Pharmacognostical and Physicochemical evaluation of the leaves of *Mikania micrantha* kunth(Asteraceae) with reference to its *in vitro* and *in vivo* Antidiabetic potential

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CERTIFICATE OF APPROVAL

This is to certify that research work embodied in this thesis entitled – **Pharmacognostical and Physicochemical evaluation of the leaves of *Mikania micrantha* kunth(Asteraceae) with reference to its *in vitro* and *in vivo* Antidiabetic potential** was carried out by **SUMI BARMAN** ,(Exam Roll No.-**M4PHG2301**;Registration No.**160247 of 2021-22** for the partial fulfillment of degree of Master of Pharmcy, to be awarded by Jadavpur University. This research work has been carried out under my guidance and supervision and it is up to my satisfaction.

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DECLARATION

I hereby declare that the Dissertation report entitled – **Pharmacognostical and Physicochemical evaluation of the leaves of *Mikania micrantha* kunth(Asteraceae) with reference to its *in vitro* and *in vivo* Antidiabetic potential** is a bonafide and genuine research work carried out by me under the guidance of Prof(Dr.) Subhash C. Mandal, Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032. The work is original and has not been submitted in part or in any form to any other university for the award of any Degree or Diploma or Fellowship. The results reported here are the findings of work carried out by me and plagiarized data have not been incorporated.

I also declare that, as required by the rules and conduct, I have fully cited and referenced all the materials and results that are not original to this work.

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Sl No.	Abbreviations	Full Form
1	%	Percentage
2	µg	Microgram
3	Ml	Microlitre
4	G	Gram
5	C	Celsius
6	Fig	Figure
7	No.	Number
8	Sl. no.	Serial number
9	Abs.	Absorbance
10	ROS	Reactive oxygen species
11	RNS	Reactive nitrogen species
12	cm	Centimetre (s)
13	DPPH	2,2-diphenyl-1-picrylhydrazyl
14	GAE	Gallic acid equivalent
15	IC_{50}	Half maximal inhibitory concentration
16	kg	Kilogram
17	l	Litre(s)
18	M	Metre
19	M	Molarity
20	mg	Milligram(s)

21	min	Minute(s)
22	mM	Millimolar
23	no.	Number
24	QE	Quercetin equivalent
25	pH	Potential of hydrogen
26	SD	Standard deviation
27	SEM	Standard error of mean
28	UV- Vis	Ultraviolet visible
29	v/v	Volume by volume
30	v/v	Volume by volume
31	Conc.	Concentrated
32	$FeCl_3$	Ferric chloride
33	H_2SO_4	Sulfuric acid
34	STZ	Streptozotocin
35	WHO	World Health Organization
36	nm	Nanometer
37	µl	Microliter
38	i.e.	That is
39	*Et.al*	Et alia
40	w/w	Weight/weight
41	TLC	Thin Layer Chromatography
42	HPLC	High Performance Liquid Chromatography

43	ppt	Precipitate
44	Nacl	Sodium chloride
45	DM	Diabetes Mellitus
46	mmol	Millimolar
47	L.	Linneaus
48	Linn.	Linnaeus
49	h	Hour
50	HCl	Hydrochloric Acid
51	Na	Sodium
52	NaOH	Sodium Hydroxide
53	FCR	Folin-Ciocalteu Reagent
54	TPC	Total Phenolic Content
55	TFC	Total Flavonoid Content
56	w/v	Weight by volume
57	β	Beta
58	α	alpha
59	R_f	Retention factor
60	ANOVA	Analysis of Variance
61	<	Less than
62	±	Plus- minus
63	O_2	superoxide
64	OH	Hydroxyl
65	LD_{50}	Lethal dose

66	e.g.	Exempli gratia (for example)
67	&	And
68	DNS	3,5-Dinitrosalicylic acid
69	*B.*	*Bunge*

1.1: Introduction

The world is largest source of natural treatments for treating human problems. In addition to the three basic needs of man—food, clothing, and shelter—plants have also given humans access to natural products that have the power to treat all illnesses and sufferings brought on by the extreme susceptibility of humans to infection and disease (Thomas *et al*., 2000).

In the present era, there is a herbal "renaissance" taking place all over the world (Hussain *et al*., 2008).

Nowadays, plants are utilized to cure practically all illnesses, including gastrointestinal problems, skin problems, skeletal problems, bronchitis, cardiovascular problems, etc. (Harborne 1998; Dass *et al*., 2009).

Humans have employed herbal medications since long before written records were created. Man-made species and herbs have also reportedly been found to have therapeutic qualities (Tapsell *et al*., 2006). Regardless of the underlying philosophical basis, the use of plants in all of the major medical systems serves as an example of the universal function that plants play in the treatment of sickness. Written evidence demonstrates that the Sumerians, who produced clay tablets with lists of hundreds of therapeutic plants, were the first people to study herbs more than 5,000 years ago. The numerous records from around the world offer a wealth of important information that has given man the medical expertise he has today (Sumner 2000).

