Therapeutic Potential of Extract of Black Catechu against Skin Problems

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ABSTRACT

Skin problems, such as dermatitis, eczema, and acne, are common dermatological conditions affecting a significant portion of the global population. The use of natural plant-based remedies has gained attention due to their potential therapeutic benefits and fewer side effects. Black catechu (Acacia catechu), a medicinal plant widely used in traditional medicine, has been attributed to various pharmacological properties. The percentage of heat-induced hemolysis and Inhibition of albumin denaturation were evaluated. Black catechu was collected, and the aqueous extract was obtained using standard extraction methods. Phytochemical screening was performed to identify the presence of various secondary metabolites, such as alkaloids, flavonoids, tannins, saponins, and phenolic compounds. The findings of this study suggest that black catechu extract possesses promising pharmacological properties, including antioxidant and anti-inflammatory activities. These results support its potential use as a natural remedy for various skin problems. Further investigations, including in vivo studies and clinical trials, are warranted to validate its safety and efficacy in human subjects. Black catechu could offer a novel and sustainable approach to complement existing treatments for skin problems, contributing to the development of effective and natural dermatological therapeutics.

Keywords: Skin problems, Acacia catechu, Black catechu, flavonoids, Soxhlet extraction.

1. INTRODUCTION

About 75 to 80 percent of the world's population, mostly in underdeveloped nations, still relies heavily on herbal medicine for primary healthcare.[1] This is partly due to the widespread misconception that herbal medications have no adverse effects other than being affordable and easily accessible.[2] The World Health Organisation (WHO) reports that the usage of herbal therapies is two to three times greater than that of conventional medications worldwide.[3] Plants have been used for therapeutic purposes since the dawn of time, and much of contemporary medicine has its roots in this tradition. The majority of the few effective treatments from a century ago were plant-based, therefore many standard drugs now have plant origins. Examples include morphine, digoxin from foxglove, quinine from cinchona bark, and aspirin from willow bark.[4]

Descriptions of people using herbs to treat the ailing members of society may be found throughout medical history dating back to the beginning of time. However, we saw the emergence of allopathic medicine concurrent with the start of the industrial revolution. Even though it was less popular, herbal medicine was a powerful kind of treatment.Herbal remedies were abandoned from mainstream medicine in the middle of the 20th century, not necessarily because they were unsuccessful but rather because they were less financially successful than the more recent synthetic medications.[6] As scientific methods evolved and gained favour in the early 19th century, the use of plant remedies was scorned as quackery.Interest in "natural health" and the use of herbal products developed in the 1960s as a result of worries about the iatrogenic consequences of traditional treatment and a desire for greater independence. The National Institutes of Health in the USA established the office of Alternative Medicine in 1992 in response to the growing use of herbal medicines and other non-conventional treatments. When the WHO urged developing nations to employ traditional plant medicine to address needs not supplied by modern systems, herbal medicine received a boost globally.[7]

1.1 Herbal Medicine

The WHO recently described traditional medicine (including herbal medications) as consisting of therapeutic practises that were in use before the creation and dissemination of modern medicine and are still in use now, frequently for hundreds of years. Traditional medicine is a culmination of generations of indigenous system of medicine practitioners' therapeutic experiences. Traditional remedies include organic material, minerals, and medicinal plants. Only traditional medications that use medicinal plant

preparations as their main therapeutic component are classified as herbal pharmaceuticals. Their use is first documented in manuscripts from around 5000 years ago in Indian, Chinese, Egyptian, Greek, Roman, and Syrian languages. The Rigveda, Atharvaveda, CharakSamhita, and SushrutaSamhita are some examples of classical Indian literature. Therefore, the traditional medicines and herbal remedies have their roots in the rich traditions of ancient civilizations and in scientific history.[8-9]

Herbal medicine has been used for centuries in various cultures for skin care and wound healing. Here are some key roles of herbal medicine in these areas:

Anti-inflammatory properties: Many herbs used in traditional medicine possess anti-inflammatory properties. When applied topically or used in skincare products, these herbs can help reduce inflammation, redness, and swelling associated with skin conditions like eczema, psoriasis, and acne. Examples of herbs with anti-inflammatory properties include chamomile, aloe vera, calendula, and turmeric.[10]

Antimicrobial and antiseptic effects: Several herbs have natural antimicrobial and antiseptic properties, which can help prevent infection in wounds and promote wound healing. These herbs may have broad-spectrum antimicrobial activity against bacteria, fungi, and other microorganisms. Some commonly used herbs with antimicrobial properties include tea tree oil, lavender, neem, and thyme.[11]

Wound healing promotion: Certain herbs contain compounds that promote wound healing and tissue regeneration. They can enhance the production of collagen, accelerate the formation of new blood vessels, and improve the overall healing process. Examples of herbs with wound healing properties include aloe vera, gotu kola, comfrey, and yarrow.[12]

Moisturizing and soothing effects: Many herbal extracts and oils are used in skin care products due to their moisturizing and soothing properties. These herbs can help hydrate the skin, improve its elasticity, and soothe dry, irritated, or sensitive skin. Popular examples include lavender, chamomile, rosehip oil, and shea butter.[13]

Antioxidant activity: Herbal medicine often utilizes plants rich in antioxidants. Antioxidants help protect the skin from oxidative stress caused by free radicals, which can lead to premature aging and skin damage. By neutralizing free radicals, herbal antioxidants can support skin health and promote a youthful appearance. Common herbs with antioxidant properties include green tea, rosemary, grape seed extract, and pomegranate.[14]

1.2 Mechanisms of Herbal Medicine

Herbal medicines can exert a variety of beneficial effects on wound healing. The specific mechanisms of action vary depending on the herb or plant used, but some common mechanisms include:

1. Anti-inflammatory effects: Many herbal medicines contain compounds that have anti-inflammatory properties, which can help to reduce swelling and inflammation at the site of the wound. Examples of herbs with anti-inflammatory properties include aloe vera, chamomile, and turmeric.[15]

2. Antimicrobial effects: Some herbal medicines contain compounds that have antimicrobial properties, which can help to prevent infection and promote healing. Examples of herbs with antimicrobial properties include tea tree oil, echinacea, and garlic.[16]

3. Antioxidant effects: Oxidative stress can impede wound healing by damaging the tissue and slowing down the healing process. Herbal medicines that contain antioxidants, such as green tea, ginseng, and grape seed extract, can help to reduce oxidative stress and promote healing.[17]

4. Stimulation of collagen synthesis: Collagen is a key component of the extracellular matrix, which provides structural support to the tissue. Some herbal medicines, such as Centellaasiatica (Gotu kola) and calendula, have been shown to stimulate the synthesis of collagen and improve the strength and elasticity of the tissue.[18]

5. Angiogenesis promotion: Some herbal medicines, such as ginseng and echinacea, have been shown to promote angiogenesis, which is the formation of new blood vessels. Angiogenesis is a critical step in wound healing as it helps to bring oxygen and nutrients to the site of the wound.[19]

1.3 Introduction to Human Skin

The human skin is an incredible organ that serves as a protective barrier between our body and the external environment. It is the largest organ in the human body, covering an average surface area of about 2 square meters in adults. The skin plays a crucial role in maintaining our overall health and well-being.[20]

The skin consists of three primary layers: the epidermis, dermis, and hypodermis (subcutaneous tissue). Each layer has distinct functions and contributes to the overall structure and function of the skin.[21]

