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A Review on *in vitro* pharmacological activities of some crude drugs

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Abstract
This review explores the *in vitro* pharmacological activities of various crude drugs, focusing on their carminative, antidiabetic, anti-inflammatory, anticoagulant, and anti-cholesteremic properties. These studies provide valuable insights into the therapeutic potential of herbal extracts such as *Citrus maxima* for gas relief, *Caesalpinia digyna* for managing blood glucose levels, and *Zingiber officinale* for blood clot prevention. The findings highlight the importance of *in vitro* experiments as a foundation for understanding the molecular mechanisms behind these bioactivities, contributing to the early stages of drug development for chronic conditions like cardiovascular diseases, diabetes, and inflammation.

Keywords: Pharmacological activities, *in vitro* studies, crude drugs, herbal extracts, anti-inflammatory, antidiabetic, anticoagulant, carminative.

Introduction
In vitro studies of herbal extracts have garnered significant attention due to their potential therapeutic benefits, particularly in the areas of carminative, antidiabetic, anti-inflammatory, anticoagulant and anti-cholesteremic activities. These studies are crucial in the early stages of drug development, providing insights into the biochemical and molecular mechanisms through which plant-based compounds exert their effects.
Carminative properties, which help alleviate digestive discomfort by reducing gas formation, have been attributed to various aromatic plants traditionally used in herbal medicine *Citrus maxima*. Antidiabetic activity is another key area, where extracts from plants such as *Ceaesalpinia digyna* and Gymnema sylvestre have demonstrated the ability to lower blood glucose levels by influencing insulin secretion or glucose metabolism.
Anti-inflammatory activity, vital for managing chronic conditions such as arthritis, is often linked to the presence of phenolic compounds and flavonoids in plants like *Casia auriculata*, Curcuma longa and *Boswellia serrata*.
These compounds inhibit pathways that lead to inflammation, thereby offering potential relief from pain and swelling.
Anticoagulant and anti-cholesteremic activities are equally important, particularly in the prevention of cardiovascular diseases. Herbal extracts like those from *Zingiber officinale*, *Allium sativum* and *Ginkgo biloba* have been shown to inhibit platelet aggregation and reduce cholesterol levels, respectively, through various mechanisms including antioxidant activity and modulation of lipid metabolism.
Overall, *in vitro* studies serve as a valuable platform for understanding the multifaceted roles of herbal extracts in health management, paving the way for their potential use in clinical practice.

In vitro carminative activity
What is gas and bloating?
Gas and bloating refer to symptoms commonly experienced in the abdomen, characterized by the presence of excess gas and a feeling of fullness, tightness, or distension. Gas, or flatulence, occurs when air accumulates in the digestive tract through swallowing or the breakdown of undigested foods by bacteria in the colon.

This air is then expelled through belching or passing gas. Bloating, on the other hand, is the sensation of increased abdominal pressure or fullness, often accompanied by visible swelling or distension of the abdomen. These symptoms can be caused by various factors, including dietary habits (such as consuming gas-producing foods like beans or carbonated beverages), swallowing air while eating or drinking, gastrointestinal conditions like irritable bowel syndrome (IBS) or lactose intolerance and certain medications. In some cases, bloating may also be related to hormonal fluctuations, particularly in women during menstruation or pregnancy.

Management of gas and bloating typically involves identifying and addressing the underlying cause. This may include dietary modifications (such as reducing gas-producing foods or increasing fiber intake), lifestyle changes (like eating more slowly and avoiding carbonated drinks), and, in some cases, the use of over-the-counter medications to relieve symptoms. Persistent or severe symptoms should be evaluated by a healthcare professional to rule out more serious underlying conditions and to determine the most appropriate treatment approach.

What is carminative?

A carminative is a substance or drug that helps to relieve or prevent gas and bloating in the digestive tract. Carminatives work by promoting the expulsion of gas from the stomach or intestines, thereby reducing discomfort associated with flatulence, indigestion and abdominal distension.

Carminatives are generally considered safe for most people when used appropriately and in recommended amounts. They are commonly used in traditional medicine systems worldwide and are available over-the-counter in various forms, including teas, capsules, and tinctures. However, individuals with gastrointestinal conditions such as gastro esophageal reflux disease (GERD), peptic ulcers or allergies to specific herbs or spices should exercise caution and consult healthcare professionals before using carminative remedies.

