

***Datura metel* AS A POTENTIAL NATURAL ANAESTHETIC AGENT FOR AQUATIC ANIMALS**

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Abstract: Anaesthetic agents play an important role in enabling scientific research, by facilitating safe handling for procedures and ensuring animal welfare. This study reviews the natural anaesthetic agent extracted from *Datura metel* leaves as an alternative that replaces commercial synthetic anaesthetic agents. The *Datura* plant is rich in bioactive phytochemicals including alkaloids, phenols, flavonoids, saponins, tannins, and sterols. Alkaloid atropine, hyoscine, and scopolamine derivatives are interesting candidates for use as anaesthetic agents in aquatic animals due to their pharmacological properties. These anaesthetic agents for aquatic animals were judged by understanding the administration and absorption of the substances or drugs, and the physiological and environmental impact.

Keywords: *Datura metel*, anaesthetic, scopolamine, aquaculture, aquatic animals.

Introduction

Studies on aquatic organisms and routine aquaculture practice by the biologists and aquaculturists involve multiple procedures that may threaten the health and welfare of aquatic animals (Huntingford *et al.*, 2006; Ak *et al.*, 2022). Biometric measurements for species identification, tagging and tracking for genetic population study, implantation of sampling devices for bile, blood and urine collection, health monitoring and vaccination, artificial breeding, grading and sorting as well as packaging and transportation are some of the common practices in aquaculture management that distress aquatic organisms (Nascimento *et al.*, 2020).

Therefore, anaesthetic agents for aquaculture activities has been introduced to minimise physiological stress, prevent handling injuries and ensure the optimum quality and health. However, anaesthetic agents for aquacultural activities are limited compared with anaesthetic agents for veterinary practices (Nascimento *et al.*, 2020).

Anaesthetics agents can be divided into synthetic and natural products (Hoseini *et al.*, 2018). Synthetic anaesthetic agents

that are commonly used for aquacultural purposes are tricaine methane sulfonate (MS-222), benzocaine (ethyl 4-amino- benzoate), quinaldine, and 2-phenoxyethanol (Priborsky & Velisek, 2018). However, various researchers documented adverse impacts of synthetic anaesthetic agents in aquatic organisms. Imjai *et al.* (2021) documented that the residue from synthetic agents may accumulate in the tissues of aquatic organisms and as such may be unsafe for human consumption.

Meanwhile, Uma *et al.* (2020) stated that MS-222 may lower the pH level of water creating an acidic condition that may aggravate aquatic organisms. Brown (2011) reported that MS-222 is a dangerous chemical that causes eyes and neurological disorders. In addition, MS-222 also may cause hyperglycaemia and induce oxidative stress (Carter *et al.*, 2011). Vieira *et al.* (2021) emphasised that synthetic agents may alter the physiological and biochemical nature of aquatic animals after exposure.

Synthetic anaesthetic agents not only harm aquatic animals but humans as well. These substances are volatile compounds that may pose an allergenic and or carcinogenic risk to

individuals. Misuse and misconduct of synthetic agents such as administering an inappropriate dosage and exposure time as well as applications to unspecified target species gives adverse effects on both aquatic animals as well as their handlers.

Additionally, the lack of proper wastewater management including inappropriately treated and untreated water discharge routines by the aquaculture industry harms the marine environment. This might result in significant environmental and ecological changes such as eutrophication, or the introduction of excessive amounts of nitrogen and phosphorus into water resources. Draining this wastewater involves polluting open water sources, which makes them unsafe for human consumption or use, even for recreational purposes. Contaminated water sources cause a significant death toll on other marine life.

Natural anaesthetic agents have potential as an alternative to synthetic agents for aquacultural activities. Studies by Souza *et al.* (2018) recommended that extracts of plant origin have various bioactive compounds that are valuable for anaesthetics purposes. Aydın and Barbas (2020) added that the anaesthetic properties of bioactive compounds had a selective effect in fish species (Neiffer & Stamper, 2009). Most common plant-based extract used on aquatic animals is clove oil. This essential oil is extracted from clove trees, *Eugenia aromatica* and *Eugenia caryophyllata* that has a strong and effective anaesthetic effect. Clove oil contains between 85% and 95% of eugenol and isoeugenol that have anaesthetic effects. However, Li *et al.* (2018) discovered that eugenol had a slower recovery and possible mortality rate at high temperatures and dosages on small shrimp.

Studies by Ak *et al.* (2022) suggested chamomile oil (*Matricaria chamomilla*) at 100 ml L⁻¹ is an effective natural anaesthetic with a high induction time and lower adverse effects on the overall health of rainbow trout, *Oncorhynchus mykiss*. Nascimento *et al.* (2020) documented that *Lippia alba* essential oil has a

significant effect as an anaesthetic agent for both Nile tilapia, *Oreochromis niloticus* and Brazilian fish, *Astyanax lacustris* with no genotoxic damage. Interestingly, Wan Adnan *et al.* (2021) revealed *Cymbopogon citratus* essential oil showed a promising natural anaesthetic agent for *Macrobrachium rosenbergii*. They also noted that *C. citratus* essential oil significantly reduced the glycogen level and AChE activity, which reduces stress. The current study aims to evaluate the potential of *Datura metel* extract as a natural anaesthetic for aquatic animals.

