

A REVIEW ON PHYTOCHEMICAL INVESTIGATION, FORMULATION AND EVALUATION OF PIMPINELLA ANISUM FOR SMOOTH MUSCLE RELAXATION: A NOVEL APPROACH TO NATURAL THERAPY

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ABSTRACT

Pimpinella anisum (anise) is known as traditional medicine for its flavor and therapeutically used as different properties like anti-inflammatory, digestive, expectorant, antispasmodic, smooth muscle relaxing, antimicrobial, diabetes and arthritis. The collection of aniseed is very much reliable throughout west bengal and easy to grow at home. Aqueous and Hydro-alcoholic extracts, especially above 55% ethanol have shown in vitro relaxation of rat anococcygeus organ bath for isolated muscle or any rodent animal's estrous cycle, JQF, model and normal control group, Yuanhu zhitong tablet group, attributed to nitric oxide, cGMP pathway activation and study of various hormonal treatment through aniseed solution. However, optimal phytochemical profiling is done by analytical determining active compounds, after which the standardized formulation should be carried out, and in vivo efficacy remains underexplored through induced ileus and dysmenorrhea with rodent models by biochemical pathway. This isolates active constituents, develops a standardized topical formulation, evaluates its efficacy in vitro and in vivo, and elucidates detailed mechanisms of smooth muscle relaxation. After determination of the formulation, it can be sent for OECD guidelines for toxicity testing and ICH guidelines for pharmaceutical registration across different regions ultimately leading to safety and higher effectivity in products.

Introduction

Aniseed has been a source of medicinal agents for lots of years, and an impressive number of modern drugs have been isolated from natural sources under Indian spices, many of which are based on their use in traditional medicine. These plant-based traditional medicines continue to play an essential role in health care, with about 80% of the hydroalcoholic extract used on traditional medicines for their primary health care. To develop a standardized Pimpinella anisum based formulation with various

activities and elucidate its mechanism of action equivalent to their active constituent like anethole, volatile oils, many more.

Material

Plant of Pimpinella anisum (aniseed) & raw seed, water, Ethanol, acetylcholine, histamine, high concentrated KCl, L-NAME, ODQ, verapamil.

Collection of Plant

Aniseed, also known as MOURI, comes from dried ripe fruits, aerial plant of Pimpinella

anisum, family- Umbelliferae (also known as Apiaceae). The fruits are mainly harvested in the autumn when it becomes ripe and then dried for use as an indian spice. We have to procure authenticated Pimpinella anisum seeds and plant them. Collect the aerial plant like leaves or whole stem and prepare extracts with solvents (aqueous, hydro-alcoholic 40–80%), optimize for bioactivity, hold or place those solutions for 2 hours in a proper container.

Different types of Method Extraction

Extraction methods mainly categorised into two parts, one is liquid-liquid extraction & the other is solid-liquid extraction. Under Pimpinella anisum plant it goes through solid-liquid extraction by different methods along with a few solvents like aqueous extraction and hydroalcoholic extraction.

10gm of Aniseed > fine coarse powder making > pore into 250ml conical flask & add 100ml water solvent > Mixed with the help of rotary shaker at 190-220rpm for 24hours > filtration done by whatman no.1 filterpaper > collection of filtrate> Evaporated to dryness in water bath at 80degree C to get dried material for further study

Aqueous Extraction of Aniseed

The solid-liquid extraction methods come through maceration, percolation, soxhlet extraction,ultrasound-assisted extraction, microwave-assisted extraction, pressurized liquid extraction. Maceration involves by soaking the aniseed coarse powder in a water for a period of time to dissolve the desired compounds.

The other process is Percolation which has a shorter period compared to maceration, Soxhlet extraction uses a reflux system to continuously cycle solvent through the solid material of plant, lastly two UAE, PLE & MAE are used rarely for extraction. Gulcin et al., (2003) reported the antioxidant properties of water and methanolic extracts of aniseed

using different antioxidant tests, including reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, and metal chelating activities. Water and ethanol extracts at the rate of 20 µg/ml exhibited 99.1 and 77.5% inhibition of peroxidation in the linoleic acid system, which was greater than the same concentration of α -tocopherol (36.1%). These antioxidant activities were compared with synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and α -tocopherol. The water extract of Pimpinella anisum L. exhibited greater antioxidant capacity than that of ethanol.