Crude Drugs used as carminative

Peppermint, Ginger, Lemon balm, Fennel, Chamomile, Turmeric, Cardamom, Coriander, Cumin, Cinnamon, Dill, Caraway, Aniseed, Rosemary, Marshmallow, Pineapple sage, Liquorice Root, Slippery elm, Basil, Clove, Bhenda, Agheda, Sarala, Karli, Agheda, Onion, Korphad, Suran, Shopa, Sheku, Phanas, Neem, Rakta Chandan, Saur, Slap Hali, Panfutti, Palas, Bhang, Lal Mirch, Ajmoda, Deva Daru, Mexican tea etc.

Allopathic Medicines

Simethicone, Activated Charcoal, Dicyclomine, Dimethicone, Alpha-Galactosidase, Lactase Supplements, Beano (Alpha-Galactosidase), Sorbitol Solutions, Digestive Enzyme Supplements, Proton Pump Inhibitors (PPIs) Example: rabeprazole, lansoprazole, omeprazole, pantoprazole, Antacids as Mylanta and Tums (Calcium Carbonate, Magnesium Hydroxide, Magnesium Hydroxide), H2 blockers, such as Tagamet HB (cimetidine), Pepcid AC (famotidine), Axid AR (nizatidine), and Zantac 75 (ranitidine), hamper acid production etc.

Determination of *in vitro* carminative activity of *Citrus maxima*: *Citrus maxima*

It consists of the dried leaves of the plant *Citrus maxima* belonging to the family Rutaceae. The leaves of *Citrus maxima* have several distinctive characteristics. The tree produces broad, oval-shaped leaves, that are glossy and dark green on the upper surface, with a lighter, less shiny underside. These leaves are arranged alternately on the Branches and have a distinctive winged petiole, a feature typical of many citrus species. When crushed, the leaves release a strong citrus fragrance, indicative of the essential oils contained within. Beyond their aesthetic contribution to the tree, the leaves of *Citrus maxima* have been valued in traditional medicine for their anti-inflammatory and antioxidant properties. They are also occasionally used in culinary applications for flavoring. The overall appearance and aroma of the leaves enhance the acceptance. The pomelo tree's appeal as a decorative plant in gardens and landscapes.

Culinary uses

- The flesh of the pomelo is eaten fresh, often as a dessert or snack. It can be segmented and added to salads,both fruit and savory.
- The juice from the pomelo is consumed as a refreshing drink and can be used in cocktails and other beverages
- The zest from the thick peel is used to flavor dishes, desserts and Beverages.

Medicinal uses

- The leaves, peel and fruit have been used in traditional medicine to treat various ailments. The leaves are believed to have anti-Inflammatory and antioxidant properties.
- The fruit is known to aid digestion and is used to treat digestive issues such as constipation.
- Rich in Vitamin C, pomelo helps boost the immune system and fight off infections.

Ornamental uses

- The pomelo tree is grown as an ornamental plant in gardens and landscapes due to its attractive foliage and large fragrant fruits.
- The tree provides ample shade due to its large canopy and broad leaves.

Other Uses

- **Aromatherapy:** The essential oils extracted from the leaves and peel are used in aromatherapy for their refreshing and uplifting scent.
- **Cultural Significance:** In some cultures, pomelos are used in religious and cultural ceremonies, especially during the Chinese mid-autumn. Festival plant in gardens and landscapes due to its attractive foliage and large fragrant fruits.

***In vitro* carminative activity of *Citrus maxima* by acid-base titration technique: Requirements**

- **Chemicals and drugs**: Methanol, sodium hydroxide, standard HCl, phenolphthalein, methyl orange, hydrochloric Acid, sodium carbonate, *Citrus maxima* leave extract and distilled water.
- **Apparatus:** Conical flask, Plastic container, Aeration system and Burette

