditis elegans*, and showed no efficacy at the tested doses (Atjanasuppat *et al.*, 2009). Beloin *et al.* (2005) reported anthelmintic activity against *Caenorhabditis elegans* at 500 μg/ml. Marie-Magdeleine *et al.* (2009) showed that aqueous, methanolic, and dichloromethane extracts of *C. moschata* seed greatly inhibited (> 90%) larval development of *Haemonchus contortus in vitro*. The dichloromethane and methanolic extracts had a marked effect on adult worm motility *in vitro* (inhibition of motility > 59% after 24 h of incubation). Atjanasuppat *et al.* (2009) evaluated the activity of methylene chloride and methanolic extracts of *C. moschata* seeds against *Paramphistomum epiclitum*, and found no efficacy at the tested doses. Seeds administered *per os* at a dose of 80 g/person showed high antischistosomial activity (TRAMIL, 1999). Clinical trials in Thailand confirmed that seed extracts were effective against schistosomes and tapeworms (Grubben & Denton, 2004). The use of aqueous extracts of pumpkin seeds in the treatment of puppies experimentally infected with heterophyasis gave promising results, with even better results when combining extracts of areca nut and pumpkin seeds than when giving either extract alone. An effect was reported at the minimum inhibitory concentration of 23 g of pumpkin seeds in 100 ml of distilled water in preclinical studies (Caili *et al.*, 2006).

Some of the bioactive compounds present in the pumpkin seeds thus appear to possess anthelmintic properties, prompting further studies. Pumpkin seed oil contains 9.5–13% palmitic, 6–7.93% stearic, 0.04% arachidic, 37–39% oleic, and 44% linoleic acid. The seed also contains a wide range of bioactive compounds.

The vermifugal active principle of pumpkin seeds has for many years eluded a large number of investigators (Mihranian & Abou-Chaar, 1968). In 1931, it was shown that this bioactive compound is soluble in 75% ethanol but not in petroleum ether, that it is dialyzable, and that its activity is reduced by boiling in dilute sulfuric acid. In 1937, it was also established that the compound is soluble in water and heat resistant. A purified deproteinized aqueous extract of the seeds of *C. moschata* was used as an anthelmintic in humans by Veen and Collier (1949). In 1961, after a comparative morphological, historical, and clinical study (Mihranian & Abou-Chaar, 1968), it was found that pharmacologically active strains of pumpkin seeds should be administered in doses of about 500 g.

The secondary metabolites suspected to be responsible for anthelmintic activity in *C. moschata* seed (Marie-Magdeleine, 2009; Marie-Magdeleine *et al.*, 2009) are a triterpenic compound named cucurbitacin B, a non-proteic amino acid named cucurbitin (3-amino-pyrrolidine-3-carboxylic acid), saponins, and sterols (Mihranian & Abou-Chaar, 1968; TRAMIL, 1999), but other compounds might also be involved, such as cucurmosin, a ribosome-inactivating protein present in the sarcocarp of the pumpkin and also in the seed (Morton, 1981). Researchers are currently working on the structural properties and function of bioactive compounds of pumpkin (Caili *et al.*, 2006) in order to elucidate their modes of action.

The role of non-proteic amino acids in plants is to protect seeds by intoxicating predators via an antimetabolite action; interference between non-proteic and normal amino acids during the biosynthesis of proteins by the predator causes toxicity because the enzymatic system of the predators cannot distinguish these non-proteic amino acids from the normal amino acids, due to their isostery. Consequently, the proteins biosynthesized by the predator may not be functional.

The non-proteic amino acid cucurbitin, which is only present in the seeds (0.4—0.84% of the whole seed; Mihranian & Abou-Chaar, 1968), has been focused on as the active principle responsible for anthelmintic, notably taenicidal and schistosomicidal, activity (Fang *et al.*, 1961). Cucurbitin is also used as an anti-allergen for the preparation of cosmetics and pharmaceutical, particularly dermatological, products. The chemical structure of this amino acid is shown in Figure 110.3A.

In 1965, a study of the amino acids of the *Cucurbitaceae* in several species of *Cucurbita* (in Mihranian & Abou-Chaar, 1968) revealed that cucurbitin was present in all species examined. A further study by Mihranian and Abou-Chaar (1968) confirmed that it appears to be confined to the genius *Cucurbita*, a matter of chemotaxonomic importance. Cucurbitin was associated with a large number of free amino acids in the seed. The free water-soluble amino acid cucurbitin was isolated from *C. moschata* seed, and showed inhibition of the growth of immature *Schistosoma japonicum in vivo* (Fang *et al.*, 1961). Preliminary human research conducted in China and Russia has shown that it may also help in the treatment of tapeworm infestations (Plotnikov *et al.*, 1972). Nevertheless, a high dose of cucurbitin appears to be necessary for efficient anthelmintic action (Mihranian & Abou-Chaar, 1968).