50gm of Aniseed aerial parts of plant > dried powder making > pore into 250ml conical flask & add 300ml hydroalcoholic solvent > Mixed with the help of rotary shaker at 190-220rpm for 10min at 50 degree C > filtration done by whatman no.1 filterpaper > collection of filtrate > Evaporated by rotary evaporator and then transfer to lyophilizer to get dried material for further study

Alcoholic Extraction of Aniseed

Phytochemical Study

Phytochemical study goes through some preliminary methods like evaporation, filtration, separation and condensation of natural bioactive material determined from the extraction process. Pimpinella anisum, commonly known as aniseed, is known to contain a variety of phytochemicals including alkaloids, flavonoids, saponins, steroids, tannins, and phenolic compounds like volatile oils. These components give different medicinal properties such as antioxidant, antimicrobial, analgesic, antiinflammatory, antispasmodic, muscle relaxant, antidiabetics and expectorant activities. Different extracts collected from ethanol or aqueous solutions, may vary in their phytochemical composition: **Alkaloids**, These nitrogen-containing compounds are known for their diverse biological activities, including potential medicinal properties.

Flavonoids: These are powerful antioxidants with potential health benefits, including anti-inflammatory and anti-cancer properties.

Saponins: These compounds can exhibit foaming properties and have been studied for their potential roles in various biological activities.

Steroids: These compounds are involved in various physiological processes and have been investigated for their potential medicinal properties.

Tannins: These compounds are known for their astringent properties and potential antioxidant activity.

Phenolic compounds: This group includes a wide range of compounds, many of which act as antioxidants and have been linked to various health benefits.

Volatile oils: Aniseed contains a volatile oil that contributes to its characteristic aroma and flavor, with trans-anethole being a major component.

Lipids: Aniseed also contains lipids, rich in fatty acids like palmitic and oleic acids.

Carbohydrates: Aniseed contains carbohydrates, which serve as an energy source. Few methods of analysis incorporate with aniseed like, Perform GC-MS for essential oils and HPLC for phenolics/flavonoids. Quantify anethole, estragole, quercetin, and others like constituents by various chemical tests.

Standard phytochemical tests: Techniques like the foam test for saponins, alkaline reagent test for flavonoids, and ferric chloride test for tannins are used to identify the presence of these compounds.

Spectrophotometry: This technique is used to quantify the total phenolic content and flavonoid content.

Chromatography (HPLC and GC): High-performance liquid chromatography (HPLC) and gas chromatography (GC) are used to identify and quantify specific volatile compounds like trans-anethole and other essential oil components.

Antioxidant and antimicrobial assays: Various assays, like DPPH (2,2-diphenyl-1-picryl -hydazyl) radical scavenging activity, are used to evaluate the antioxidant potential of aniseed extracts, while agar disk diffusion methods are used to assess antimicrobial activity.

Formulation Development

After several phytochemical tests active constituents are gone through pH, viscosity, particle size, zeta potential, encapsulation efficiency. Then choice for formulation mainly depends on different dosage forms which design as nano-emulsion gels.

Animal Study

The animal study appears to investigate the various effects of different compounds on smooth muscle relaxation in various tissues, likely focusing on the NO or cGMP specific signaling pathways, using animal rodent models of ileus and dysmenorrhea to understand their potential effects over therapeutic implications.

Organ bath experiments done by isolated tissues (ileum, jejunum, uterus, vascular tissues) are placed in an organ bath to observe their contractile responses to agonists like acetylcholine and histamine. Relaxation in response to these agonists is measured, allowing researchers to assess the muscle's reactivity to stimuli. Inhibitors like L-NAME (nitric oxide synthase inhibitor), ODQ (soluble guanylate cyclase inhibitor), and verapamil (calcium channel blocker) are

added to elucidate the specific pathways involved in relaxation, such as the nitric oxide-cGMP pathway or calcium signaling. **Animal models,** Rodent models of ileus (intestinal obstruction) and dysmenorrhea (painful menstruation) are used to study the effects of potential treatments. Motility indices (frequency and strength of contractions), behavioral parameters (pain responses, anxiety-like behavior), and smooth muscle tonus are measured to assess the impact of interventions. Blood and gut tissue samples are collected for biochemical analysis to investigate changes in specific signaling pathways involved in smooth muscle relaxation. Potential application may lead to development of new therapeutic strategies over the same pathways.

Mechanistic Study

Aniseed has been studied for its different medicinal properties, including its potential to act as an antispasmodic, analgesic, anti-inflammatory, antioxidant, antimicrobial, gastrointestinal and neuroprotective agent. **Antimicrobial Activity:** Aniseed's essential oil exhibits concentration-dependent fungicidal activity and antibacterial effects against various pathogens, including *Staphylococcus aureus* and *Escherichia coli*. The mechanism involves disrupting microbial cell membranes and interfering with their metabolic processes. **Antioxidant Effects:** Aniseed is rich in antioxidants, which can neutralize free radicals and protect cells from oxidative damage. This mechanism is crucial in preventing chronic diseases and reducing inflammation. **Anti-inflammatory Properties:** Studies suggest that aniseed can reduce inflammation by modulating the production of inflammatory mediators and promoting the activity of antioxidant enzymes. **Neuroprotective Effects:** Aniseed extracts have been shown to