The epidermis is the outermost layer of the skin and acts as a protective barrier against physical, chemical, and microbial threats. It is primarily composed of keratinocytes, which produce the protein keratin, giving the skin its strength and waterproof properties. The epidermis also contains melanocytes that produce the pigment melanin, responsible for skin color.[22]

Beneath the epidermis lies the dermis, a thicker layer that provides structural support to the skin. It contains a network of collagen and elastin fibers that give the skin its elasticity and strength. The dermis houses various structures, including blood vessels, nerves, hair follicles, sweat glands, and sebaceous glands.[23]

The deepest layer of the skin is the hypodermis, also known as the subcutaneous tissue. It consists of fat cells (adipocytes) that provide insulation, cushioning, and energy storage for the body.[24]

The skin serves several vital functions. Firstly, it acts as a physical barrier, preventing the entry of harmful microorganisms, toxins, and excessive water loss. It also helps regulate body temperature through the process of sweating and blood vessel dilation or constriction. The skin plays a role in sensory perception, allowing us to feel touch, pain, temperature, and pressure. Additionally, it participates in vitamin D synthesis when exposed to sunlight.[25]

1.4 Structure and Composition of the Skin

The structure and composition of the skin are complex and involve multiple layers and components. Here is an overview of the structure and composition of the skin:

1. Epidermis: The epidermis is the outermost layer of the skin and acts as a protective barrier. It is composed of several layers of specialized cells, including:[26]

- Stratum Corneum: The outermost layer of the epidermis consists of dead, flattened skin cells called corneocytes. These cells are filled with keratin, a tough protein that provides strength and water resistance.[27]
- Stratum Granulosum: This layer contains granular cells that produce lipids, which help to further strengthen the skin barrier.[28]
- Stratum Spinosum: It consists of several layers of actively dividing cells known as keratinocytes. These cells produce keratin and provide structural support to the skin.[29]
- Stratum Basale: The innermost layer of the epidermis contains basal cells that continuously divide and give rise to new keratinocytes. It also houses melanocytes, which produce the pigment melanin responsible for skin color.

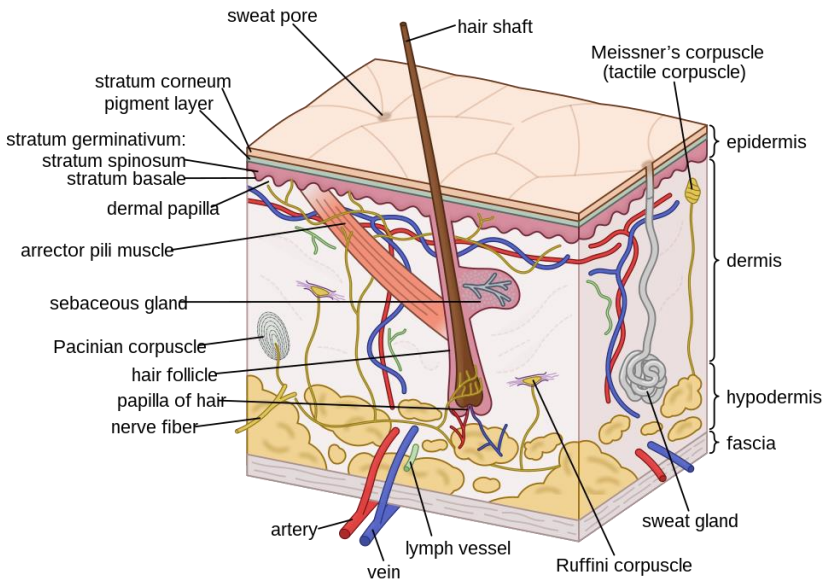


Figure 1.1: Human Skin Structure

2. Dermis: The dermis is the middle layer of the skin and provides structural support. It consists of connective tissue and contains various components, including:[30-34]

- Collagen: The dermis is rich in collagen fibers, which provide tensile strength and elasticity to the skin.
- Elastin: Elastin fibers allow the skin to stretch and recoil, maintaining its flexibility.
- Blood vessels: The dermis contains a network of blood vessels that supply nutrients and oxygen to the skin cells and help regulate body temperature.
- Hair follicles: Hair follicles are present in the dermis and give rise to hair growth.
- Sweat glands: The dermis contains sweat glands, including eccrine and apocrine glands, which produce sweat for thermoregulation.
- Sebaceous glands: These glands secrete sebum, an oily substance that lubricates and moisturizes the skin.

3. Hypodermis (Subcutaneous Tissue): The hypodermis is the deepest layer of the skin, composed mainly of adipose (fat) tissue. It provides insulation, cushioning, and energy storage for the body.[35]

2. LITERATURE REVIEW

Shenefelt et al.,(2011) reported that "Many herbal therapies have been used for centuries, which show good anecdotal results. A few randomized, controlled trials have also demonstrated significant results in the use of herbal therapies for the treatment of dermatologic disorders. Some countries, such as Germany, now require standardization of herbal preparations and specific recommendations as to the use and efficacy of herbs in the treatment of disease. It is important to know what common herbal alternatives exist and which potential adverse effects or interactions can occur to permit more effective counseling of patients."[36]

Tsioutsiou et al.,(2022) reported that "The analysis showed that 967 taxa belonging to 418 different genera and 111 different families are used in the treatment of skin related problems. The majority of the plants belong to the families of Asteraceae (11.7%), Lamiaceae (7.4%), Rosaceae (6.7%), Plantaginaceae (5.4%), and Malvaceae (3.8%). Their usage is internal or external to treat ailments such as wounds and burns (22.1%), hemorrhoids (14.7%), boils, abscesses, and furuncles (8.2%). Beside specific skin disorders, numerous species appeared to be used for their antifungal, antimicrobial, and antiseptic activity (9.1%). Literature evaluation highlighted that, the most commonly used species are Plantago major L. (Albania, Turkey), Hypericum perforatum L. (Greece, Turkey), Sambucus nigra L. (Cyprus, Greece), Ficus carica L. (Cyprus, Turkey), Matricaria chamomilla L. (Cyprus, Greece), and Urtica dioica L. (Albania, Turkey), while many medicinal plants reported by interviewees were common in all four countries. Finally, to relate this ethnopharmacological knowledge and trace its expansion and diversification through centuries, a comparison of findings was made with the use of the species mentioned in Dioscorides' "De Materia Medica" for skin disorders."[37]

Ayurvedic formulations. The seeds of the plant are reported to possess hypoglycemic activity in rats. Acacia catechu also shows hypotensive effect. The water decoction of Acacia catechu is widely consumed as health drink especially in Kerala and other south Indian states. It is believed that the water decoction can purify blood, improve skin texture and boost body's defence mechanism (personal communication). Since, the plant is widely used for treatment of various ailments and is a constituent of many formulations."[38]

