Pharmacological And Phytochemical Screening of *Achillea Millefolium* Extract for Its Anti-Diabetic Activity

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Abstract

The aim of this study is to investigate the pharmacological and phytochemical properties of *Achillea millefolium* extract for its anti-diabetic activity. Diabetes mellitus is a group of disease that results in too much sugar in the blood (high blood glucose). The extractive values for the *Achillea millefolium* leaves extract are as follows: Methanol extractive value: 15.55% (w/w), Ethanol extractive value: 11.20% (w/w).The *Achillea millefolium* leaves and fruit extract has a moisture content of 2.5%, 1.84% of foreign organic matter, and an extractive value of 16.35%. Findings indicate that the methanol extract of *Achillea millefolium* leaves has the highest total phenolic content, while the ethanol extract exhibits the highest DPPH scavenging activity. The crude extract shows the strongest α-amylase
inhibitory activity among the tested extracts. The petroleum ether extract has the lowest total phenolic content and its DPPH scavenging activity is not significant. These results provide valuable information about the antioxidant and α-amylase inhibitory properties of *Achillea millefolium* leaves extracts, which can have potential applications in natural product research, herbal medicine, and the development of functional foods.

Keywords: Diabetes mellitus, Achillea millefolium, α-amylase, Phenolic Content, Petroleum Ether.

1. Introduction

1.1 Diabetes mellitus

It is a syndrome that interferes with the body's ability to turn food into energy, causing an overabundance of glucose in the blood.[1] This might happen due to inadequate insulin production from the pancreas (Type 1 diabetes) or a body that has developed an immunity to insulin's effects (Type 2 diabetes). Gestational diabetes can occasionally develop during pregnancy and go away after giving delivery. If untreated, diabetes mellitus can have a substantial negative impact on general health and quality of life since it increases the risk of issues affecting the heart, kidneys, eyes, neurological system, and other organs and systems when blood sugar levels are consistently raised.[2] A nutritious diet and regular exercise are only a couple of the lifestyle changes that go into managing and treating diabetes effectively. Other factors include prescription drugs, insulin therapy, and ongoing blood glucose testing.

The long-term consequences linked to diabetes mellitus must be avoided or minimised by having an early diagnosis, routine monitoring, and adherence to an extensive care plan.[3]

1.2 Prevalence and Impact on Global Health of Diabetes mellitus

With its incidence gradually rising over time, diabetes mellitus has become a serious worldwide health problem. A predicted 700 million persons will have diabetes by 2045, according to the International Diabetes Federation (IDF), up from an estimated 463 million in 2019. Diabetes has a huge negative influence on global health since it raises the burden of illness, death, and healthcare costs.[4]

Numerous reasons, such as population expansion, ageing, urbanisation, sedentary lifestyles, and bad eating habits, are to blame for the increase in the incidence of diabetes. Because of the quick socioeconomic and lifestyle changes they experience, developing nations are especially impacted. But diabetes does not only affect certain geographical areas or socioeconomic classes. It has far-reaching effects on people, families, and communities and affects people of all ages, especially children.[5]

Diabetes has a wide range of health effects, some of which can be very serious if not well controlled. Heart attacks and strokes are among the cardiovascular conditions that diabetes makes more likely. It also results in side effects include diabetic foot ulcers, which may need lower limb amputations, diabetic retinopathy, a primary cause of blindness, diabetic nephropathy, which damages the kidneys, diabetic neuropathy, which damages the nerves. Additionally, diabetes significantly raises the chance of acquiring other chronic diseases including obesity and hypertension.[6]

Diabetes has a significant financial impact on healthcare systems. Diabetes-related healthcare expenditures are a huge burden on people, families, and healthcare budgets. These costs include direct medical spending as well as indirect costs from lost productivity and incapacity. According to IDF estimates, more over $760 billion was spent globally on diabetes-related healthcare in 2019.[7]

At several levels, coordinated efforts are required to combat the incidence of diabetes and its effects on world health. Implementing preventative measures including encouraging healthy lives, early diagnosis through routine screenings, and guaranteeing access to cost-effective, high-quality healthcare are all part of this. To increase awareness, enhance diabetes management, and lessen the impact of the illness on people and society, public health efforts, education programmes, and legislative interventions are essential.[8]

1.3 Types of Diabetes Mellitus

A. Type 1 Diabetes mellitus

The beta cells of the pancreas, which are in charge of making insulin, are destroyed in type 1 diabetes, an autoimmune condition. These beta cells are incorrectly targeted and attacked by the immune system, which results in a considerable decrease or cessation of insulin production. It is believed that a combination of genetic predisposition and environmental conditions causes this autoimmune damage.[9]

People with type 1 diabetes become dependent on exogenous insulin for survival as a result of the loss of beta cells. By enabling glucose to enter cells for use as fuel, the hormone insulin controls blood sugar levels. Without enough insulin, circulatory glucose builds up and causes hyperglycemia.[10]

Type 1 diabetics require ongoing insulin therapy for the rest of their lives, typically delivered through several daily injections or the use of insulin pumps. To keep steady glucose control, they must carefully check their blood sugar levels and modify their insulin dosages as necessary. For the purpose of avoiding difficulties brought on by high or low blood sugar levels, constant monitoring is essential.[11]

Although it can occur at any age, type 1 diabetes is commonly diagnosed in children, teenagers, or young adults. Although the precise aetiology is unknown, it is thought to be a result of a mix of genetic predisposition and environmental triggers, such as viral infections or exposure to certain chemicals.[12]

A balanced diet, frequent exercise, and careful monitoring of carbohydrate consumption are all necessary components of regulating blood sugar levels when living with type 1 diabetes. Additionally, support groups, diabetes education, and regular medical treatment are beneficial for people with type 1 diabetes in order to provide the best possible disease control and lower the risk of complications.[13]

B. Type 2 Diabetes mellitus

A metabolic condition known as type 2 diabetes is characterised by both insulin resistance and beta cell dysfunction. When the body's cells develop insulin resistance, they are less receptive to the effects of the hormone, which reduces their ability to absorb glucose from the bloodstream. Hyperglycemia, a condition where there are increased levels of glucose circulating in the blood, results from this.[14]

The inability of the pancreas to release enough insulin in response to high blood glucose levels is referred to as beta cell malfunction. The mismatch between insulin production and insulin resistance may worsen over time as the beta cells gradually lose their capacity to generate insulin.[15]

The risk of type 2 diabetes is significantly increased by variables related to lifestyle, including being sedentary, eating poorly, and having too much body weight, especially in the abdomen. The likelihood of a person having type 2 diabetes is also influenced by genetic variables.[16]

While type 2 diabetes is increasingly being seen in younger people due to increased rates of obesity and sedentary lifestyles, it typically develops gradually and is frequently diagnosed in adults. Contrary to type 1 diabetes, which must be treated with insulin to survive, type 2 diabetes can initially be controlled by making changes to one's lifestyle, such as increasing regular physical exercise, eating a balanced diet, controlling one's weight, and monitoring glucose levels.[17]

Oral anti-diabetic drugs may occasionally be recommended to enhance insulin sensitivity, increase insulin production, or lessen hepatic glucose production. Insulin treatment may be necessary when the condition worsens in order to reach and maintain target blood sugar levels.[18]

When hormonal changes and insulin resistance cause blood glucose levels to rise during pregnancy, gestational diabetes develops. Although it normally goes away after giving delivery, women who have experienced gestational diabetes are more likely to subsequently acquire type 2 diabetes. Monitoring blood glucose levels, eating a balanced diet, getting regular exercise, and, in certain situations, utilising insulin treatment to maintain ideal blood sugar control are all part of managing gestational diabetes.[19]

1.4 Causes and Risk Factors of Diabetes mellitus

A combination of genetic predisposition and a variety of environmental and lifestyle variables contribute to the development of diabetes mellitus. It is essential to comprehend these causes and risk factors in order to develop efficient preventative and control techniques.