Methods

The decoctions constituted 5 and 10 milliliters and were placed into conical flasks, and 100 milliliters of distilled water were added. Approximately 100 ml of NaOH solution [1M, formerly standardized with oxalic acid] were poured into a plastic container. *Citrus maxima* are suspended within a reaction vessel that is equipped with an aeration system. After stirring the flask manually for 45 minutes and placing it in a room through the night, the flask will begin to evolve. A container for collecting CO_2 gas discharged from the reaction vessel was let into the reaction vessel. Excess NaOH has been absorbed in the form of Na2Co3 (sodium carbonate) and converted to an equivalent quantity. A mixture containing an abundance of sodium hydroxide (NaOH) and sodium carbonate (Na_2CO_3), salts of which were titrated with standard HCl by using phenolphthalein as an indicator. First, to dope out the first end point, and then simultaneously it is important to riddle out the second end point using methyl orange as the indicator. To estimate the concentration of carbon dioxide (CO_2) per gram of sample, use the variation in milliliters between the first and second end points. Volume of titrant x molarity of standard. Determine the carminative activity of *Citrus maxima* by acid-base titration technique with the following formula;

[Acid x mol. wt. of CO_2 = mass of CO_2 in gm]

***In vitro* antidiabetic activity**

What is diabetes mellitus?

Diabetes mellitus is a chronic metabolic disorder characterized by high levels of glucose in the blood due to defects in insulin production, insulin action, or both. Insulin, a hormone produced by the pancreas, is essential for regulating blood sugar levels by facilitating the uptake of glucose into cells for energy production. In diabetes mellitus, this regulatory mechanism is impaired, leading to persistent hyperglycemia.

Types of Diabetes Mellitus

Diabetes Mellitus are of following types

Type 1 Diabetes: An autoimmune condition where the body's immune system attacks insulin-producing cells in the pancreas. It typically appears in childhood or adolescence. Type 1 diabetes is an autoimmune condition where the body's immune system mistakenly attacks and destroys the insulin-producing beta cells in the pancreas. This leads to little or no insulin production, necessitating lifelong insulin therapy for individuals with this type. It commonly develops in children and young adults but can occur at any age. The exact cause is not fully understood, but genetic and environmental factors, such as viral infections, are believed to play a role.

Type 2 Diabetes: The body becomes resistant to insulin or doesn't produce enough insulin. It's often associated with obesity and tends to develop in adults over 45, but younger people are increasingly at risk. Type 2 diabetes is the most common form, accounting for about 90-95% of all diabetes cases. It is characterized by insulin resistance, where the body's cells do not respond effectively to insulin, and a relative deficiency of insulin. Initially, the pancreas compensates by producing more insulin, but over time, it cannot keep up, leading to elevated blood glucose levels. This type is strongly associated with lifestyle factors such as obesity, physical inactivity and poor diet, as well as genetic predisposition. It typically develops in adults over 45, but increasing numbers of younger people, including children and adolescents, are being diagnosed.

Gestational Diabetes

A specific type of diabetes includes gestational diabetes, which occurs during pregnancy and usually resolves after childbirth, but increases the risk of developing Type 2 diabetes later in life. There are also various monogenic forms of diabetes, which are caused by single gene mutations, and secondary diabetes, which can result from other medical conditions or treatments, such as pancreatitis or steroid use.

Managing diabetes involves a combination of lifestyle changes such as diet and exercise, monitoring blood sugar levels and sometimes medications or insulin therapy.

Common symptoms of diabetes mellitus include frequent urination, excessive thirst unexplained weight loss, extreme hunger, fatigue, blurred vision, slow-healing sores and frequent infections. These symptoms are due to hyperglycemia and the body's inability to use glucose effectively [7].

What is antidiabetic activity?

Antidiabetic medications, often simply referred to as antidiabetics, encompass a diverse group of Pharmaceutical agents used to manage and treat diabetes mellitus. These medications work through various mechanisms to help regulate blood glucose levels, either by increasing insulin production, improving insulin sensitivity, reducing glucose absorption from the intestines or promoting glucose excretion through the kidneys. They are crucial in controlling hyperglycemia, the hallmark of diabetes and in preventing its associated complications such as cardiovascular disease, nerve damage, kidney dysfunction, and vision impairment.

Antidiabetic drugs are categorized into several classes, each with unique mechanisms of action. These include insulin and insulin analogs, which are essential for individuals with Type 1 diabetes and some with Type 2 diabetes who require supplemental insulin due to inadequate production by the pancreas. Other classes include biguanides like metformin, which reduce hepatic glucose production and improve insulin sensitivity; sulfonylureas, which stimulate insulin secretion from pancreatic beta cells; and newer classes such as dipeptidyl peptidase-4 (DPP-4) inhibitors and sodium-glucose co-transporter 2 (SGLT2) inhibitors, which target different pathways in glucose metabolism.