Ahsan et al.,(2021) reported that "Areca catechu, commonly known as supari, consists of dried ripe nuts that came under Arecaceae family, which is cultivated in the tropical region of India and Southeast Asia. It is a prevalent traditional herbal medicine that is chewed to separate collected fluid in the alimentary canal and for killing worms. Areca catechu seed contains alkaloids (arecoline, arecaine, arecaidine, guvacoline, guvacine, and choline), tannin, gallic acid, gum, and various minerals such as copper, calcium, phosphorus, and iron. The chemi- cal constituents of this plant have been used as antidiabetic, stomatitis, bleeding gums, gingivitis, conjunctivitis, glaucoma, leucorrhoea, urinary disorders, anorexia, diarrhea, blood pressure regulating activity, antiulcerogenic, antioxidant activity, anticonvulsant activity, central nervous system stimulant activity, antifertility, oxytocic activity, antivi- ral activity, anthelmintic, and foul breath. It showed a dose-dependent toxicity profile, and various research has been done regarding its safety analysis and it would be con- sidered safe when administered in the prescribed dose. The purpose of the present paper is to make available an up-to-date review on the ethnic, traditional description, morphology, phytochemistry,

and the pharmacological and toxicological profile of this plant."[39]

Maver et al.,(2015) reported that "Herbs have been integral to both traditional and non-traditional forms of medicine dating back at least 5000 years. The enduring popularity of herbal medicines may be explained by the perception that herbs cause minimal unwanted side effects. More recently, scientists increasingly rely on modern scientific methods and evidence-based medicine to prove efficacy of herbal medicines and focus on better understanding of mechanisms of their action. However, information concerning quantitative human health benefits of herbal medicines is still rare or dispersed, limiting their proper valuation. Preparations from traditional medicinal plants are often used for wound healing purposes covering a broad area of different skin-related diseases. Herbal medicines in wound management involve disinfection, debridement, and provision of a suitable environment for aiding the natural course of healing. Here we report on 22 plants used as wound healing agents in traditional medicine around the world. The aim of this review is therefore to review herbal medicines, which pose great potential for effective treatment of minor wounds."[40]

3. PLANT PROFILE (*BLACK CATECHU*)

Black catechu, also known as cutch, acacia catechu, or khair, is a deciduous tree belonging to the Fabaceae (Leguminosae) family. It typically grows up to 10-15 meters tall, with a dense, spreading crown and crooked branches. The bipinnate leaves are feathery in appearance, with small leaflets arranged in pairs along the stem. The small, pale yellow flowers are borne in spherical heads on short peduncles, while the elongated pods are flat and woody, containing small, dark brown, kidney-shaped seeds. The bark of the black catechu tree is dark brown to blackish, with deep fissures, and exudes a red-brown juice when cut.

Figure 3.1: Black catechu

1. Common name: Black catechu, cutch, acacia catechu, khair
2. Scientific name: Acacia catechu
3. Family: Fabaceae (Leguminosae)

3.1 Habitat:

- Native range: the Indian subcontinent, Southeast Asia, and China.
- Ecological requirements: grows well in well-drained soils in tropical and subtropical regions, with average rainfall.
- Distribution: found in India, Pakistan, Bangladesh, Sri Lanka, Myanmar, Thailand, Cambodia, Vietnam, and Indonesia.

3.2 Cultivation:

- Propagation: from seeds or cuttings.
- Growing conditions: prefers full sun, well-drained soil, and moderate watering.
- Harvesting: the bark is harvested from the tree, and then boiled, sun-dried, and ground into a powder.

3.3 Traditional uses:

- Medicinal uses: used in Ayurvedic and traditional medicine to treat a range of ailments, including diarrhea, dysentery, cough, sore throat, bronchitis, asthma, skin diseases, wounds, and burns.
- Culinary uses: used as a flavoring and coloring agent in traditional Asian cuisines.
- Other uses: used as a dye for textiles and leather, as a tanning agent, and in the production of ink and varnish.

3.4 Chemical constituents:

Active compounds: contains tannins, catechins, flavonoids, alkaloids, and phenolic compounds, which are responsible for its medicinal properties.

4. MATERIALAND METHODS

4.1 Collection and Authentication of the Plant

Due to their significant pharmacological effects, minimal toxicity, and economic viability, plants have been studied for their therapeutic characteristics all over the world in light of contemporary scientific findings. The current study evaluates the activity of Black catechu based on data gathered from local vaidhyas and other traditional medicine practitioners.

For six days, the aerial portion of Black catechu seeds were washed, rinsed, and dried. The ground-up dry plant material was combined into a coarse powder and stored for upcoming research at room temperature.

Table 4.1 Instrument List

INSTRUMENT	INSTRUMENT DETAIL
Round-bottom flask	Corning
Condenser	Chemglass
Heating mantle or hot plate	Cole-Parmer
Electric heater	Yamato Scientific
Digital pH meter	Mettler Toledo
Optical microscope	Zeiss
Filtration apparatus	MilliporeSigma
Rotary evaporator	Yamato Scientific
Refrigerator	Thermo Fisher Scientific
Digital balance	OHAUS
Magnetic stirrer	Corning
Vernier caliper	Mitutoyo
Graduated cylinder	Corning
Volumetric pipette	Corning
Test tubes	Corning

Table 4.2 Material Used

Material Name	Company Name
Black catechu seeds	Botanical garden
Methanol	Methanex Corporation
Sodium chloride	Morton Salt
Acetone	Honeywell
Ethanol	Valero Energy Corporation
Chloroform	Fisher Scientific
Folin-Ciocalteu reagent	Fisher Scientific
HCl	Fisher Scientific
DPPH, α-glucosidase	Fisher Scientific
Starch	Fisher Scientific
Ethanol	Fisher Scientific
Chloroform	Fisher Scientific
Other chemicals used were of analytical grade	

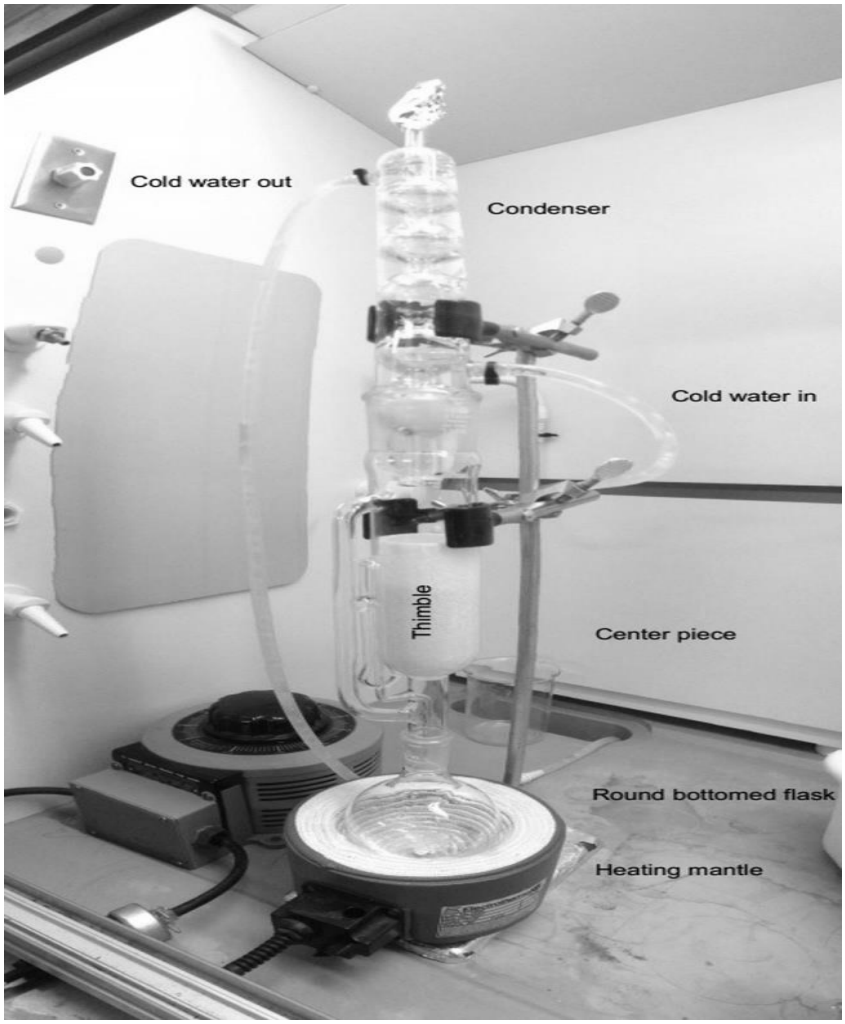
4.2 Soxhlet Extraction

Grind the black catechu seeds into a fine powder to increase the surface area available for extraction. Ensure that the powder is homogeneous. Choose a suitable solvent for the extraction process. Common solvents used for Soxhlet extraction include ethanol,