Botanical and Biological Properties of *Datura metel*

The genus *Datura* originated from the *Solanaceae* family which consists of 10-12 species (Geeta & Gharaibeh, 2007). *Datura* is native to tropical Asia and found throughout Asia, Africa, North and South America, Europe, and Australia (Sharma *et al.*, 2021). However, only *D. metel* and *D. stramonium* are found in South-East Asia. *Datura* species are herbaceous and grow to approximately 1.5 m in height and have branching stems. The leaves have a simple structure, alternately posed, deep green between 10 cm and 20 cm in length, and between 5 cm and 18 cm in width show. The flowers are singular, large and bell-shaped, between 5 cm and 20 cm in length, and between 4 cm and 12 cm in width (Islam *et al.*, 2023). Most flowers are white but they may also be yellow, purple, lavender, pink, or red. The *Datura* fruit is between 4 cm and 10 cm in length and between 2 cm and 6 cm in width. Seeds are released when the ripe fruit is split open and naturally spread on land.

Datura plants are rich in bioactive phytochemicals (Cinelli & Jones, 2021). Vadlapudi and Kaladhar (2012) documented *D. metel* has approximately six functional groups including alkaloids, phenols, flavonoids, saponins, tannins, and sterols extracted from various parts including the leaves, roots, and shoots. Islam *et al.* (2023) listed five functional groups including alkaloid, saponins, tannins, flavonoids, and terpenes with approximately 30 bioactive compounds were extracted from

the leaves. Ayoola *et al.* (2008) noted that tannins, saponins, and flavonoids possess anti-hypoglycaemic and anti-inflammatory properties. Meanwhile, terpenes are effective analgesics and improve central nervous system activity. Passos and Mironidou-Tzouveleki (2016) emphasised that all plants' parts contain alkaloids atropine, hyoscyne, and scopolamine, which have psychoactive potential and can cause respiratory depression, arrhythmias, fever, delirium, hallucinations, anticholinergic toxidrome, psychosis, and even death if consumed.

A study by Lim *et al.* (2020) found that *D. metel* fruit extracts have antibacterial properties that protect against pathogenic bacteria, *Staphylococcus aureus* and *Escherichia coli*. Okoye *et al.* (2010) added that *D. metel* extract possesses antimicrobial properties that successfully suppressed pathogenic bacteria that caused infectious disease. Furthermore, Bachheti *et al.* (2018) discovered that *D. metel* seed oil possessed antibacterial protection against *Lactobacillus delbrueckii subsp lactis*, *Pseudomonas aeruginosa*, and *S. aureus*. Okwu and Igara (2009) proved that an ethanolic extract from *D. metel* leaves had positive antibacterial effects on *B.subtilis*, *K.pneumoniae*, *P. aeruginosa*, *P. mirabilis*, *S. aureus*, and *S. typhi*. Bachheti *et al.* (2018) added that *D. metel* seed oil has antifungal properties, which work well on *Candida albicans* MTCC227 and *Candida tropicalis* MTCC230. Previously, Sharma (2002) reported that *D. metel* extracts have antifungal properties that protect against *A. fumigatus*, *A. niger*, and *A. flavus*. *D. metel* leaf extracts also have potential as an antifungal agent that protects against *Rhizoctonia solani* (Kagale *et al.*, 2004).

Meanwhile, Bachetti *et al.* (2018) reported that *D. metel* seed oil is an effective antioxidant. Interestingly, Bhardwaj *et al.* (2016) reported that the total phenolic content in *D. metel* leaf and seed methanolic extracts were recorded at between 46.09 ± 0.43 and 61.93 ± 0.69 mg GAE g⁻¹, respectively. Meanwhile, the DPPH was between 180.97 ± 5.49 and 199.34 ± 6.29 g ml⁻¹.

The added *D. metel* seed methanolic extract was observed to have the lipid peroxidation inhibition activity. Mbida *et al.* (2022) noted that *D. metel* seed extracts had higher anti-free radical levels compared with *D. metel* leaf extracts.

Moreover, Cespedes-Mendez *et al.* (2021) reported that *D. metel* leaf extract have insecticidal and repellent potential especially against small insects via direct contact. The application also has widened to be used as herbicidal against noxious weed parthenium. *D. metel* extracts are also able to inhibit the weed germination and stunt the stem development after two weeks (Ibrahim *et al.*, 2018). Javaid *et al.* (2010) added that *D. metel* root extracts are able to inhibit the *Phalaris minor* germination.

***Datura metel* as an Anaesthetic Agent for Aquatic Animals**

Datura metel extracts have potential for application as an aquatic anaesthetic agent for aquacultural use. Anaesthesia is a state that involves an induction of substances or drugs that cause a loss of sensation by suppressing central nervous system activity (Uma *et al.*, 2020). It ranges from light sedation during handling and non-invasive procedures for reducing stress to loss of consciousness during surgery and larger interventions to avoid pain (Ross & Ross, 2008; Neiffer & Stamper, 2009; Ackerman *et al.*, 2017).