The chemical structure of the cucurbitin compound is similar to that of the nematicidal compound named kainik acid (Figure 110.3B). Kainik acid has a neurodegenerative action on nematodes by substituting for glutamate. The analogy in chemical structure may underlie the similar mode of action of cucurbitin as an anthelmintic (Marie-Magdeleine *et al.*, 2009). Several methods of detection, quantitative determination, and extraction of this amino acid from pumpkin seeds have been used: a two-dimensional paper chromatographic method, a separation method on ion-exchange cellulose phosphate paper, and HPLC and gas chromatography-mass spectrometry (Fang *et al.*, 1961; Mihranian & Abou-Chaar, 1968; Schenkel *et al.*, 1992).

Despite the focus on cucurbitin as the active principle, other secondary metabolites present in the seeds of *C. moschata* might be considered as anthelmintics.

The ribosome-inactivating protein (RIP) cucurmosin is a RNA N-glycosidase that inactivates ribosomes via site-specific deadenylation of the large ribosomal RNA (Marie-Magdeleine *et al.*, 2009). Ribosome-inactivating proteins are also capable of inactivating many non-ribosomal nucleic acid substrates. Some RIPs are able to promote antitumor or antiviral activities. The cucurmosin RIP, which may be present in the seed, could also have an

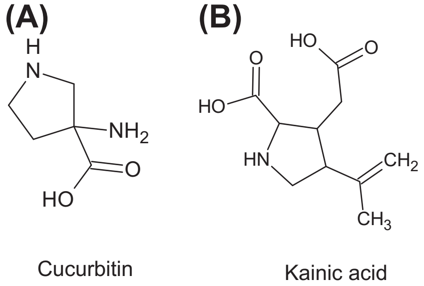

FIGURE 110.3
Comparison of chemical structures of (A) the amino acid cucurbitin (3-amino-pyrrolidine-3-carboxylic acid) **and (B) the nematicidal compound kainik acid.** The similarity in chemical structure (the pyrrolidine ring) may underlie the similar mode of action of cucurbitin as an anthelmintic.

anthelmintic action by inhibiting the synthesis of proteins and stopping development in helminths.

Terpenoid compounds (e.g., essential oils, saponins) are known to be active against a large range of organisms. Some of them are bioactive, whereas others can affect physical variables. Because they are a complex mixture of compounds, terpenoids can be effective against several targets. The triterpenic cucurbitacins can be toxic, purgative compounds, and are involved in insect resistance (Marie-Magdeleine *et al.*, 2009). Terpenoids reduced the mobility and the consequent migration ability of ovine nematode larvae (Marie-Magdeleine *et al.*, 2009). Furthermore, triterpenoids, saponins, and sterols are all antibacterial, antimicrobial, anticarcinogenic, and antifungal (Marie-Magdeleine *et al.*, 2009). The complexity of the nature of these compounds and their chemical structures could enable interaction with multiple molecular targets at the various developmental stages of the parasites, and be responsible for the anthelmintic activity of the *C. moschata* seeds. The mode of action of these groups of compounds against nematodes and helminths may involve synergy (Marie-Magdeleine *et al.*, 2009).

ADVERSE EFFECTS AND REACTIONS (ALLERGIES AND TOXICITY)

Ingestion of the internal part of seed given *per os* to 105 children infected with *Enterobius vermicularis* was effective, and had no side effects (TRAMIL, 1999). No side effects were observed when testing cucurbitin compared to a placebo on 53 persons for prostate hyperplasia over 3 months (TRAMIL, 1999). Low-level toxicity in dogs and humans was described for cucurbitin (TRAMIL, 1999).

SUMMARY POINTS

- The pumpkin *Cucurbita moschata* is an annual dicotyledonous vegetable, belonging to the *Cucurbitaceae* family. It is commonly cultivated worldwide.
- The seeds and fruits of *C. moschata* are edible; they are used as a medicinal plant for prostate and bladder problems, and as an anthelmintic, a galactogogue, and an anti-emetic.
- Pumpkin seed is used as a vermifuge in several countries, and *C. moschata* seed contains a wide range of bioactive compounds, some of which could possess anthelmintic properties, prompting experimental studies.
- Biological assays showed that the *C. moschata* seed has nematicidal, trematodicidal, taenicidal, and schistosomicidal effects.
- The non-proteic aminoacid cucurbitin (3-amino-pyrrolidine-3-carboxylic acid) is suspected to be the active principle. Cucurbitin was mostly reported to have no side effects, and only a weak level of toxicity was described in dogs and humans.

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