methanol, or a mixture of both. The choice of solvent depends on the specific compounds you are targeting for extraction. Assemble the Soxhlet apparatus, which consists of a round-bottom flask, a Soxhlet extractor, a condenser, and a collection flask. Place the black catechu seed powder in the Soxhlet extractor. Pour the chosen solvent into the round-bottom flask and heat it. As the solvent vaporizes, it rises into the condenser and drips onto the black catechu seed powder in the Soxhlet extractor. The solvent gradually dissolves the target compounds from the seeds and gets collected in the round-bottom flask. The solvent in the round-bottom flask gradually fills up and reaches a siphoning level, causing it to flow back into the extraction chamber. This continuous extraction process allows for efficient extraction of the target compounds. The extraction process typically takes several hours, and it is often performed overnight. The duration can vary depending on factors such as the solvent used, the sample size, and the desired level of extraction efficiency. Once the extraction is complete, the collected extract in the round-bottom flask is concentrated. This can be done by evaporating the solvent using techniques such as rotary evaporation or vacuum drying.

The % Yield in different solvents plant extracts were calculated by using the following formula:

$$\%\ Yield = \left(\text{Net weight of powder in gram after extraction} / \text{Total weight of powder in gram taken for extraction}\right) \times 100$$



4.1 Soxhlet apparatus

4.3 Physicochemical Evaluation

Physicochemical evaluation is a broad term that encompasses various analytical tests and measurements performed to assess the physical and chemical properties of a substance or material. These evaluations provide valuable information about the composition, structure, and characteristics of a sample. While the specific tests conducted can vary depending on the

nature of the material being evaluated, here are some common physicochemical evaluations:

Figure 4.2: SEEDS

4.3.1 Loss on drying

Mass loss as a percentage of mass/matter is referred to as loss on drying. A Petri plate containing 5–6g of medication powder is precisely weighed and stored in a hot-air oven with the temperature set at 105°C for 4-5 hours. Each case's weight loss was noted after chilling in a dessicator. The process was repeated until the weight remained consistent.

Loss on drying (%) = loss in weight X 100/ W

W= weight of the drugs in grams.

4.3.2 Determination of Ash Value

Ash value is a method for evaluating the purity and quality of unprocessed powdered pharmaceutical materials. Since the natural matter was burnt, which tells us more about the existence of the active ingredient in the unprocessed medicine, the debris is actually the rough material without the natural matter.

4.3.3 Total ash value

Take 2 to 3g of precisely weighed powdered extract and place it in a platinum or silica plate that has been tarred, lighted, and weighed. Disperse the medication powder on the plate's base. Increase the heat gradually, never reaching dull red heat, and incinerate until the material is free of carbon. Then let it cool and weigh it. If a carbon-free ash cannot be produced in this manner, the charred mass should be extinguished with hot water, the residue collected on an ashless filter paper, the filtrate added, the residue evaporated, and the residue ignited at a low temperature. First, an empty silica crucible was placed in the muffle furnace and heated to 600 °C for 30 minutes to ignite it. 2g of powdered medication was put to the silica crucible after it had been removed and weighed. After that, you put it in a muffle furnace for a couple of hours at 500–6000°C to make it white. finally weighed it. With an air-dried sample, the percentage of total ash was determined.

Total ash value = (z-x/y) × 100

Where,

X = weight of the silica crucible

Y = weight of the drug powder (g)

Z = weight of the silica cruicible with powder ash

4.3.4 Acid-insoluble ash

The insoluble material was removed from the ash after it had been heated for 10 to 15 minutes with around 30 millilitres of diluted hydrochloric acid. It was lit, cleaned with hot water, and weighed. In order to quantify the proportion of acid-insoluble ash, the air-dried medication was used as a reference. To 25 ml of dil HCl, the ash produced by the aforementioned technique was added. For five minutes, boil it. The residue was then collected on filter paper with less ash. Then, it was heated to 560^0C for 1 hour in a muffle furnace. With reference to the sample that had been air dried, the percentage of acid-insoluble ash was estimated.

Acid insoluble ash value % = (A/Y) × 100

where,

A = weight of the remaining residue

Y = weight of crude powder taken (g)

4.3.5 Water-soluble ash

The ash from the total ash value was boiled in 25 cc of water for 5 minutes. The insoluble material was added to filter paper with no ash. Then, a low temperature ignition was used to maintain a steady weight. By deducting the weight of water insoluble ash from the overall ash value, the weight of water soluble ash was calculated. Calculations were used to determine the amount of water-soluble ash in relation to air-died value.

4.3.6 Extractive Value

Procedure 100 ml of solvent (chloroform, ethanol, and water) was macerated with 5 gm of coarsely powdered, air-dried medication for 24 hours, shaking regularly for 6 hours, and allowing to stand for 18 hours. After that, it was quickly filtered to prevent alcohol loss. A 25 ml sample of the filtrate was dried to dryness in a shallow dish with a flat bottom, dried at 1050°C, and weighed. The proportion of extractive that is soluble in alcohol was estimated using the air-dried medication as a base. The amount of soluble components needed for extraction in that particular solvent is referred to as the extractive value.

Extractive value was determined using the formula

$$\text{Extractive value}(\%) = \frac{weightofresidue}{weightofdrypowder} \text{ x } 100$$

4.4 Phytochemical Screening

Standard procedures were followed for the phytochemical analysis of the powdered seed and/or aqueous seed extract (made by Soxhlet extraction). The next sections give a quick explanation of the methodologies. Three copies of every analysis were performed.