The choice of antidiabetic medication depends on factors such as the type and severity of diabetes, individual patient characteristics and considerations of side effects and patient preference. Often, treatment involves a combination of medications tailored to achieve optimal blood glucose control while minimizing adverse effects. Alongside medication, lifestyle modifications including diet, exercise and weight management play a crucial role in diabetes management.

Regular monitoring of blood glucose levels is essential to adjust treatment and reduce the risk of complications. Antidiabetic therapies continue to evolve with ongoing research aimed at improving efficacy, safety and overall outcomes for individuals living with diabetes.

Crude drugs used for antidiabetic activity
Momordica charantia (Bitter Lemon), Gymnema sylvestre (Gurmar), Trigonella foenam-graecum (Fenugreek), *Allium sativum* (Garlic), Panax gingseng (Gingseng), Ocimum sangtum (Holy Basil), Cinnamomum cassia (Cinnamom), Aloe vera, Syzygium cumini (Jamun), Berberis vulgaris (Beribery)

Allopathic drugs used for antidiabetic activity
A. Enhance Insulin secretion
1. K ATP Channel Blockers
Sulphonyl Ureas e.g. Tolbutamide, Glibenclamide, Glipizide, Glicazide, Glimiperide, Meglitenide/ Phenylalanine Analogue, Rapaglinide, Nataglinide,
2. Dipeptidyl peptidase Inhibitor Sitagliptin, Vildagliptin, Saxaglipyin, Alogliptin, LInagliptin

B. Overcome insulin resistance: Biguanides, Metformin, Thiazolidinediones, Pioglitazone.

C. MIscellanious Drugs: Alpha Glucosidase inhibitor, Acarbose, Miglitol, Voglibose, Amylin analogue, Pramlintide, Dopamine D2 Agonist, Bromocriptine, Sodium Glucose Cotransport Inhibitor, Dapagliflozin.
Insulins: Rapid-acting (e.g., Lispro, Aspart), Short-acting (e.g., Regular insulin), Intermediate-acting (e.g., NPH), Long-acting (e.g., Glargine, Detemir) Ultra-long-acting (e.g., Degludec).
Determination of *in vitro* antidiabetic activity of *Caesalpinia digyna Ceasalpinia digyna*:
It is also known as Caesalpinia Bondoc or Fever Nut, belonging to the family Caesalpiniaceous. In traditional medicine, *Caesalpinia digyna* has been used to treat a variety of conditions, including fever, inflammation and gastrointestinal disorders. It is also used as an antimalarial and in the treatment of skin diseases. The plant contains several constituents, including flavonoids, tannins, saponins and glycosides. These compounds are believed to contribute to its medicinal properties.

Other uses
- The plant has been used to reduce inflammation and relieve pain in conditions such as arthritis and rheumatism.
- Extracts from *Caesalpinia digyna* have been used to treat infections and wounds.
- Used to protect against oxidative stress-related conditions.
- Employed to support liver health and treat liver disorders.
- Used in some cultures as a treatment for malaria.
- Utilized to treat digestive issues, such as diarrhea and dysentery.
- It has been used to reduce fever, so it is known as Fever Nut.
- Applied to treat various skin conditions, including eczema and rashes.

In vitro antidiabetic activity of *Caesalpinia digyna* by inhibition of alpha amylase enzyme method Requirements:
Potato starch, trichloroacetic acid, Folin-Ciocalteau reagent, 3,5-dinitrosalicylic acid, Tri's buffer, linoleic acid, ammonium molybdate, α-amylase, α-glucosidase enzymes, xanthine oxidase, quercetin, hypoxanthine, pyrocatechol, Glucose assay kit, Acarbose, ferrozine, (2'2-azobis (2-amidino propane) dihydrochloride), butylated hydroxy toluene. The roots of *Caesalpinia digyna* (CD).

Extraction of root
The shade dried *Caesalpinia digyna* roots were powdered mechanically and sieved through sieve no 20 and stored in an air tight container. The extraction was carried out by hot percolation method using Soxhlet apparatus. The solvent used was methanol. About 100 gm of powder was extracted with 600 ml of methanol. The extract was concentrated to dryness under controlled temperature 40-50℃. The extract was preserved in refrigerator till further use.