Traditional uses of herbal remedies by definition imply extensive historical use, and this is unquestionably true for many of the products marketed as such. Herbal medicine has frequently maintained its popularity for historical and cultural reasons while coexisting with such conventional practices in modern medicine. Commercially speaking, these goods are now more readily accessible, particularly in industrialized nations. Herbs have long been a part of traditional healing practices all across the world (Schulz *et al*., 2001).

A metabolic condition called diabetes mellitus is characterized by:

1. Hyperglycemia (fasting plasma glucose above or equal to 126 mg/dl and/or above or equal to 200 mg/dl two hours after 75 g of oral glucose).

2. Glycosuria

3. Hyperlipidaemia

4. Negative nitrogen balance

5. Ketonuria

Ancient Egypt and Greece both had a working understanding of diabetic mellitus. The Latin word "Mellitus" means "Sweetened with honey," which relates to thepresence of sugar in urine. The word "DIABETES" is derived from the Greek word "DIAB," meaning to pass through, referring to the cycle of intense thirst and frequent urination (Tripathi, 2008).Ancient Hindu physicians described the condition known as "Madhumeha" as one in which the patient passes delicious urine and displays sweetness in their perspiration, mucous, breath, and blood (Patel *et al*., 2012).

Two major types of Diabetes Mellitus are there –

Type 1: Insulin Dependent Diabetes Mellitus (IDDM)/ Juvenile Onset Diabetes Mellitus:

The majority of cases of beta cell loss in pancreatic islets are autoimmune (type 1A). Beta cell-destroying antibodies can be detected in blood, some of which are idiopathic (type 1B). Beta cell antibodies are not discovered. Circulating immune levels are always low or very low in type 1 cases, and patients are more likely to go into ketosis. This variety has a lower prevalence and a weaker hereditary tendency.

Type 2: Maturity-Onset Diabetes Mellitus (MODM)/Non Insulin Dependent Diabetes Mellitus (NIDDM):

There is no loss of beta cell mass or only a mild reduction, insulin levels are low, normal, or even high, anti-beta-cell antibodies cannot be seen, there is a high degree of hereditary predisposition, and the disease typically manifests late in life (after

middle age). Over 90 % cases of diabetes are type 2 Diabetes Mellitus. Possible causes include:

1. Abnormalities in beta cells' gluco-receptors

2. A decrease in the number of insulin receptors, or a decline in the sensitivity (relative resistance) of peripheral tissue to insulin

3. Obesity and excess hyperglycemic hormones (glucagon, etc.) lead to a relative insulin deficit.

Other uncommon types of diabetes mellitus include gestational diabetes mellitus (GDM, type 4), other endocrine illnesses, and those caused by unique hereditary diabetes (type 3), such as Maturity Onset Diabetes of Young (MODY). **(**Toshihiro *et al*., 2001)

1.2: Mechanism and Present Drug Therapy of Diabetes

The current approach to treating diabetes focuses on managing and bringing blood glucose levels down to a normal range. Western medications and Chinese traditional treatments both work in the same way to decrease blood sugar:

1. To induce the release of insulin from the beta cell of the pancreatic islet

2. To fend against hormones that raises blood sugar.

3. To enhance the amount of insulin receptor sites' sensitivity and affinity to the hormone.

4. To reduce glycogen leading out.

5. To ensure that the tissue and organs use glucose

6. To reduce free radicals, prevent lipid peroxidation, treat protein and lipid metabolic disorders, and enhance the body's microcirculation. **(**Li *et al*., 2004**;** Tripathi 2008**)**

On the market, there are numerous variations of anti-diabetic medications. These medications can help diabetic people with their blood sugar levels. Orally, these medication classes work well. Through various processes, many drugs are used to

treat diabetes and reduce blood sugar levels. By enhancing insulin secretion, sulfonylureas like Tolbutamide, Glibenclamide, Glipizide, and Glimipride, as well as meglitinides like Repaglinide and Nateglinide, can lower blood sugar levels. By reducing insulin resistance, biguanides like metformin and thiazolidinediones like pioglitazone lower blood sugar levels. Acarbose, miglitol, and voglibose are examples of alpha-glucosidase inhibitors that can postpone carbohydrate absorption and reduce blood sugar levels. However, all of these medications have some undesirable side effects, with hypoglycemia being the most common. As additional major side effects, oral anti-diabetic medications can potentially result in hypoglycemia coma, hypersensitivity, hepatotoxicity, and other conditions (Tripathi 2008). Because they are made from natural sources like plants, animals, or mineral parts, herbal formulations are growing in popularity and importance in the market as a way to avoid these complications of allopathic medications. These formulations are also associated with fewer or almost no side effects and adverse reactions.