For the qualitative analysis of alkaloids, carbohydrates, fixed oils, flavonoids, glycosides, phytosterol/terpenoids, saponins, and tannins/phenols, preliminary phytochemical screening was carried out following the prescribed methods. the following screening exams:

4.4.1 Test for Glycosides

- The test for glycosides is a chemical analysis performed to detect the presence of glycosides in a substance. Glycosides are a class of compounds consisting of a sugar molecule (glycone) attached to a non-sugar component (aglycone) through a glycosidic bond. They are commonly found in plants and have various biological activities and therapeutic properties.
- There are different methods and specific tests available to detect and characterize specific types of glycosides. Here is a general procedure that can be followed for the test of glycosides:
- Preparation of the sample: Obtain a representative sample of the substance that is suspected to contain glycosides. The sample should be finely ground or extracted using a suitable solvent to ensure efficient extraction of glycosides.
- Hydrolysis: Perform hydrolysis of the sample to break the glycosidic bond and release the aglycone. This step involves treating the sample with acid or enzymatic hydrolysis, depending on the specific glycoside being tested. Acid hydrolysis is commonly used, where the sample is heated with dilute acid under controlled conditions.
- Testing for aglycone: Once hydrolysis is completed, test the resulting solution or extract for the presence of the aglycone. The specific test will depend on the type of aglycone expected. Various reagents and techniques such as color reactions, precipitation reactions, or chromatographic methods may be employed for identification.
- Testing for the sugar component: After confirming the presence of the aglycone, the sugar component can be tested. This step involves performing specific tests to identify the type of sugar present in the glycoside. Common tests include the Fehling's test, Benedict's test, or specific enzymatic assays.
- Confirmation and quantification: Once the presence of glycosides is confirmed, further characterization and quantification can be performed using appropriate techniques such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), or mass spectrometry (MS).

4.4.2 Test for flavonoids

The test for flavonoids is a specific chemical analysis conducted to detect and identify the presence of flavonoids in a substance. Flavonoids are a class of naturally occurring plant secondary metabolites known for their diverse biological activities and potential health benefits.

Here is a general procedure for the test of flavonoids:

1. Preparation of the sample: Obtain a representative sample of the substance suspected to contain flavonoids. The sample can be in the form of a plant extract or a finely ground material.
2. Solvent extraction: Extract the flavonoids from the sample using a suitable solvent. Commonly used solvents include ethanol, methanol, or a mixture

of water and organic solvents. The extraction can be performed by maceration, percolation, or sonication, depending on the sample and its characteristics.

3. Preliminary identification tests: Perform preliminary tests to detect the presence of flavonoids in the extract. These tests can include color reactions, such as the Shinoda test or the magnesium hydrochloric acid test, which produce characteristic color changes in the presence of flavonoids. These tests provide initial indications of the presence of flavonoids but are not specific for individual compounds.

4. Chromatographic analysis: To further confirm and identify specific flavonoids, chromatographic techniques such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), or gas chromatography (GC) can be employed. These techniques separate the components of the extract based on their chemical properties, allowing for the identification and quantification of individual flavonoids.

5. Spectroscopic analysis: Spectroscopic techniques such as ultraviolet-visible (UV-Vis) spectroscopy, infrared (IR) spectroscopy, or nuclear magnetic resonance (NMR) spectroscopy can be used to obtain structural information and confirm the identity of the flavonoids present in the sample. These techniques help in the characterization and differentiation of various flavonoid compounds.

6. Reference standards and controls: To ensure accurate identification and quantification, it is essential to compare the results obtained from the sample with those obtained from authentic reference standards. These standards are purified compounds with known structures and characteristics.

4.4.3 Test for Alkaloids

The test for alkaloids is a chemical analysis conducted to detect and identify the presence of alkaloids in a substance. Alkaloids are a class of naturally occurring nitrogenous compounds with diverse biological activities, commonly found in plants.

Here is a general procedure for the test of alkaloids. Preliminary identification tests: Perform preliminary tests to detect the presence of alkaloids in the extract. These tests can include color reactions, precipitation reactions, or formation of specific crystals. Commonly used tests include the Dragendorff's test, Mayer's test, or Wagner's test. These tests provide initial indications of the presence of alkaloids but are not specific for individual compounds.

4.4.4 Inhibition of albumin denaturation

Through the use of an inhibition of albumin denaturation approach and other adjustments, the anti-inflammatory efficacy of black catechu was investigated. A sample of 1% aqueous bovine serum albumin was obtained along with the test extract. The mixture's pH is kept constant by adding a little quantity of 1 N HCl. The sample extract was heated to 510C for 20 minutes after being left at 370C for 20 minutes. Cool the sample after that, and turbidity at 660 nm was measured (UV visible spectrophotometer). For the correctness of the results, the experiment was carried out three times.

The following formula was used to compute the percentage inhibition of protein denaturation:

Percentage inhibition= (Abs control - Abs sample) × 100 / Abs control

4.4.5 Heat induced Haemolysis

It was carried out using a reaction mixture containing 1 ml of 10% RBC suspension and 1 ml of test sample with concentrations ranging from 100 to 500 g/ml. Only saline was added to the test tubes used as controls, whereas the reaction mixture for the test sample contained drug extract solution. Diclofenac was a commonly prescribed medication. All of the reaction mixture-containing tubes underwent a 30-minute incubation in a water bath at 560C. The tubes were then placed under flowing water to cool. All of the reaction mixture-containing tubes were spun at 2500 rpm for five minutes, and 560 nm absorbance measurements were made (UV Spectrophotometer). There were three duplicates of each experiment. This is how the percentage inhibition of hemolysis was determined.

Percentage inhibition = (Abs control - Abs sample) × 100 / Abs control

5. RESULT AND DISCUSSION

The study of pharmacognosy and the use of medicinal plants, which form the basis of conventional medicine, is known as herbal medicine (also known as herbalism). The safety and effectiveness of plants used in 21st-century herbalism, which typically doesn't set standards for purity or dose, are subject to a paucity of scientific data. The range of herbal medicine frequently includes minerals, shells, and various animal parts in addition to fungi and bee products. Phytomedicine or phytotherapy are additional names for herbal medicine.

In the hunt for novel medicines, plants are helpful. Around 80% of the world's population relies on conventional medical procedures because they lack access to Western medicine. Plant-based ingredients in food or botanical powders have been utilised to treat and prevent disease with various degrees of effectiveness. The prevalence of naturally occurring substances with therapeutic capabilities has been linked to the widespread usage of herbal medicines and healthcare preparations that are derived from frequently used traditional herbs and medicinal plants, including those recorded in ancient writings like the Vedas.

The study of natural products is the analysis of their structure, creation, usage, and function in the organism. Natural products, as the title indicates, are those chemical substances obtained from living creatures like plants, animals, and insects. Even a subfield of chemistry is devoted to the study of the chemical properties of the chemical components created by living things, including their separation, identification, structural elucidation, and analysis. Typically, secondary metabolites and their derivatives—which nowadays must be pure and thoroughly described compounds—are used to make pharmaceuticals that are derived from natural sources.

Natural product research continues to be popular since more than 25% of the medications used today are derived from them. The usage of natural products was primarily restricted to simple plant preparations before the early 20th century and was largely based on empirical findings. The chemistry of the plant being employed was not well recognised or understood. According to estimates from the World Health Organization (WHO), 80% of people in several Asian and African nations already utilise herbal medications for some part of basic healthcare. Herbal remedies, in contrast, may be produced from seeds or collected from nature for little to no expense. Plants contain a wide range of pharmacological activities and are abundant in phytoconstituents. Secondary metabolites having antioxidant capabilities that are extensively dispersed include flavones and flavanoids. Natural substances formed from plants can come from any part of the plant, including the bark, leaves, flowers, roots, fruits, and seeds.