Methods
A total of 500 µl of test samples and standard drug (100-1000 µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25 ℃ for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25 ℃ for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represents 100% enzyme activity and were conducted in similar way by replacing extract with vehicle.

Inhibition of alpha glucosidases enzyme
The inhibitory activity was determined by incubating a solution of starch substrate (2% w/v maltose or sucrose) 1ml with 0.2 M Tris buffer pH 8.0 and various concentration of plant extract for 5 min at 37 ℃. The reaction was initiated by adding 1ml of α-glucosidase enzyme (1U/ml) to it followed by incubation for 10 min at 37 ℃. Then, the reaction mixture was heated for 2 min in boiling water bath to stop the reaction. The amount of liberated glucose is measured by glucose oxidase peroxidase method and calculate 50% Inhibitory Concentration (IC50) The concentration of the plant extracts required to scavenge 50% of the radicals (IC50) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I%) was calculated by the following formula.

$$I\% = (Ac-As)/Ac \times 100$$

Where Ac is the absorbance of the control and as is the absorbance of the sample.

***In vitro* anticoagulant activity**
What is coagulation?
Coagulation is the process by which blood changes from a liquid to a gel, forming a blood clot. It's a critical part of the body's mechanism to stop bleeding when a blood vessel is injured. The process involves a series of steps, including the activation of clotting factors (proteins in the blood) that ultimately lead to the formation of a fibrin mesh, which stabilizes the clot and prevents further bleeding. It is basically done internally and externally by two mechanisms:

1) Intrinsic mechanism

The intrinsic mechanism of blood clotting is a pathway that is triggered by damage to the blood vessel wall. It involves the activation of clotting factors present in the blood itself, without the need for external tissue factors. Here's an overview of the process:

- **Vascular Injury:** When a blood vessel is injured, collagen and other substances are exposed to the blood flow.
- **Activation of Factor XII:** The exposure to collagen activates Factor XII (Hageman factor), which then activates Factor XI.
- **Activation of Factor IX:** Activated Factor XI (Factor Xia) activates Factor IX.
- **Formation of Tenase Complex:** Activated Factor IX (Factor IXa) combines with Factor VIIIa (which is activated by thrombin) on the surface of platelets to form the tenase complex.
- **Activation** of Factor X: The tenase complex then activates Factor X to Factor Xa.
- **Prothrombin Conversion:** Factor Xa, in conjunction with Factor Va, forms the prothrombinase complex, which converts prothrombin into thrombin.
- **Formation of Fibrin Mesh:** Thrombin converts fibrinogen into fibrin strands, which weave through the platelet plug and solidify the clot.

The intrinsic pathway is crucial for clotting and works alongside the extrinsic pathway (which is triggered by external trauma) to ensure effective hemostasis.

2) Extrinsic mechanism

The extrinsic mechanism of blood clotting is a pathway that is triggered by external trauma to blood vessels. It involves a series of steps that lead to clot formation, primarily involving tissue factors from outside the blood. Here's a brief overview of the process:

Tissue Injury: When a blood vesselis damaged, tissue factor (TF), also known as tissue thromboplastic, is released from the damaged tissue into the bloodstream.

- **Activation of Factor VII:** Tissue factor binds with Factor VII, activating it to Factor VIIa.
- **Activation of Factor X:** The tissue factor-Factor VIIa complex then activates Factor X to Factor Xa.
- **Prothrombin Conversion:** Factor Xa, together with Factor Va, forms the prothrombinase complex, which converts prothrombin into thrombin.
- **Formation of Fibrin Mesh:** Thrombin converts fibrinogen into fibrin, leading to the formation of a fibrin mesh that stabilizes the platelet plug and forms a blood clot.
- The extrinsic pathway acts quickly and is the primary mechanism for clotting in response to acute injury, complementing the intrinsic pathway to achieve effective hemostasis.

Crude Drugs that show anticoagulant activity: Garlic, Ginger, *Ginkgo biloba*, Dans hen, Bromelain, Turmeric, Willow bark, Horse chestnut

Anticoagulants: Basically, used in dialysis process E.g. sodium citrate, heparin

Allopathic drugs used for anticoagulant activity

Warfarin (Coumadin), Heparin, Rivaroxaban (Xarelto), Abixaban (Eliquis), Dabigatran (Pradaxa), Enoxaparin (Lovenox), Fondaparinux (Arixtra), Acenocoumarol, Edoxaban (Savaysa), Dalteparin (Fragmin).