A. Genetic Predisposition: Genetic Predisposition refers to an individual's increased likelihood of developing a particular trait or condition due to genetic factors inherited from their parents. These genetic factors can make certain individuals more susceptible to certain diseases, behavioral patterns, or physical characteristics.

Genes are segments of DNA that encode specific traits, and variations or mutations in these genes can affect an individual's susceptibility to certain conditions or traits. Some traits with a strong genetic basis include eye color, hair color, and height. Additionally, certain diseases and disorders, such as cystic fibrosis, sickle cell anemia, and certain types of cancer, have a well-established genetic component.

It is important to understand that genetic predisposition does not mean that a person is guaranteed to develop a specific condition or exhibit a particular trait. Environmental factors, lifestyle choices, and other external influences can also play a significant role in determining whether a genetic predisposition manifests into an actual condition or trait The likelihood of acquiring diabetes is significantly influenced by genetics. People who have diabetes in their families are more likely to get it themselves. Although carrying these genes doesn't ensure developing the condition, they are linked to an increased vulnerability to diabetes. An individual's total risk is influenced by both genetic and environmental variables.[20]

B. Lifestyle Factors:

1. 1. Sedentary Lifestyle: Lack of routine exercise and extended periods of inactivity raise the risk of getting diabetes. Enhancing insulin sensitivity. Regular exercise lowers the chance of getting type 2 diabetes.[21]

   Unhealthy diet: Poor diets that are heavy in processed foods, sugar, unhealthy fats, and little fibre are associated with an increased risk of developing diabetes.[22]

C. Obesity and Body Weight: The risk of acquiring type 2 diabetes is greatly increased by excess body weight, especially abdominal obesity. Insulin resistance is facilitated by the release of inflammatory compounds from fat cells, particularly those in the area of the belly. The risk of diabetes must be reduced by eating a balanced diet and engaging in frequent physical activity.[23]

D. Other Contributing Factors: Other factors that can contribute to the development of diabetes include:[24-27]

- Ethnicity: Some ethnicities are more predisposed to diabetes than others, including African Americans, Hispanics, Native Americans, and Asians.
- High Blood Pressure: Insulin resistance and hypertension frequently combine, raising the risk of diabetes and cardiovascular problems

IV. Symptoms and Complications

Depending on the kind of diabetes and the individual, symptoms of diabetes mellitus might change. However, both type 1 and type 2 diabetes share certain comparable symptoms. Over

time, issues affecting several organ systems might develop as a result of chronically high blood sugar levels.[28]

A. Common Symptoms:[29-32]

a. Frequent urine (Polyuria): The kidneys work harder to remove extra glucose from the circulation, which results in increased thirst and urine.
b. Excessive Thirst (Polydipsia): Dehydration brought on by high blood sugar levels might result in excessive thirst.
c. Fatigue: Improper glucose utilisation and metabolic abnormalities can lead to decreased energy levels and chronic fatigue.
d. Weight Gain or Loss: Type 1 diabetes may result in unexplained weight loss as the body uses stored fat as fuel. Contrarily, people with type 2 diabetes may gain weight or find it challenging to lose weight as a result of insulin resistance and metabolic abnormalities.

B. Acute Complications:[33-34]

1. Diabetic ketoacidosis (DKA): People with type 1 diabetes are more susceptible to this potentially fatal illness. Ketones are produced when there is a significant insulin deficit, which causes the breakdown of lipids for energy. Deep, quick breathing, fruity breath, nausea, vomiting, stomach discomfort, and bewilderment are a few symptoms.
2. Hyperglycemic Hyperosmolar State (HHS): Predominantly affecting those with type 2 diabetes, HHS is marked by excessively high blood sugar levels and severe dehydration. Excessive thirst, dry mouth, disorientation, visual abnormalities, seizures, and in severe cases, coma are among the symptoms.

C. Long-term Complications:

1. Cardiovascular Disease: Diabetic patients are far more likely to develop cardiovascular disease, such as coronary artery disease, heart attacks, and stroke. Blood arteries can be harmed by high blood sugar levels, which increases the risk of atherosclerosis and cardiovascular disease.[35,36]
2. Neuropathy: Several different forms of neuropathy can be brought on by high blood sugar levels damaging the nerves all throughout the body. In addition to digestive difficulties, urinary troubles, and sexual dysfunction, symptoms may include tingling, numbness, or discomfort in the limbs.[37][38]