5.1 Physical Test of Crude Drugs (Table 5.1)

Insights on the nature, colour, odour, and taste of Black catechu seeds extract can be gained from knowledge of its physical features in their basic pharmacological form. The Black catechu seeds extract physical test findings are as follows:

The Organoleptic properties of the plant extract were evaluated for Black catechu seeds extract

S.no	Parameter	Result
1.	Odour	Distinct aroma
2.	Powder as such	Fine
3.	Colour	Brownish black color
4.	Texture	Rough and grainy
5.	Taste	Bitter and astringent
7.	Solubility	Methanol

Figure 5.1: Black catechu seeds extract powder form

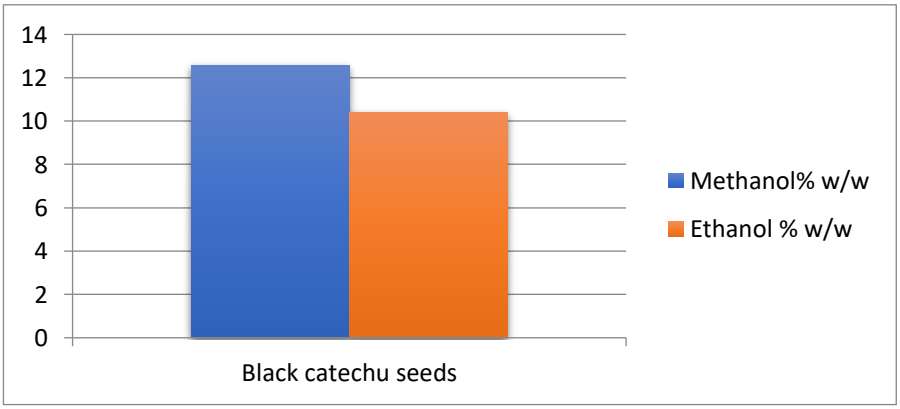
Extractive Values (Table 5.2)

The Extractive Values of the plant extract were evaluated for alcoholic and aqueous solutions Black catechu seeds

Crude drugs	Methanol% w/w	Ethanol % w/w
Black catechu seeds Extract	12.55	10.40

Graph of the Extractive Values

The extractive values provide a quantitative measure of the solubility and extractability of various chemical compounds present in the plant material. These values can be useful in determining the quality and potency of the extract and can be used as a basis for standardization and quality control in herbal medicine or other applications where the extract is utilized.



Loss on Drying And Foreign Organic Matter Black catechu seeds extract (Table 5.3)

S.No	Physical Constant	Values (% W/W)
1.	Moisture content	1.5
2.	Foreign matter	1.26
3.	Extractive value(%w/w)	15.05

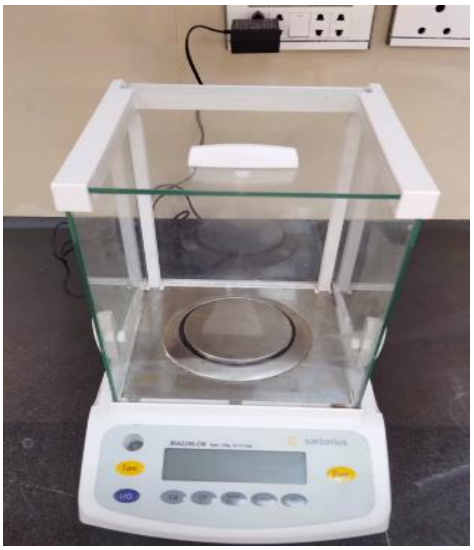


Figure 5.2 Before and after weighing of the powder for moisture content

Graph of Loss on Drying & Foreign Organic Matter Black catechu seeds extract

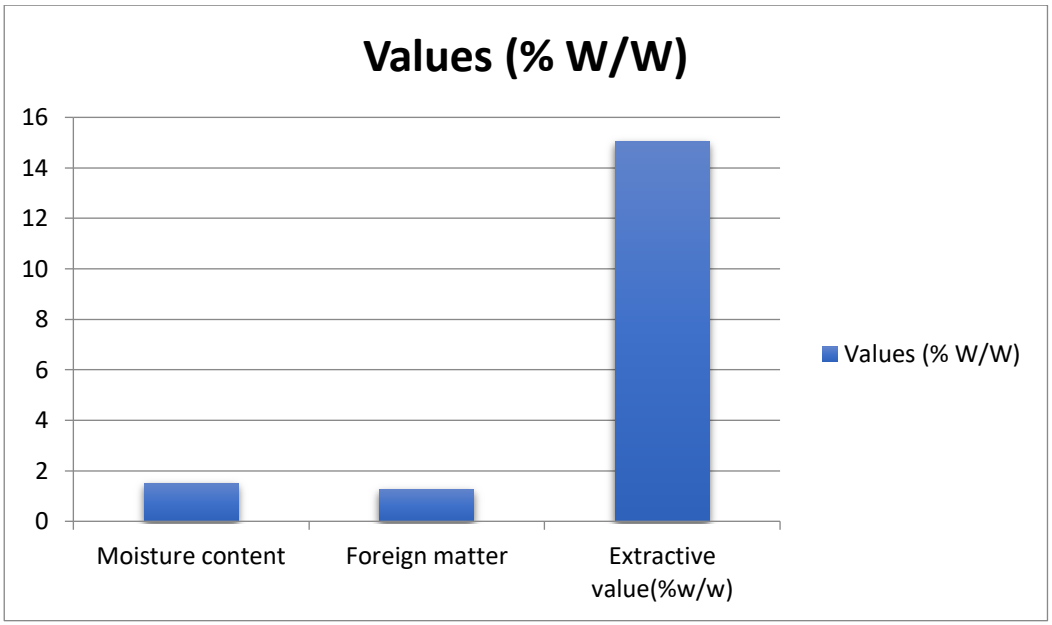
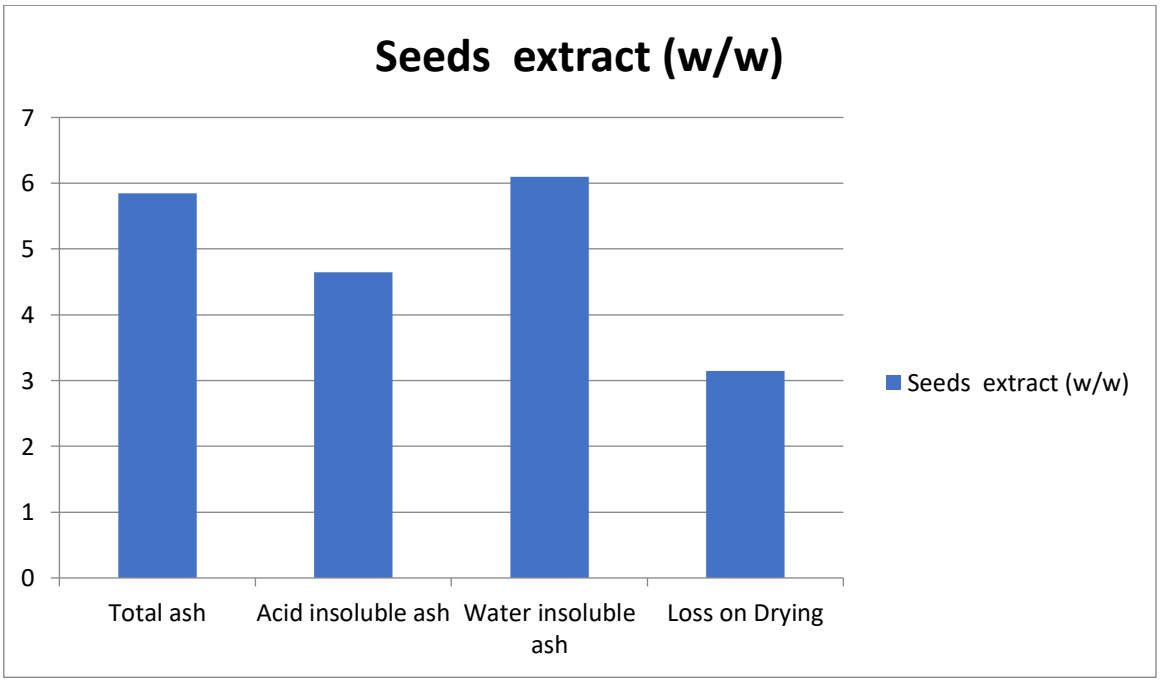