Determination of *in vitro* anticoagulant activity of ginger Ginger

Which obtained from the rhizome of the plant *Zingiber officinal* belongs to the family Zingiberaceae.

Ginger has many medicinal properties like anti-coagulant, anti-inflammatory, anti-oxidant, anti-nausea, digestive aid, analgesic, antimicrobial activity etc.

***In vitro* anti-coagulant activity of ginger by Prothrombin time assay method Requirements**

1) Plasma Samples

Obtain plasma samples from the individuals

2) Ginger Aqueous Extract

Prepare four different concentrations (25 µL, 50 µL, 75 µL, and 100 µL) of the ginger aqueous extract.

3) Calcium/Thromboplastin Reagent

Use, stable, liquid, combined calcium/thromboplastin rabbit brain reagent as the standard.

4) Pipettes and Pipette Tips

For accurate measurement and transfer of samples and reagents

5) **Water Bath:** For gentle shaking and maintaining sample temperature.

6) **Stopwatch:** To measure the time of clot formation (prothrombin time).

Preparation of *Zingiber officinale* extract

Dried Ginger (*Zingiber officinale*) rhizomes can be purchased from the local vegetable Market. The dried rhizomes, ground into a fine Powder and five grams of the powder were weighed using sensitive balance and then suspended in 100 ml of distilled water in a conical flask with continues shaking for twenty-four hours.

The supernatant of *Zingiber officinale* extract filtrated using filter Paper size 42 mm. The final aqueous extract (5%) of *Zingiber officinale* was used for an *in vitro* testing of its possible anticoagulant activity in blood samples of normal. Individuals using the principles of prothrombin time test. The 5 g of ginger was added with 500 ml of 96% ethanol and 500 ml of deionized distilled water and continuously stirred at room temperature for 72 hours.

Methods

Preparation of Plasma Samples: Divide the plasma sample from each individual into five groups of 50 µL each.

Control Group: Test Group 1 (N=30) to determine the normal prothrombin time (PT) using the calcium/ thromboplastin reagent. This group serves as the positive control.

Treatment Groups: Add different volumes of ginger aqueous extract (25 µL, 50 µL, 75 µL, and 100 µL) separately to the remaining four groups of plasma samples.

Mix gently in a water bath with gentle shaking to ensure proper mixing.

Addition of Thromboplastin Reagent: Add 100 µL of thromboplastin reagent separately to each mixture of plasma and ginger extract using a pipette with volume adjustment.
Thromboplastin reagent is used to counteract the sodium citrate in the Plasma and allow clotting to proceed

Measurement of Prothrombin Time (PT): Use a stopwatch to measure the time taken for clot formation after the addition of the thromboplastin reagent.
Record the prothrombin time for each sample.

Data Analysis: Compare the prothrombin times of the ginger extract-treated groups with the control group to determine the anticoagulant effect of *Zingiber officinale*.

Steps to conduct the assay

- Take five test tubes in which 50 ml of blood is being taken in each of the Test tubes
- Control group is being selected and the remaining test tubes are to be taken as treatment group
- Control group: blood + thromboplastin reagent (100 ml)
- Clotting time recorded and taken as the reference
- Treatment group: blood + ginger extract (25 ml, 50 ml, 75 ml, 100 ml) + Thromboplastin reagent
- Prothrombin time is noted for each of the test tubes
- Data analysis

***In vitro* anti-Cholesterimic activity**

Cholesterol

Cholesterol is a waxy, fat-like substance found in the body. It is an essential component of cell membranes and is involved in the production of hormones, vitamin D, and bile acids. While cholesterol is necessary for good health, high levels of cholesterol can lead to serious health problems.
Three types of cholesterol are: Low-Density Lipoprotein (LDL), High-Density Lipoprotein (HDL) and Very Low-Density Lipoprotein (VLDL)

Balance and Regulations

The body maintains a delicate balance of cholesterol through complex mechanisms involving synthesis, absorption and excretion. When this Balance is disrupted due to genetic predispositions, dietary habits or other factors cholesterol levels can become elevated, potentially leading to health problems such as atherosclerosis (hardening of the arteries) and cardiovascular diseases.
Managing cholesterol levels involves a combination of dietary Adjustments, regular physical activity, maintaining a healthy weight and sometimes medication under the guidance of healthcare professionals. Regular monitoring through blood tests is essential to assess cholesterol levels and make informed decisions about treatment and lifestyle modifications.