Anaesthesia plays an important role as a sedative and analgesic as well as to stimulate the hypnotic, anti-anxiety, and muscle relaxation in aquatic animals (Bin *et al.*, 2021). In addition, general anaesthesia involving both full and partial inhibition of nerve response activity and consciousness, which prevents noxious stimulation and pain (Bin *et al.*, 2021). Ideal anaesthetic agents are recommended to be effective within three minutes after exposure and fully conscious within five minutes in recovery conditions; non-toxic; suppressing the physiology and movement; easily excreted; and cost-effective (Park, 2019).

Anaesthesia has multiple stages; including sedation, partial anaesthesia, full anaesthesia, and death (Coyle *et al.*, 2005; Uma *et al.*, 2020). These stages depend on the dosage and duration of exposure (Uma *et al.*, 2020). In the initial stages after exposure, aquatic animals become hyperactive and then lose consciousness. The second stage is the period just after the loss of consciousness and may involve agitated movements and delirium. The third stage is divided into four substages: Light anaesthesia, reflection such as eyes blinking and gasping, followed by the loss of the ability to blink, and regular respiration at the surgical anaesthesia substage. The third substage involves the loss of respiration and the ability to breathe, followed by a complete loss of the ability to breathe in the fourth substage. Stage four is defined as when the aquatic animal faces respiratory arrest and death. A desired stage of anaesthesia is achieved by reducing the dosage and observing the behaviour of the sedated animals over the maintenance periods (Uma *et al.*, 2020). Rapid breathing is an obvious indicator of overexposure. Aquatic animals should be removed from the exposure tank and transferred to a tank with normal stable conditions as soon as rapid breathing can be observed (Uma *et al.*, 2020).

At the recovery stage, the anaesthetic substances or drugs need to be removed entirely, and normal conditions need to be restored with the aquatic animals placed in anaesthetic-free water after induction (Uma *et al.*, 2020). Recovery time is the duration immediately after the aquatic animals are placed in a tank with normal water conditions until it recommences full balanced motion. Uma *et al.* (2020) emphasises that in the early stages of recovery, aquatic animals will respond to the sensory stimulation within a few minutes of being transferred to the tank with normal water conditions. Ross and Ross (2008) added that aquatic animals need anywhere from a few minutes to a few days to achieve a full recovery. Uma *et al.* (2020) added rapid admission of anaesthetic substances or drugs, and minimal handling of aquatic animals may shorten the recovery phase.

A study by Monira and Munan (2012) added that *Datura* sp. constitutes tropane alkaloids, which includes, hyoscyamine, hyoscyne, littorine, acetoxytropine, valtropine, fastusine, fastusinine, several with anolides, and various triglycol esters of tropine and pseudotropin. Hyoscyamine and scopolamine are the primary tropane alkaloids in *Datura*. Hyoscyamine can pass through the blood-brain barrier (Kohnen-Johannsen & Kayser, 2019) and affecting the central and peripheral nervous systems. This non-selective muscarinic acetylcholine receptor (mAChR) antagonist inhibits the binding of the physiological neurotransmitter acetylcholine (Schlesinger *et al.*, 2021). The mAChR consists of five subtypes that are able to stimulate the receptor and generate cytosolic calcium transients, inhibit the adenylyl cyclase, and involved in smooth muscular contractions. It blocks the action of acetylcholine at parasympathetic sites and the central nervous system (Ally & Mohanlall, 2020) and thus elicits hallucinations and has psychoactive effects (Kohnen-Johannsen & Kayser, 2019).

Scopolamine also known as hyoscyne, differs from hyoscyamine by the presence of an epoxide bridge in the tropane ring of scopolamine (Schlesinger *et al.*, 2021). Like hyoscyamine, scopolamine also affects cognitive processes, sensory function, and stress responses. Meanwhile, atropine can function like hyoscyamine and scopolamine. Atropine is stable and able to bind to muscarinic receptors that block the parasympathetic cholinergic neurons (Ally & Mohanlall, 2020) and act on both peripheral and central muscarinic receptors (Ally & Mohanlall, 2020).

Atropine may also act as an antidote to organophosphates and carbamates (Pillay & Sasidharan, 2019) and is known for its anticholinergic capabilities. The abundance of tropane alkaloids, including scopolamine, hyoscyamine, and atropine in *D. metel* leaves extract may serve as a potential anaesthetic substance for aquatic animals. The exploration of dosages and exposure rates reveals *D. metel* is

an efficient natural anaesthetic agent for aquatic animals, which can benefit the aquaculture industry.

Conclusions

The use of *Datura metel* as an anaesthetic agent for aquatic animals presents a growing interest in natural and sustainable alternatives to synthetic chemicals in the aquaculture industry. The alkaloids in *Datura metel*, particularly atropine, scopolamine, and hyoscyamine may have promising anaesthetic applications with regard to aquatic organisms, including crustaceans. A further study on the concentration, rate of exposure and recovery time may improve the safety and effectiveness of *Datura metel* as an anaesthetic agent.

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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