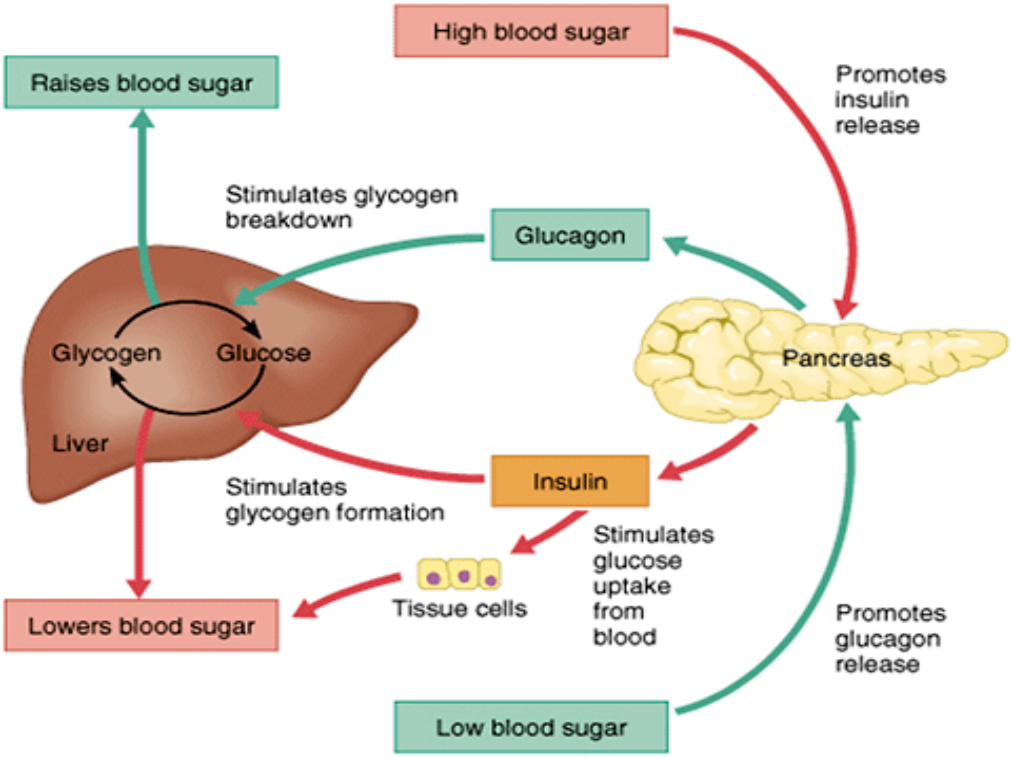


Figure 1.1: Regulation of blood glucose

1.5 Role of herbal medicine in diabetes management

Due to the potential advantages certain herbs may provide, the use of herbal medicine in the management of diabetes has attracted considerable interest and investigation. Traditional medical practises have long relied on herbal treatments to treat diabetic symptoms and promote general health. It's critical to stress that herbal medicine should never be used in place of traditional therapy for diabetes; rather, it should be viewed as an adjunctive strategy used together with conventional medicines and under the supervision of healthcare specialists.

Numerous herbal treatments have demonstrated promise in their ability to control blood glucose levels. For instance, the efficacy of herbs like bitter melon, fenugreek, cinnamon, and gymnema sylvestre to reduce blood sugar levels or increase insulin sensitivity has been researched. Additionally, many herbal treatments include a lot of antioxidants, which help lower oxidative stress and neutralise dangerous free radicals, both of which are factors in diabetic problems.

Aloe vera and ginseng are two plants that have been studied for their ability to increase insulin production, which may have advantages for people with diabetes. Additionally, some herbal remedies with anti-inflammatory characteristics, such as turmeric and ginger, may help to control inflammation brought on by diabetes.

Additionally, certain herbal treatments may help with weight management, which is important for managing and preventing diabetes. These herbs can contribute to better health outcomes by supporting weight reduction efforts or lowering the chance of acquiring type 2 diabetes.

Additionally, the effects of several herbal remedies on the health of the organs, notably the liver, which is particularly susceptible to damage in diabetes, have been researched. For instance, milk thistle's silymarin has demonstrated potential as a liver protector.
Despite the promise of herbal treatment, prudence is advised. Herbal medicines' effectiveness can vary, and some may be harmful or ineffectual. Additionally, herbal remedies may interact negatively or lessen the efficacy of prescription drugs when used together. Therefore, diabetic patients should seek advice from their doctors before using any herbal supplements or treatments in their treatment regimen. Better results for people with diabetes may be fostered by healthcare professionals regularly monitoring their impacts and ensuring that these complimentary therapies are in line with the overall diabetes management plan.

1.6 Literature Review

1.6.1 Diabetes mellitus

Abdel et al.,(2010) reported that "Understanding of hepatic anatomy has evolved greatly over the past 50 years. Greater knowledge of vascular anatomy along with advancement of technologies for intraoperative mapping and parenchymal transection have made liver surgery safer and more efficacious. Recognition of the presence of a dual blood supply and dependence of hepatic tumors on arterial bloody supply have made feasible various interventional techniques allowing directed chemotherapy and radioactive particles via the hepatic artery with simultaneous embolization to minimize tumoral blood supply as treatment options for various tumor types. The complexities and nuances of liver anatomy require continual respect and lifelong learning by liver surgeons." [39]
Bilal et al.,(2018) reported that "Diabetes mellitus is a group of metabolic disorders associated with the endocrine system that resulted in hyperglycemic conditions. Metabolic disorders can cause many complications such as neuropathy, retinopathy, nephropathy, ischemic heart disease, stroke, and microangiopathy. Traditional botanical therapies have been used around the world to treat diabetes. Among several medications and different medicines, various herbs are known to cure and control diabetes; also have no side effects. History has shown that medicinal plants have long been used for traditional healing around the world to treat diabetes. More than 800 plants around the world are shown by ethnobotanical information as traditional remedies for the treatment of diabetes. Several parts of these plants have been evaluated and appreciated for hypoglycemic activity. Medicinal plants have been found to be more effective than conventional drug compounds with no/fewer side effects and relatively inexpensive."[40]

1.6.2 *Achillea millefolium*

Kooti et al.,(2016) reported that "Plants are natural antioxidants and effective herbal medicines, in part due to their anti-diabetic compounds, such as flavonoids, tannins, phenolic, and alkaloids that improve the performance of pancreatic tissues by increasing the insulin secretion or decreasing the intestinal absorption of glucose. More researches are needed in order to separate the active components of plants and molecular interactions of their compounds for analysis of their curative properties. These factors are mostly responsible for the reduction or elimination of diabetes complications. It is worth noting that in this study,

STZ rats are the most common animal model used to investigate anti-diabetic activity of plant extracts."[41]

Saeidnia et al.,(2011) reported that "*Achillea L*. (Compositae or Asteraceae) is a widely distributed medicinal plant throughout the world and has been used since ancient time. Popular indications of the several species of this genus include treatment of wounds, bleedings, headache, inflammation, pains, spasmodic diseases, flatulence and dyspepsia. Phytochemical investigations of *Achillea* species have revealed that many components from this genus are highly bioactive. There are many reports on the mentioned folk and traditional effects. Although, the medicinal properties of *Achillea* plants are recognized worldwide, there are only one review article mainly about the structures of the phytochemical constituents of *Achillea*. The present paper reviews the medicinal properties of various species of *Achillea,* which have been examined on the basis of the scientific in vitro, in vivo or clinical evaluations. Various effects of these plants may be due to the presence of a broad range of secondary active metabolites such as flavonoids, phenolic acids, coumarins, terpenoids (monoterpenes, sesquiterpenes, diterpenes, triterpenes) and sterols which have been frequently reported from *Achillea* species."[42]

1.7 PLANT PROFILE [*Achillea millefolium*]

Achillea millefolium, commonly known as yarrow, is a flowering perennial herb that belongs to the Asteraceae family. It is native to Europe and Asia but is now widely distributed in many other regions around the world.

Figure no.1.2: ***Flowers of Achillea millefolium Linn***

1.7.1 Scientific Classification

Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Eudicots
Clade	Asterids
Order	Asterales
Family	Asteraceae
Genus	Achillea
Species	*A .millefolium*

2.Material And Methods

Table 2.1 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 2.2 Material Used

Material Name	Company Name
Achillea millefolium leaves	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

- The plant has finely split, feathery-looking leaves and a thin stem that can reach a height of 1 metre. Small, white or pinkish Achillea millefolium blooms are grouped near the apex of the stem in flattened clusters. From June through September, the plant blooms.
- Achillea millefolium has been traditionally utilised for medical reasons from the time of the ancient Greeks and Romans. Its medicinal benefits are caused by a number of bioactive substances, including as flavonoids, alkaloids, sesquiterpenes, and triterpenes.
- The plant has long been used to cure a variety of illnesses, including menstrual irregularities, respiratory infections, skin wounds, and gastrointestinal issues. Additionally, it has been demonstrated to have analgesic, antibacterial, anti-inflammatory, and antioxidant effects.