Effects of High Cholesterol: Heart Disease, Stroke, Gallstones, Liver Damage, Risk Factors for Other Diseases

Anti-Cholesterimic Activity: Anti-cholesterimic activity refers to the ability of a substance to reduce or inhibit cholesterol levels in the body. Cholesterol is a lipid molecule that is essential for various bodily functions, including cell membrane structure and hormone production. However, excessive cholesterol levels, particularly low-density lipoprotein (LDL) cholesterol, can lead to atherosclerosis and cardiovascular diseases.
The capacity of a substance to lower or inhibit cholesterol levels in the body is anti-cholesterimic agent. It helps to prevent atherosclerosis and cardiovascular diseases caused by excessive cholesterol.
Particularly, it targets reduction of low-density lipoprotein (LDL) cholesterol. The primary reason for studying and developing anti-cholesterimic agents is to manage and reduce the risk of cardiovascular diseases (CVDs), which are a leading cause of morbidity and mortality globally. Common mechanism of anti-cholesterimic activity include inhibition of HMG-CoA reductase, Enhancement of LDL receptor activity, inhibition of cholesterol absorption and promotion of bile Acid secretion.

Natural anti-cholesterimics: Herbal remedies like garlic, Ginger, turmeric and green tea, Dietary supplements such as Plant Sterols, Omega-3 fatty acid and red yeast, Marine-derived compounds have also shown promise like seaweed, microalgae and certain marine invertebrates
Determination of *in vitro* anti-cholesterimic activity of *Allium sativum Allium sativum* (Garlic).
Garlic (*Allium sativum*) belongs to the family Amaryllidaceae. It contains bioactive compounds responsible for its distinctive smell, taste and numerous health benefits. This family is well-known for its characteristic bulbous plants, which Include onions, leeks, shallots and chives. Garlic contains allicin, which can reduce cholesterol synthesis in the liver and increase the breakdown of cholesterol. It may lower total cholesterol and low-density lipoprotein (LDL) cholesterol levels.
Allicin is the chemical constituent of *Allium sativum* formed when garlic is chopped or crushed, responsible for strong aroma and has potent antibacterial, antifungal and antiviral properties. Sulfur Compounds present in garlic include diallyl disulfide, diallyl trisulfide and S-allyl cysteine, contributing to garlic's therapeutic effects. Flavonoids and Polyphenols of garlic act as antioxidants that help to combat oxidative stress and inflammation. Vitamins B6 and minerals such as manganese, selenium and small amounts of essential nutrients present in garlic.
Benefits of *Allium sativum*: It helps to reduce blood pressure, lower Cholesterol levels and prevent atherosclerosis. Boost antimicrobial properties, helps to fight infections and boost the immune system. Due to antioxidants, it helps to reduce inflammation. It may help to reduce the risk of certain cancers by inhibiting cancer cell growth. Enhances detoxification by increasing production of liver enzymes. It may help to improve bone health by increasing estrogen levels in females. Crushing or chopping fresh garlic activates the enzyme alliinase, which converts alliin to allicin, the compound responsible for most of garlic's health benefits. Letting crushed garlic sit for about 10 minutes before consuming or cooking can enhance allicin formation. Cooking garlic can reduce its allicin content. However, lightly cooking or adding garlic towards the end of the cooking process can help retain some of its beneficial compounds. It is available in various forms such as garlic powder, garlic oil, aged garlic extract, and garlic Pills. Aged garlic extract, in particular, is noted for its

enhanced antioxidant properties and reduced pungency, making it a popular choice for supplementation. Garlic oil made by infusing garlic in oil, can be used for cooking or as a topical application for its antimicrobial properties.

In vitro anti-cholesterimic activity of *Allium sativum* by HMG-CoA reductase inhibition assay method: Requirements: Garlicextract, HMG-CoA (substrate), NADPH, HMG-CoAreductaseenzyme and Spectrophotometer

Methods

HMG-CoA reductase inhibition assay method is used to study the *In vitro* anti-cholesterimic activity of garlic extract. Prepare a reaction mixture containing HMG-CoA, NADPH and the enzyme in a suitable buffer. Add different concentrations of Garlic extract to the reaction mixture. Incubate at 37 ℃ for 30 minutes. Measure the decrease in NADPH absorbance at 340 nm using a spectrophotometer. Calculate the percentage inhibition of HMG-CoA reductase activity by comparing the rate of NADPH consumption in the presence and absence of garlic extract.