Figure 5.3 Removing of Moisture Content

Total Ash, Acid Insoluble Ash And Water Soluble Ash Values Black catechu seeds extract (Table 5.4)

S.no	Properties	Seeds extract (w/w)
1.	Total ash	5.85
2.	Acid insoluble ash	4.65
3.	Water insoluble ash	6.10
4.	Loss on Drying	3.15



5.3 Phytochemical Screening

Phytochemical screening was performed on the fraction of the dry extract using the technique developed by Trease, Evans, and Harbourne. Alkaloids, saponins, glycosides, proteins, phytosterols, flavonoids, triterpenoids, tannins, fixed oil, and fats were all tested for throughout the phytochemical screening. Below is a list of outcomes:

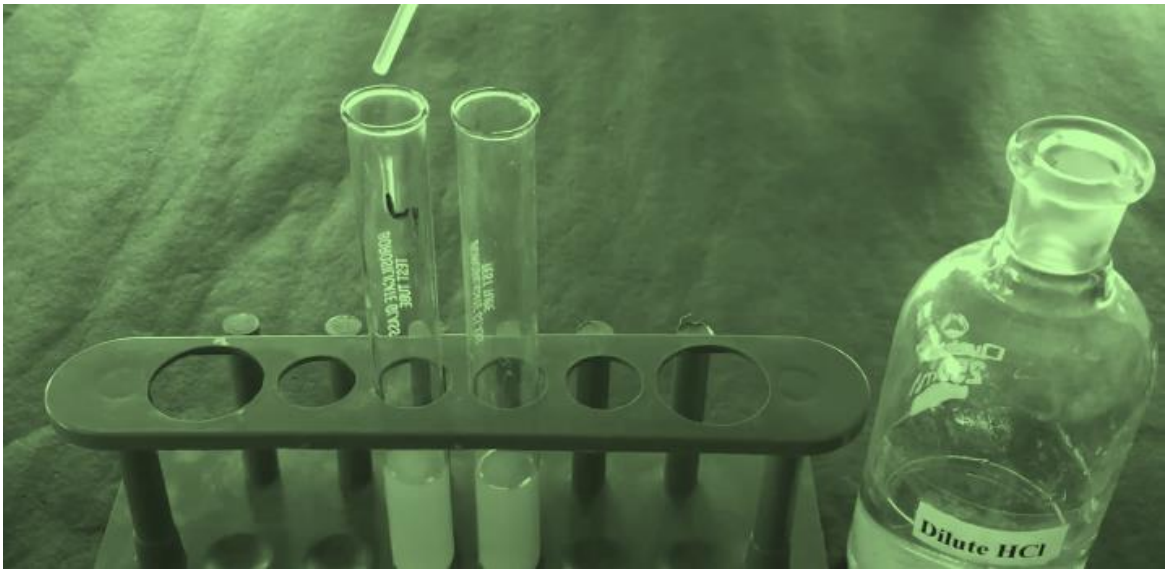


Figure 5.4: Phytochemical screening

Phytochemical screening for extract of Black catechu seeds Extract (Table 5.5)

S.No	Chemical Tests	Black catechu seeds Extract
1.	Tests for Steroids and Triterpenoids:	
	• Liebermann's Burchard Test	-
	• Salkowski Test	-
2.	Test for Saponins:	
	• Foam Test	+
3.	Tests for Alkaloids:	
	• Hager's Test	+
	• Mayer's Test	+
4.	Tests for Glycosides:	
	• Borntrager's Test	+
	• Keller Killiani Test	
5.	Tests for Tannins and Phenolic compounds:	
	• Gelatin Test	+
	• Ferric Chloride Test	+
	• Lead Acetate Test	+
	• Dilute Nitric acid Test	+
6.	Tests for Flavonoids:	
	• Ferric chloride Test	+
	• Alkaline reagent Test	+
	• Lead acetate Test	+
7.	Tests for Proteins:	
	• Biuret Test	-
	• Xanthoproteic Test	-

8.	Test for Carbohydrates:	
	• Fehling Test	+

"+"Found

"-" Not Found

5.4 Inhibition of albumin denaturation

Through the use of an inhibition of albumin denaturation approach and other adjustments, the anti-inflammatory efficacy of Black catechu seeds was investigated. A sample of 1% aqueous bovine serum albumin was obtained along with the test extract. The mixture's pH is kept constant by adding a little quantity of 1 N HCl. The sample extract was heated to 510C for 20 minutes after being left at 370C for 20 minutes. Cool the sample after that, and turbidity at 660 nm was measured (UV visible spectrophotometer). For the correctness of the results, the experiment was carried out three times.

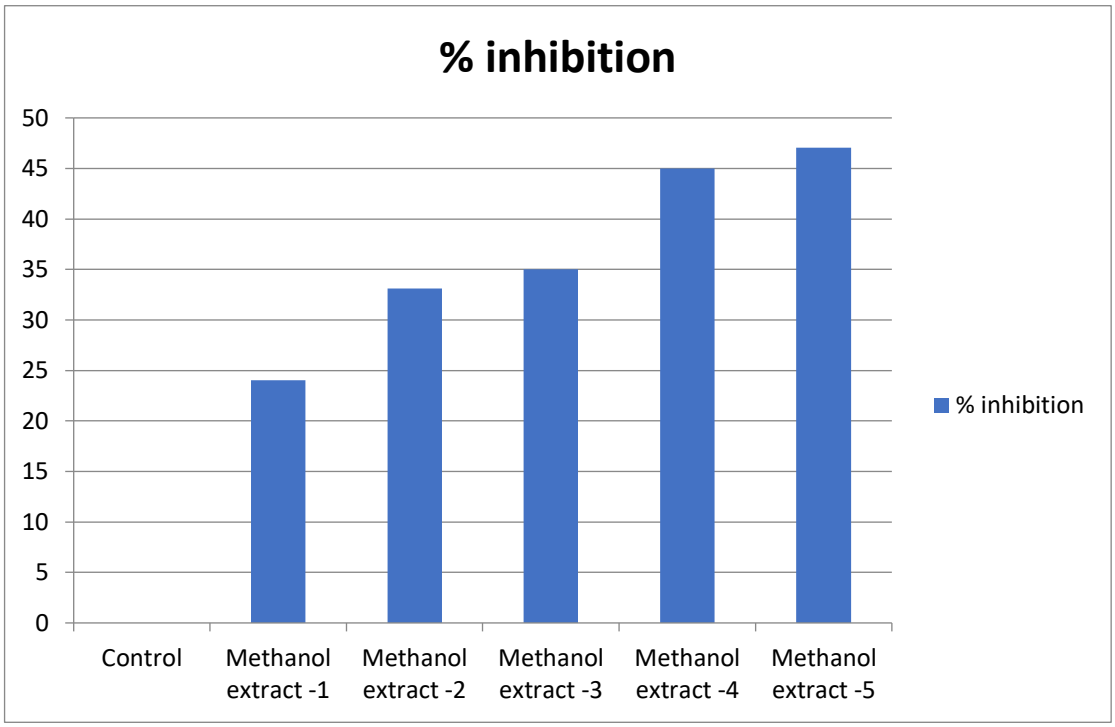
The following formula was used to get the protein denaturation inhibition percentage:

Percentage inhibition= (Abs control - Abs sample) × 100 / Abs control

Table 5.6: Effect of methanol drug extract on inhibition of albumin denaturation

S. No	Sample	Concentration (µg/ml)	Absorbance at 660nm	% inhibition
1	**Control**	**-**	**0.365**	**-**
2	**Methanol extract -1**	**100**	**0.250**	**24.01**
3	**Methanol extract -2**	**200**	**0.260**	**33.10**
4	**Methanol extract -3**	**300**	**0.245**	**35.03**
5	**Methanol extract -4**	**400**	**0.254**	**44.98**
6	**Methanol extract -5**	**500**	**0.180**	**47.05**

Effect of methanol drug extract on inhibition of albumin denaturation



5.5 Heat induced Haemolysis

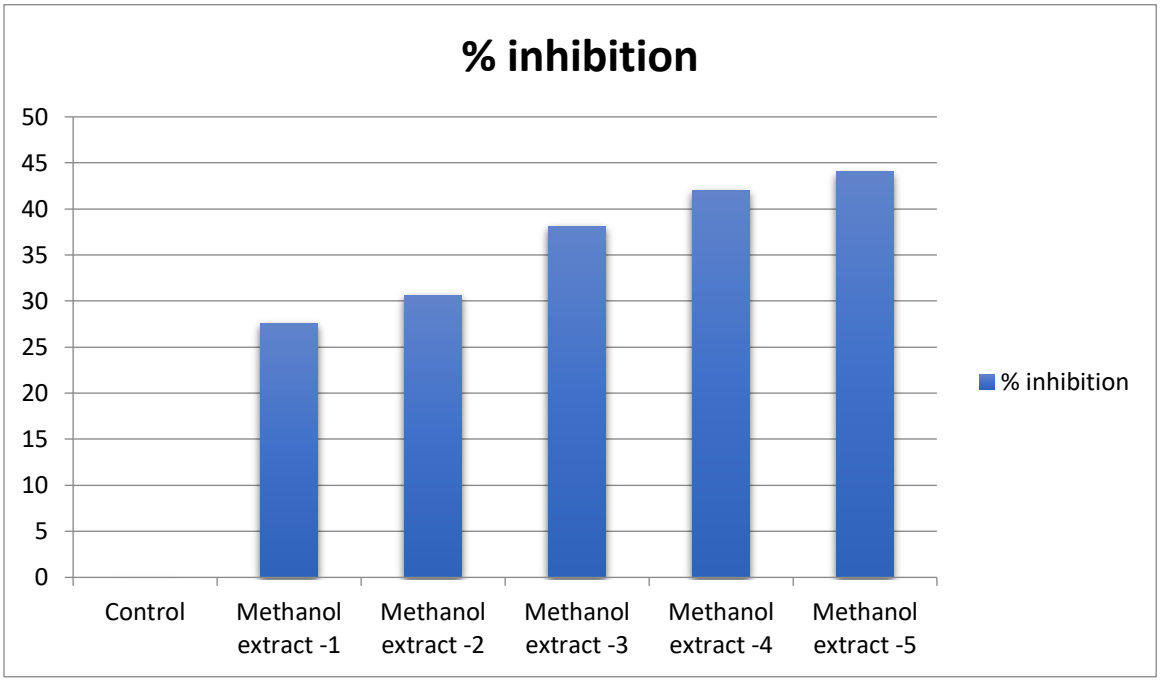
It was carried out using a reaction mixture that contained 1 ml of test sample at various concentrations ranging from 100-500 g/ml and 1 ml of 10% RBC suspension. Only saline was added to the test tubes used as controls, whereas the reaction mixture for the test sample contained drug extract solution. Diclofenac was a commonly prescribed medication. All of the reaction mixture-containing tubes underwent a 30-minute incubation in a water bath at 560C. The tubes were then placed under flowing water to cool. All of the reaction mixture-containing tubes were spun at 2500 rpm for five minutes, and 560 nm absorbance measurements were made (UV Spectrophotometer). There were three duplicates of each experiment. This is how the percentage inhibition of hemolysis was determined.

Percentage inhibition = (Abs control - Abs sample) × 100 / Abs control

Effect of methanol drug extract on heat induced haemolysis of erythrocyte (Table 5.7)

S. No	Sample	Concentration (µg/ml)	Absorbance at 660nm	% inhibition
1	Control	-	0.365	-
2	Methanol extract -1	100	0.250	27.55
3	Methanol extract -2	200	0.260	30.65
4	Methanol extract -3	300	0.245	38.12
5	Methanol extract -4	400	0.254	42.05
6	Methanol extract -5	500	0.180	44.04

Effect of methanol drug extract on heat induced haemolysis of erythrocyte



6. SUMMARY AND CONCLUSION

We summarized these points from study:

- The organoleptic evaluation of the black catechu seeds extract revealed that it possessed a distinct aroma, fine powder form, brownish black color, rough and grainy texture, bitter and astringent taste, and solubility in methanol. These findings provide insights into the sensory properties of the extract, which can be important considerations for its potential applications in various fields, such as herbal medicine, cosmetics, or food industry.
- The extractive values of the black catechu seeds extract were found to be 12.55% w/w in methanol and 10.40% w/w in ethanol. These values indicate the percentage of the desired constituents that were successfully extracted from the black catechu seeds using the respective solvents.
- The black catechu seeds extract had a moisture content of 1.5% w/w, 1.26% w/w of foreign matter, and an extractive value of 15.05% w/w. These physical constants provide important information about the quality, purity, and potency of the extract, which are crucial factors to consider for its use in various applications, including herbal medicine, pharmaceuticals, or other industries.
- The black catechu seeds extract had a total ash content of 5.85% w/w, acid insoluble ash of 4.65% w/w, water insoluble ash of 6.10% w/w, and a loss on drying of 3.15% w/w. These values provide insights into the inorganic mineral content and moisture content of the extract, which are important parameters to consider for its quality, stability, and potential applications in various industries, such as herbal medicine or pharmaceuticals.
- The methanol extract of the drug demonstrated concentration-dependent inhibition of albumin denaturation. As the extract concentration increased, the absorbance decreased, indicating a greater protection against albumin denaturation. These findings suggest that the methanol extract may have potential anti-denaturation properties and could be further explored for its therapeutic applications in preventing protein denaturation-related disorders.
- The methanol extract of the drug demonstrated concentration-dependent inhibition of heat-induced haemolysis of erythrocytes. As the extract concentration increased, the absorbance decreased, indicating a greater protection against haemolysis. These findings suggest that the methanol extract may have potential cytoprotective properties and could be further explored for its therapeutic applications in conditions related to erythrocyte damage or haemolysis.

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