- In addition to being used medicinally, Achillea millefolium is also utilised as a food and beverage seasoning. The perfume and cosmetic industries utilise its essential oil.A valuable medicinal plant with a long history of conventional usage and significant promise for contemporary therapeutic uses, Achillea millefolium is generally regarded as.

2.1 Collection and Authentication of the Plant

- "In light of recent scientific discoveries, plants have been explored for their medicinal properties all over the world due to their strong pharmacological effects, low toxicity, and economic feasibility. Based on information acquired from neighbourhood vaidhyas and other traditional medicine practitioners, the current study assesses Achillea millefolium's efficacy."
- "Achillea millefolium leaves' aerial section was washed, rinsed, and dried over the course of six days. The dried plant material was pulverised into a coarse powder and kept at room temperature for future investigation."

2.2 Soxhlet Extraction

- Accurately weigh the appropriate quantity of dried or powdered Achillea millefolium leaves using an analytical balance. Keep a weight log.
- Assemble the Soxhlet apparatus: Join the Soxhlet extractor to the flask with a circular bottom. Make sure the junction is airtight and secure. The Soxhlet extractor and condenser should be connected.

- Make the solvent ready: Select an appropriate extraction solvent, such as ethanol or methanol. Ensure that the solvent is highly pure.

Figure 1.3: Soxhlet apparatus

- The round-bottom flask of the Soxhlet apparatus should be filled with the weighted Achillea millefolium leaves.

- Add the solvent: Pour the selected solvent into the flask with a circular bottom, making sure it completely covers the leaves. There should be enough solvent to support several extraction cycles.

- Place the round-bottom flask on a hot plate or heating mantle to begin the extraction process. Warm up slowly to start the extraction process. As the solvent begins to boil, vapours will emerge from the condenser.

- The Achillea millefolium leaves in the Soxhlet extractor will get wet when the vapours condense in the condenser and drip over them. The extraction of chemicals from the leaves is facilitated by this cycle of solvent reflux and drainage.

- Define the extraction time: Based on the properties of the compounds you intend to extract, choose the desired extraction time. The typical extraction time is between 4 and 8 hours, however this might change based on the particular molecules of interest.

- Collect the extract: Take out the flask with a circular bottom that contains the extract when the extraction procedure is finished. These extracted chemicals will be present in this solution.

- Extract concentration: If required, the extract can be made more concentrated by rinsing off the solvent. Use a vacuum distillation device or transfer the extract to a rotary evaporator to evaporate the solvent at a lower pressure. An extract that has been concentrated will be the result.

- Filtration (if required): You might need to filter the extract to get rid of any solid residues or particle materials, depending on the nature of the extract. To get a clear extract, use an appropriate filtering device, such as filter paper or a syringe filter.

- Transfer the filtered and concentrated extract to a suitable container for storage, preferably an amber glass vial or an airtight storage bottle. To keep the extract stable, keep it in a cold, dark location.

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

% Yield = ***(*****Net weight of powder in gram after extraction /Total weight of powder in gram taken for extraction*****)*** ***×100***

2.3 Physicochemical Evaluation

The subsequent Physicochemical characteristics of powdered Achillea millefolium plant material were evaluated using traditional methodology.

2.3.1 Loss on drying

The LOD test typically follows a standardized procedure, which includes the following steps:

- Weighing: Using an analytical balance, a representative sample of the substance is precisely weighed. It is noted what the sample weighs.
- Drying: The sample is heated to a specified temperature in an oven and spread out in a thin layer or placed in a suitable container. The type of material being evaluated determines the drying temperature and time required.
- Cooling: To avoid moisture absorption from the environment, the sample is allowed to cool to ambient temperature in a desiccator after the drying process.
- A second weigh: The sample is weighed using the same analytical balance after it has cooled. The dried sample's weight is noted.
- Calculation: The weight of the dried sample is subtracted from the sample's initial weight to determine the loss on drying. The amount of moisture or volatile compounds contained in the sample is indicated by this number.

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

W= weight of the drugs in grams.

The proportion of the sample's initial weight that is lost during drying is the measurement of the loss. It offers important details on a substance's quality, stability, and shelf life. Certain items with a high moisture content may experience microbial development, chemical deterioration, or changed physical qualities. LOD testing is therefore essential for quality control, formulation development, and regulatory compliance in a variety of sectors.

2.3.2 Determination of Ash Value

The ash value test involves the following steps:

- • Weighing: Using an analytical balance, a representative sample of the substance is precisely weighed. It is noted what the sample weighs.
- • Incineration: The sample is heated to high temperatures in a muffle furnace while being contained in an appropriate vessel, such as a crucible. The type of material being evaluated determines the incineration's temperature and timeframe. During incineration, the sample's organic components are burned away, leaving just the inorganic residue.
- After incineration, the container containing the leftover ash is allowed to cool in a desiccator until it reaches room temperature before being weighed. The container containing the ash is reweighed using the same analytical balance when it has cooled. It is noted how much the ash weighs.
- Calculation: The weight of the container is subtracted from the total weight of the container and the ash to determine the ash value. This number indicates how much inorganic material or residue there is in the sample.

2.3.3 Total ash value

The determination of total ash value follows a similar process as described for ash value, with a slight difference in the calculation. Here are the steps involved:

- Weighing: Use an analytical balance to precisely weigh a representative sample of the substance. Keep track of the sample's weight.
- Incineration: Put the sample in an appropriate container, such a crucible, and heat it up in a muffle furnace. The kind of material affects the temperature and length of incineration. Incineration guarantees that all organic materials are completely burned, leaving just the inorganic residue.
- After incineration, let the container containing the leftover ash to cool in a desiccator until it reaches room temperature before weighing it. Use the same analytical balance to reweigh the container with the ash when it has cooled. Keep track of the ash and container weights.
- Calculation: Subtract the container's weight from the total weight of the container and the ash to determine the total ash value. To represent the total ash value as a percentage of the sample weight, multiply this number by the weight of the initial sample and divide the result by 100.

Total Ash Value (%) = [(Weight of Ash + Container) - Weight of Container] / Weight of Sample) x 100

Total ash value analysis may be used to determine a substance's overall mineral composition as well as its quality, purity, and impurity or foreign matter content. It is often employed in a variety of sectors, including those related to agriculture, food, medicines, and herbal medicine. When doing total ash value testing, it's crucial to stick to the pertinent industry standards and detailed instructions.