***In vitro* anti-inflammatory activity**

Inflammation

Inflammation is the body's natural response to injury, infection or harmful stimuli, it is protective mechanism for tissue repair and healing process. Inflammation can be acute or chronic.

Acute inflammation

The initial, rapid response to injury or infection, characterized by redness, heat, swelling, pain and loss of function.

Chronic inflammation

When the inflammatory response persists over a longer period, potentially leading to tissue damage.

Anti-inflammation: The process of reducing or preventing inflammation. Anti-inflammatory agents or treatments work by counteracting the body's inflammatory response.

Crude drugs for anti-inflammatory activity: Turmeric, Ginger, Green Tea, Pepper, Rosemary etc.

Allopathic drugs used for anti-inflammation: Nonsteroidal anti-inflammatory drugs (NSAIDs), Corticosteroids, Disease modifying anti-rheumatic drugs (DMARDs), Cox-2 inhibitors etc.

Determination of *in vitro* anti-inflammatory activity of *Cassia auriculata* by protein denaturation assay Method.

Cassia auriculata

Cassia auriculata, commonly known as the tanner's cassia or avaram, is a flowering plant belongs to family Fabaceae. It is used in traditional medicine, especially in Ayurveda and siddha. In traditional medicine, *Cassia auriculata* has been used to treat conditions like arthritis, wounds, and other inflammatory conditions.

The flowers, leaves and seeds are used for various therapeutic purposes such as treating diabetes, skin diseases and fever.

Requirements

Bovine serum albumin (BSA), Phosphate buffer (pH6.3), *Cassia auriculata* extracts, Distilled water, Ethanol, Test tubes, Centrifuge, Water bath and UV-Visible spectrophotometer

Methods

Preparation of BSA solution: Prepare a 5% (w/v) solution of BSA in phosphate buffer (PH 6.3).

Preparation of plant extract solutions: Dissolve the crude extract of *Cassia auriculata* in distilled water or ethanol to obtain different concentrations (e.g., 50, 100, 200, 400 and 800 µg/ml.

Sample and control preparation: In a series of test tubes, mix different concentrations of *Cassia auriculata* extract (e.g., 50, 100, 200, 400, 800 µg/ml) with 1 ml of BSA solution. Prepare a control tube containing only 1 ml of BSA solution and an equal volume of solvent used to dissolve the extract (without the extract itself). Incubation: Incubate all the test tubes at 37 ℃ for 15 minutes.

Induction of denaturation: Place the test tubes in a water bath and heat them at 60℃ for 10 minutes to induce protein Denaturation.

Cooling

Allow the test tubes to cool to room temperature.

Measurement of absorbance: Measure the absorbance of each solution using UV-Visible spectrophotometer at 660nm.

Calculation of anti-inflammatory activity: Calculate the percentage inhibition of protein denaturation using the following formula:

Inhibition% = (absorbance of control – absorbance of sample/absorbance of control) ×100

Conclusion

In vitro studies of herbal extracts provide valuable insights into the potential therapeutic properties of plants. These studies allow researchers to investigate the biological activities of various compounds under controlled conditions, offering a foundation for understanding their mechanisms of action. Through *in vitro* experiments, scientists can evaluate the efficacy, toxicity and interactions of herbal extracts at the cellular and molecular levels, which is essential for identifying promising candidates for further in vivo studies or clinical trials.

Moreover, *in vitro* studies are cost-effective and time-efficient, allowing for the screening of multiple extracts or compounds simultaneously. However, it is important to recognize the limitations of *in vitro* studies, as they do not fully replicate the complexity of living organisms. Therefore, while *in vitro* research is a crucial step in the drug discovery process, it must be complemented by in vivo studies and clinical trials to fully validate the therapeutic potential of herbal extracts. Overall, *in vitro* studies are a vital tool in the initial stages of exploring and developing plant-based medicines, contributing to the growing body of evidence supporting the use of herbal remedies in modern healthcare.

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