mitigate the neurotoxic effects of certain drugs by reducing oxidative stress and inflammation in brain tissue. **Estrogenic Effects:** Aniseed may have estrogenic effects, which can be beneficial in treating certain female disorders like postpartum after pain, menstrual pain, and menopausal symptoms. **Gastrointestinal Effects:** Aniseed has been traditionally used to aid digestion and relieve gastrointestinal discomfort. It may work by relaxing smooth muscles, reducing gas, and improving gut motility. **Other Potential Mechanisms:** Research suggests that aniseed may also have anticonvulsant, analgesic, and antidiabetic properties, but further mechanistic studies are needed to fully understand these effects. There was some example of mechanistic study of aniseed product, Metronidazole Toxicity: A study demonstrated that aniseed extracts can mitigate the neurotoxic effects of metronidazole by reducing oxidative stress and inflammation in brain tissue. **Glucose Absorption:** Aniseed oil has been shown to enhance glucose absorption in the small intestine by increasing the activity of the Na⁺-K⁺ ATPase enzyme, which is essential for glucose transport. **Diuretic Effects:** Aniseed oil can also reduce urine output by stimulating the renal Na⁺-K⁺ ATPase, leading to increased sodium and water reabsorption in the kidneys.

Safety Evaluation

A safety evaluation of aniseed involves few of its potential toxicity, identifying contaminants and establishing some quality control protocols. This includes acute or subacute toxicity studies according to OECD guidelines, check for heavy metals, pesticides, microbes. The development of QC measures like limit tests, shelf-life determination, and content uniformity assessments. Toxicity assays, under this

acute toxicity involves determining the adverse effects of a single or multiple doses of aniseed administered over a short period. The OECD Test Guideline 425 (Up-and-Down Procedure) is relevant for this. This test helps classify the substance's acute toxicity according to the Globally Harmonized System (GHS). It typically involves administering the aniseed (or its extract) to rodents (rats) and observing for any adverse effects. **Subacute-Toxicity Study:** This assesses the toxic effects of repeated doses of aniseed over a longer period, typically 28 days. The OECD guideline (Repeated Dose 28-day Oral Toxicity Study in Rodents) is applicable here. This study involves observing animals for signs of toxicity, changes in body weight, food and water consumption, hematology, clinical biochemistry, and performing necropsies. The contamination assessment goes through Heavy Metals: Testing for the presence of heavy metals like lead, cadmium, arsenic, and mercury is crucial. These can be toxic even at low levels. A few notes that areca nut (a related plant) has varying levels of heavy metals, and this analysis is needed for aniseed as well. **Pesticides:** Aniseed may be contaminated by pesticides used during its cultivation. Testing for a range of pesticides is necessary to ensure safety. **Microbial Loading:** Assessing the microbial load (bacteria, fungi, etc.) is important to ensure the product is not contaminated with harmful microorganisms. **QC protocol stands for Limit Tests:** These tests establish the maximum permissible levels of impurities or contaminants in the aniseed product. **Shelf-life Determination:** This involves assessing the stability of the aniseed product over time to determine its optimal storage conditions and expiration date. **Content Uniformity:** This ensures that the active compounds are evenly distributed throughout the product, ensuring consistent dosage.

Discussions *Pimpinella anisum* L. (Aniseed) in petroleum ether and methanol also contains the oily extract of dark brown in colour. All the extracts of aniseed were orange brown in colour. The aqueous and alcoholic extracts of aniseed were found to be active in stimulating the differentiation of osteo-blastic cells and in relaxing the pro-contracted smooth muscles of different organs in-vivo respectively.

ABBREVIATIONS

NO-cGMP- Nitric Oxide- cyclic guanosine monophosphate

JQF-jingqian zhitong fang

HPLC- High-performance liquid chromatography

GC- MS - gas chromatography- Mass spectroscopy

UV- ultra visible spectroscopy

OECD- organisation for economic cooperation and development

ICH- International council for harmonisation of technical requirements for pharmaceuticals for human use

KCL- Potassium chloride

L-NAME - N(G)-nitro- L-arginine methyl ester

ODQ- 2-oxadiazolo [4.3-a]quinoxaline-1

UAE- ultrasound-assisted extraction

PLE- pressurized liquid extraction

MAE- Microwave-assisted extraction

BHA- butylated hydroxyanisole

BHT- butylated hydroxytoluene

DPPH- 2,2-diphenyl-1-picrylhydrazyl

GHS- Globally Harmonized System

QC Protocol- Quality control

Na⁺ K⁺ATPase- Sodium Potassium ATPase

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