2.3.4 The acid-insoluble ash test

The acid-insoluble ash test involves the following steps:

- To obtain the total ash, continue the procedures of weighing, burning, cooling, and weighing as explained before in the standard technique for calculating the total ash value.
- Acid treatment: Transfer some of the entire amount of ash from step 1 into a beaker or crucible. The ash sample should be mixed with a reasonable amount of diluted hydrochloric acid (about 25 mL). On a hot plate or in a water bath, slowly warm the ingredients. While leaving behind the acid-insoluble fraction, the acid aids in dissolving the soluble inorganic components.
- Filtering: To remove the insoluble residue from the acid solution, filter the combination after acid treatment using ashless filter paper or a suitable filtering device. To get rid of any leftovers, properly rinse the residue with water.
- Transfer the insoluble residue (acid-insoluble ash) from the filter paper or filtering device to a crucible that has already been pre-weighed before drying and weighing. Dry the crucible with the residue at an appropriate temperature in the oven until the weight remains constant. When the crucible has cooled, weigh it with the acid-insoluble ash residue.
- Calculation: Subtract the weight of the empty crucible from the total weight of the crucible plus residue to determine the acid-insoluble ash value. To represent the acid-insoluble ash

value as a percentage of the sample weight, divide this number by the weight of the initial sample and multiply the result by 100.

Acid-Insoluble Ash Value (%) = [(Weight of Crucible + Residue) - Weight of Crucible] / Weight of Sample) x 100

Water-soluble ash

In 25 cc of water, the ash from the total ash value was cooked for 5 minutes. Filter paper without any ash was put before the insoluble material. Then, to keep the weight constant, a low temperature ignition was employed. The weight of water soluble ash was estimated by subtracting the weight of water insoluble ash from the total ash value. Calculations were utilised to compare the quantity of ash that is water soluble to the value of air-dried ash.

2.3.5 Extractive Value

Procedure In a closed flask, a drug that had been air dried and crushed into a coarse powder was macerated with 100 cc of solvent (chloroform, ethanol, and water) for 24 hours. The first six hours were spent shaking frequently, and the last 18 hours were spent standing. It was immediately filtered after that in order to minimise alcohol loss. In a shallow plate with a flat bottom, a 25 ml sample of the filtrate was dried to dryness, weighed, and then dried at 1050 °C. The air-dried medicine was used as a base to measure the percentage of extractive that is soluble in alcohol. The extractive value is the quantity of soluble substances required for extraction in that specific solvent. The extractive value was calculated utilizing:

$$\text{Extractive value}(\%) = \frac{weight\ of\ residue}{weight\ of\ dry\ powder} \text{ x } 100$$

2.3.6 Determination of foreign matter

The process of determining foreign matter involves the following steps:

- Sample preparation: Collect a sample that is indicative of the substance being evaluated. The sample should be sufficiently large to precisely identify and measure foreign materials. During the collecting and processing of samples, caution should be taken to avoid contamination.
- Visual inspection: Examine the sample visually using the proper lighting and, if required, magnification. Check the sample carefully for any obvious foreign substances, such as dust, fibres, insects, metal particles, or other contaminants. Keep track of any findings from the examination.
- Sieving: Depending on the sample's makeup, sieving may be required to separate the small particles from more substantial foreign materials. This procedure aids in locating and measuring foreign particles that may be identified by size.
- • Separation and identification: The sample should be properly separated from any extraneous material that is discovered during the visual inspection or sifting. The type and makeup of the impurities can then be determined by doing additional analysis on the foreign material using the relevant techniques, such as microscopy, spectroscopy, or other applicable procedures.

- Quantification: After the foreign matter has been recognised, it can be measured in terms of weight or volume, depending on the situation. Typically, the quantification is expressed as a fraction of the entire sample weight or volume.

2.4 Phytochemical Screening

For the phytochemical examination of the aqueous seed extract (produced by Soxhlet extraction), standard techniques were followed. The approaches are briefly explained in the following sections. Each analysis was done three times.

Preliminary phytochemical screening was carried out in accordance with the recommended procedures.

2.4.1 Test for Glycosides

- A chemical analysis is used in the test for glycosides to find out whether they are present in a sample. A sugar molecule (glycone) joined to a non-sugar component (aglycone) by a glycosidic link makes up the family of chemicals known as glycosides. They are often found in plants and have a range of biological functions and medicinal qualities.
- To identify and detect certain kinds of glycosides, a variety of techniques and assays are available. For the test of glycosides, the following general process might be used:
- The sample's preparation Obtain a sample that is indicative of the material that may contain glycosides. To guarantee effective glycoside extraction, the material should be finely crushed or extracted using a suitable solvent.
- Hydrolysis: Use hydrolysis to dissociate the glycosidic link and liberate the aglycone from the sample. Depending on the individual glycoside being examined, this step may entail treating the sample with acid or enzymatic hydrolysis. Acid hydrolysis, which is often employed, involves heating the sample with diluted acid under controlled circumstances.
- Checking for aglycone: After the hydrolysis is complete, check the solution or extract to see if there is an aglycone present. The precise test will depend on the kind of predicted aglycone. For identification, a variety of substances and procedures, such as colour reactions, precipitation reactions, or chromatographic approaches, may be used.
- Testing for the sugar component: The sugar component can be checked after the aglycone's existence has been established. To determine the kind of sugar contained in the glycoside, particular tests must be run at this stage. The Fehling's test, the Benedict's test, or certain enzymatic assays are frequent tests.
- Confirmation and quantification: Following the identification of glycosides, additional characterisation and quantification can be carried out using the relevant methods.

2.4.2 Test for flavonoids

- A unique chemical analysis is used in the test for flavonoids to find and confirm their existence in a sample. A family of naturally occurring plant secondary metabolites called flavonoids is well-known for its wide range of biological functions and possible health advantages.
- The general method for testing flavonoids is as follows:
- The sample's preparation Obtain a sample that is indicative of the material that is thought to contain flavonoids. The sample might be a finely pulverised substance or a plant extract.

- Solvent extraction: Using a suitable solvent, remove the flavonoids from the sample. Ethanol, methanol, or a combination of water and organic solvents are examples of commonly used solvents. Depending on the sample and its properties, the extraction can be carried out via maceration, percolation, or sonication.
- 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.
- 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.
- Spectroscopic analysis: To collect structural data and verify the identification of the flavonoids contained in the sample, spectroscopic methods can be utilised, such as ultraviolet-visible (UV-Vis), infrared (IR), or nuclear magnetic resonance (NMR) spectroscopy. The characterisation and differentiation of distinct flavonoid molecules is aided by these approaches.
- Reference standards and controls: It is crucial to compare the results from the sample with those from real reference standards in order to ensure correct identification and quantification. Purified substances with well-known structures and properties serve as these benchmarks.

2.4.3 Test for Alkaloids

- A chemical analysis is used to determine whether there are any alkaloids present in a material. A group of naturally occurring nitrogenous chemicals with a variety of biological functions, alkaloids are frequently found in plants.
- The typical process for testing alkaloids is outlined below. Initial testing for identification Perform preliminary analyses to determine whether alkaloids are present in the extract. These tests may involve colour changes, precipitation changes, or the development of certain crystals. Tests like the Dragendorff's test, Mayer's test, or Wagner's test are frequently employed. Although these tests give preliminary evidence of the existence of alkaloids, they are not specific for particular substances.

2.4.4 Determination of total phenol content

- The Folin-Ciocalteu assay's redox reaction between phenolic compounds and the Folin-Ciocalteu reagent provides the basis for determining the total phenol concentration. A blue-colored result is produced when phenolic chemicals in the sample act as reducing agents and reduce the Folin-Ciocalteu reagent.
- The phosphomolybdic-phosphotungstic acid, which functions as an oxidising agent, and catalyst-grade sodium carbonate are both components of the Folin-Ciocalteu reagent. A redox reaction takes place between the phenols and the oxidising agents in the Folin-Ciocalteu reagent when the reagent is introduced to the sample of phenolic compounds.

- The phenolic compounds in the sample transfer electrons to the oxidising agents throughout the process, decreasing them. The oxidised form of the reagent and the phenolic compounds combine to create a blue-colored complex as a result of this reduction process.

- The amount of phenolic compounds present in the sample directly relates to how intense the blue colour is generated. The content of phenolic compounds may be evaluated by using a spectrophotometer to measure the blue color's absorption at a certain wavelength.
- A calibration curve is created using a standard phenolic substance with a known concentration, such as gallic acid, in order to get quantitative data. A linear connection between concentration and absorbance is established by measuring the absorbance of various standard solutions containing various concentrations of the phenolic substance.

- The total phenol content of the sample may be determined and quantified in terms of milligrammes of gallic acid equivalents (GAE) per gramme or millilitre of the sample (mg GAE/g or mg GAE/mL) by comparing the absorbance of the sample with the calibration curve.

2.4.5 *In-vitro* antidiabetic determination

2.4.5.1 Alpha-amylase Inhibitory Activity:

To investigate the inhibitory action against a-amylase, plant extract was employed. 1 ml of a-amylase solution (0.5 mg/ml generated in 0.20 mM phosphate buffer; pH 6.9) and 1 ml of (sample) plant extract in concentration ranges of 100-500 g/ml were both added in the reaction mixture. After a 30-minute pre-incubation, the mixture was added to 1 ml of starch solution (1%) in 0.02 mol/L sodium phosphate buffer (pH 6.9), and the mixture was then incubated at 37 °C for 10 minutes. The reaction mixture was halted after adding 1ml of the 3, 5-dinitrosalicylic acid reagent (DNS), and the mixture was then heated for 5 minutes. Acarbose (100–500 g/ml) was used as a reference (positive control). The reaction mixture's absorbance at 540 nm was measured with a UV-VIS spectrophotometer. Ibrahim et al. (2017) and SLDV et al. (2017) both state that each assay was carried out in triplicate. The percentage of inhibition was calculated using the formula below:

$$\% \text{ Inhibition} = [(\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample}}) / \text{Absorbance}_{\text{Control}}] \times 100$$

3. RESULT AND DISCUSSION

3.1 Physical Test of Crude Drugs (Table 3.1)

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

The Organoleptic properties of the plant extract were evaluated for ***Achillea millefolium*** **leaves extract.**

S.No.	Parameter	Result
1.	Odour	Aromatic
2.	Powder as such	Fine
3.	Colour	Greenish-brown
4.	Texture	Semi-solid preparation
5.	Taste	Bitterness
6.	Consistency	Powdered
7.	Solubility	Water or Ethanol

Figure 1.4: *Achillea millefolium* powder form

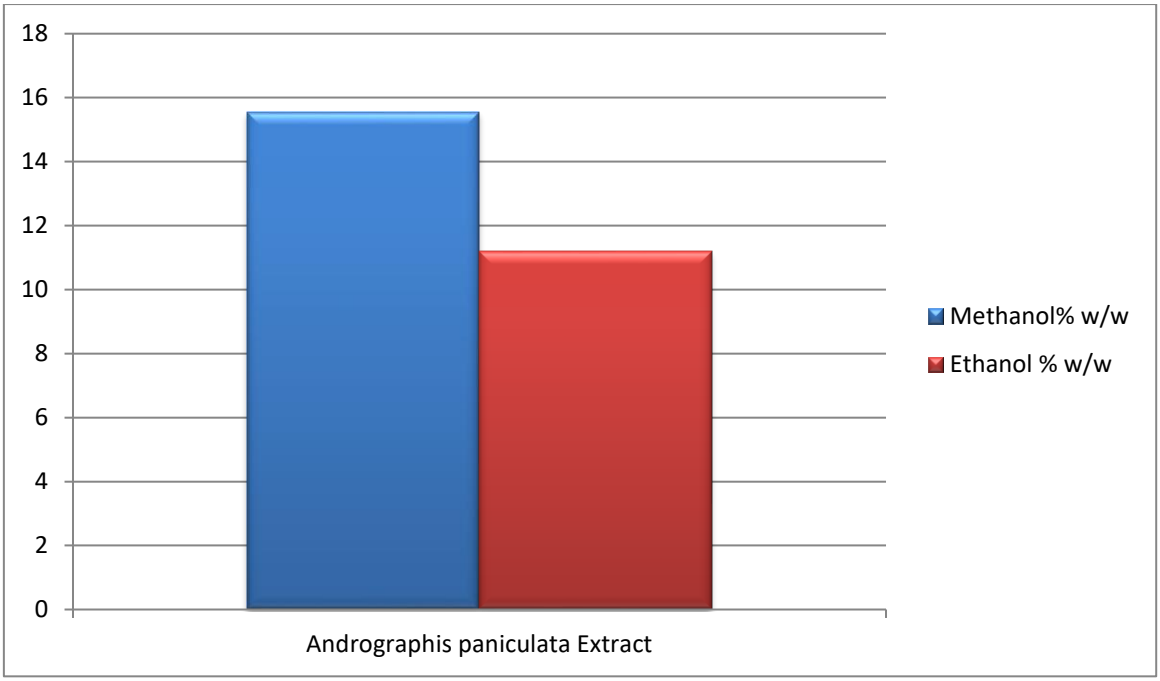
3.2 Extractive Values (Table 3.2)

The Extractive Values of the plant extract were evaluated for alcoholic and aqueous solutions Andrographis paniculata

Crude drugs	Methanol% w/w	Ethanol % w/w
Achillea millefolium leaves Extract	15.55	11.2

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.



3.3 Loss on Drying And Foreign Organic Matter *Achillea millefolium* leaves fruit extract (Table 3.3)

S.No.	Physical Constant	Values (% W/W)
1.	Moisture content	2.5
2.	Foreign matter	1.84
3.	Extractive value(%w/w)	16.35

Figure 1.5: Before and after weighing of the powder for moisture content and temp setting

Graph of Loss on Drying And Foreign Organic Matter *Achillea millefolium* leaves fruit extract

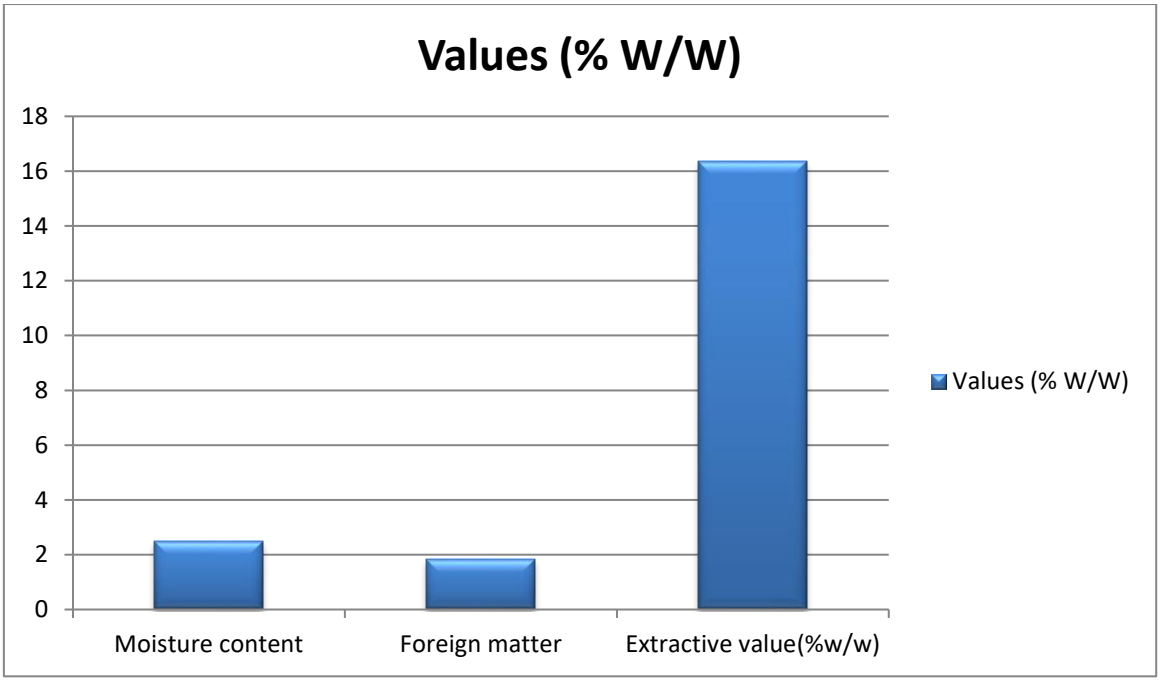
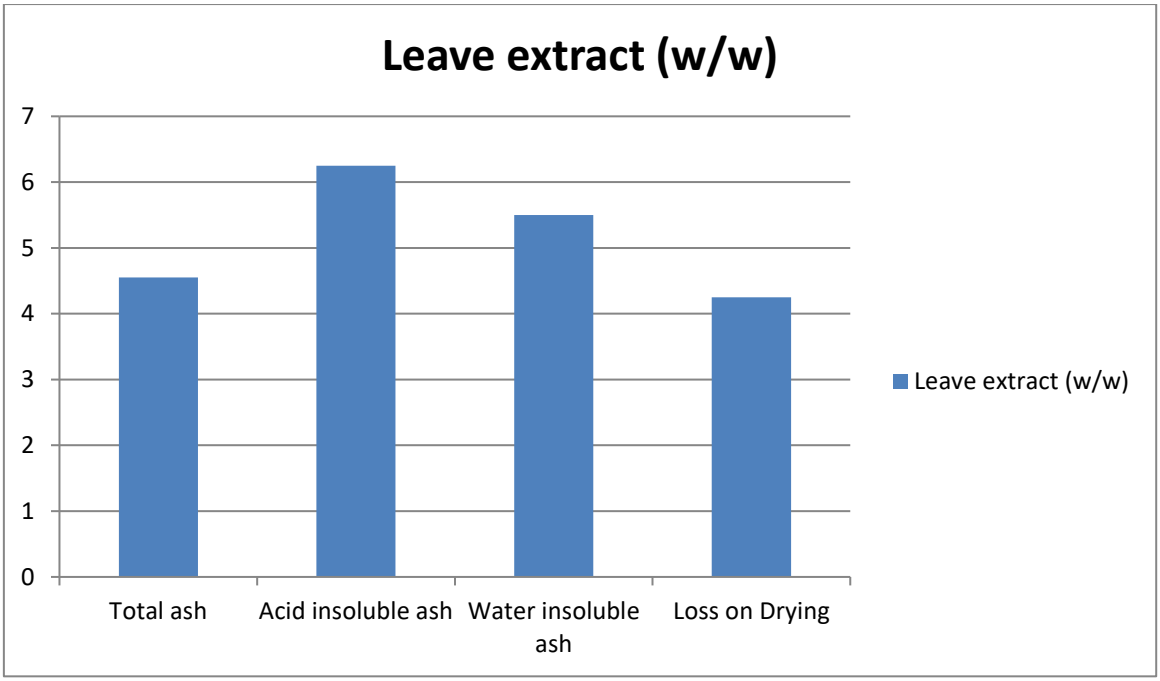


Figure 1.6: Determination of Moisture Content

3.4 Total Ash, Acid Insoluble Ash And Water Soluble Ash Values *Achillea millefolium* leaves extract (Table 3.4)

S.No.	Properties	Leave extract (w/w)
1.	Total ash	4.55
2.	Acid insoluble ash	6.25
3.	Water insoluble ash	5.5
4.	Loss on Drying	4.25



3.5 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 1.7: Phytochemical screening

Phytochemical screening for extract of *Achillea millefolium* leavesExtract (Table 3.5)

S.No.	Chemical Tests	*Achillea millefolium* leaves 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	 + - -
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

3.6 In-vitro ant-diabetic determination

The total phenolic content, DPPH scavenging activity, and α-amylase inhibitory activity were evaluated for leaves extracts of *Achillea millefolium.* The results are presented in the literature format as follows:

1. Crude Extract:

- Total Phenolic Content: The crude extract exhibited a total phenolic content of 8.51 mg gallic acid equivalents per gram of extract.
- DPPH Scavenging Activity: The crude extract demonstrated a DPPH scavenging activity with an IC50 value of 434.62 μg/ml.
- α-amylase Inhibitory Activity: The crude extract displayed α-amylase inhibitory activity with an IC50 value of 15.62 μg/ml.

2. Methanol Extract:

- Total Phenolic Content: The methanol extract showed a total phenolic content of 34.25 mg gallic acid equivalents per gram of extract.
- DPPH Scavenging Activity: The methanol extract exhibited DPPH scavenging activity with an IC50 value of 244.3 μg/ml.
- α-amylase Inhibitory Activity: The methanol extract demonstrated α-amylase inhibitory activity with an IC50 value of 560.46 μg/ml.

3. Ethanol Extract:

- Total Phenolic Content: The ethanol extract displayed a total phenolic content of 12.25 mg gallic acid equivalents per gram of extract.
- DPPH Scavenging Activity: The ethanol extract showed DPPH scavenging activity with an IC50 value of 721.12 μg/ml.
- α-amylase Inhibitory Activity: The ethanol extract exhibited α-amylase inhibitory activity with an IC50 value of 135.40 μg/ml.

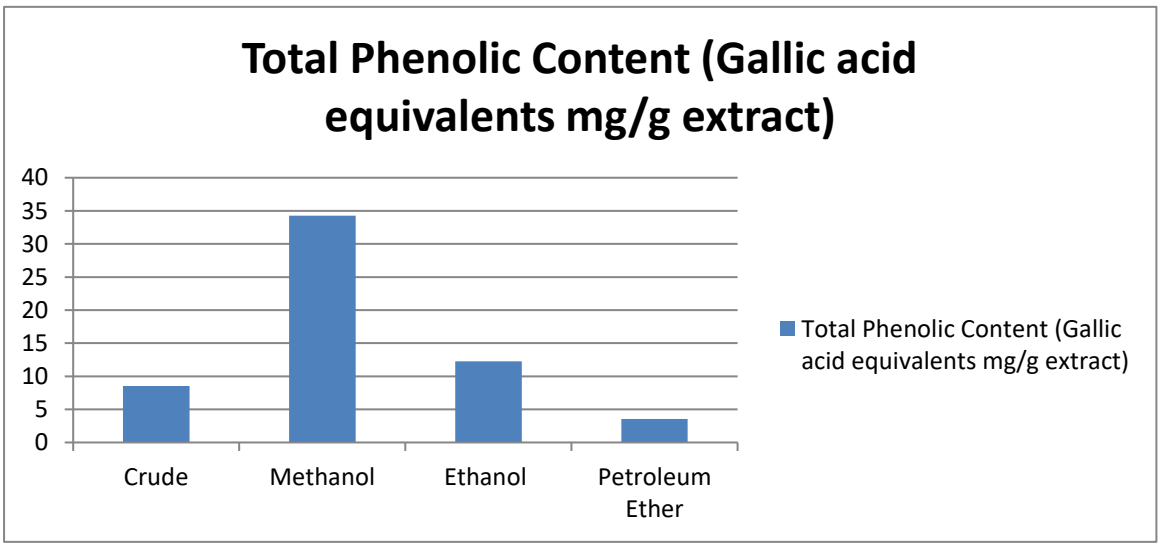
4. Petroleum Ether Extract:

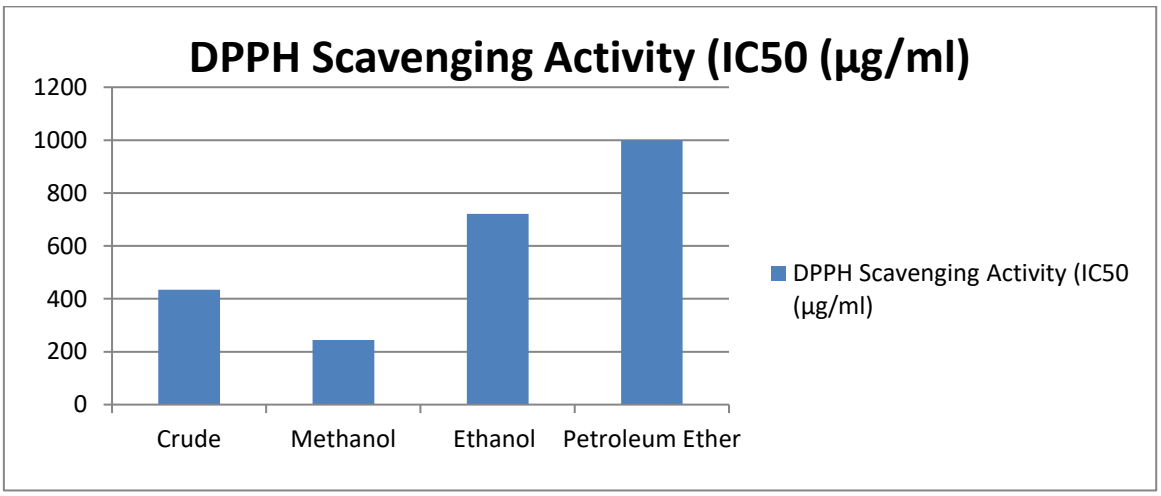
- Total Phenolic Content: The petroleum ether extract exhibited a total phenolic content of 3.56 mg gallic acid equivalents per gram of extract.
- DPPH Scavenging Activity: The petroleum ether extract showed DPPH scavenging activity with an IC50 value higher than 1000 μg/ml.
- α-amylase Inhibitory Activity: The petroleum ether extract demonstrated α-amylase inhibitory activity with an IC50 value of 213.64 μg/ml.

These results provide insights into the antioxidant capacity (DPPH scavenging activity), α-amylase inhibitory potential, and phenolic content of different extracts of *Achillea millefolium*. The information can contribute to understanding the potential health benefits and applications of these extracts in various fields, including natural product research, herbal medicine, and functional food development.

Total Phenolic Content (as Gallic acid equivalents), IC50 (µg/ml) of DPPH scavenging activity and IC50 (µg/ml) of α amylase inhibitory activity of *Achillea millefolium* leaves extract (Table 3.6)

Extract	Total Phenolic Content (Gallic acid equivalents mg/g extract)	DPPH Scavenging Activity (IC50 (µg/ml)	α-amylase inhibitory activity IC50 (µg/ml)
Crude	8.51	434.62	15.62
Methanol	34.25	244.3	560.46
Ethanol	12.25	721.12	135.40
Petroleum Ether	3.56	> 1000	213.64





4. Summary And Conclusion

We summarized these points from study

The *Achillea millefolium* leaves extract has an aromatic odour, fine powder texture, greenish-brown color, semi-solid preparation texture, bitter taste, powdered consistency, and is soluble in water or ethanol.

The extractive values for the *Achillea millefolium* leaves extract are as follows: Methanol extractive value: 15.55% (w/w), Ethanol extractive value: 11.20% (w/w).

The *Achillea millefolium* leaves and fruit extract has a moisture content of 2.5%, 1.84% of foreign organic matter, and an extractive value of 16.35%. These values provide information about the water content, presence of impurities, and the extraction efficiency of the extract.

Findings indicate that the methanol extract of *Achillea millefolium* leaves has the highest total phenolic content, while the ethanol extract exhibits the highest DPPH scavenging activity. The crude extract shows the strongest α-amylase inhibitory activity among the tested extracts. The petroleum ether extract has the lowest total phenolic content and its DPPH scavenging activity is not significant. These results provide valuable information about the antioxidant and α-amylase inhibitory properties of *Achillea millefolium* leaves extracts, which can have potential applications in natural product research, herbal medicine, and the development of functional foods.

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