1.3: Natural Mechanism from Plant Source Used For Therapy of Diabetes Mellitus

One of the top 12 countries in terms of biodiversity is India. It features a variety of climate zones as well as a flourishing and extensive ethnic medical tradition. Approximately 45,000 plant species and 81,000 animal species have been identified in India to date (Nath *et al.*, 2011).

Drugs have always been easily obtained from plants. Several plants have been employed in the treatment of various ailments and as dietary adjuvant. For generations, people have loved plants all around the world. Some herbs are said to have anti-oxidant activity, cholesterol-lowering effects, and the ability to repair the level of liver glycogen in addition to maintaining a normal blood sugar level (Missoun *et al.*, 2018). According to ethnobotanical literature, there are 800 medicinal plants that may have anti-diabetic potential, and more than 1200 plant species have been randomly selected or examined based on ethnopharmacology for activity. The majority of herbal anti-diabetic medications are classified as phytogenetically advanced substances, which include plants, marine algae, and fungi. (Toshihiro *et al.*, 2001)**.**

Table 1: List of Plants having Anti diabetic Activity

Serial No.	Scientific Name	Family	Common Name	Parts Used
1	*Acacia arabica Lam*. **(**Rajvaidhya *et al*., 1995)	Leguminosae	Indian gum Arabic tree	Seed
2	*Oryza sativa L.***(**Garris *et al.*,2005)	Poaceae	Rice	Roots
3	*Berberis aristata* **(**Komal *et al.*,2011)	Berberidaceae	Daruhaldhi	Stem bark
4	*Aegle* marmelos L.**(** Sabu and Ramadasan , 2004)	Rutaceae	Indian bael	Leaf
5	*Allium cepa L.***(** Augusti 1996)	Amaryllidaceae	Onion	Bulb
6	*Gymnema sylvestre R.***(**Saneja et al., 2010)	Apocynaceae	Gymnema	Whole plant
7	*Anacardium occidentale L.***(**Akinpelu, and Ojewole. 2001)	Anacardiaceae	Cashew	Bark
8	*Amaranthus spinosus L.*(Hussain *et al.*, 2008)	Amaranthaceae	Spiny amaranth	Stem
9	*Anemarrhena asphodeloids B.***(**	Asparagaceae	Zhi Mu	Rhizome

	Tapsell *et al*., 2006)			
10	*Ephedra distachaya L.***(** Kokate *et al.*,2005)	Ephedraceae	Sea-grape	Aerial part
11	*Momordica cochinchinensis L.***(** Aoki et al., 2002)	Cucurbitaceae	Cucurbits	Fruit
12	*Dioscorea dumentorum kunth.***(** Afoakwa *et al.*,2001)	Dioscoreaceae	Bitter yam	Tuber
13	*Musa paradisiacal L.***(** Imam *et al., 2011)*	Musaceae	Edible banana or French plantain	Flower

1.4: Free Radicals and Antioxidants

A free radical is any molecule that has an unpaired electron in an atomic orbital that is capable of supporting itself independently. Since they are unstable, they frequently operate as oxidants or reductants by donating or accepting electrons from other molecules. They damaged the DNA and other components of human cells as a result of their unstable state. One well-known example of a radical is the hydroxyl radical (HO), a compound having one unpaired electron on the oxygen atom. Free radicals and reactive oxygen species (ROS) are common in the environment and are linked to the etiology, progression, and ageing of many diseases. Many of the proteins that free radicals oxidatively alter contain side-chain carbonyl molecules, which can be used as markers for protein oxidation. The side chains of the lysine, arginine, proline, threonine, and glutamic acid residues in proteins react with free radicals such O_2 and H_2O_2 to generate carbonyl derivatives (Moskovitz et al., 2002).

A substance that is stable enough to give an unchecked free radical an electron and neutralize it, reducing the radical's potential for harm, is an antioxidant. In addition

to neutralizing free radicals, antioxidants also serve as hydrogen donors, electron donors, quenchers of singlet oxygen, decomposers of peroxide, and inhibitors of enzymes, synergists, and metal-chelating agents. Antioxidants, both enzymatic and non-enzymatic, can reduce reactive oxygen species (ROS) in the intracellular and extracellular environment. There have been two suggested primary mechanisms of action for antioxidants. The primary antioxidant initiates the first action, which breaks a chain, by donating an electron to the system's free radical. In the second method, secondary antioxidants that initiate reactive nitrogen species are removed using a catalyst that starts a quenching chain.

Numerous physiological and pathological causes give rise to oxygen free radicals and related oxidants. They are produced by conventional cellular processes, even in little quantities. In addition to intracellular and extracellular sources, exogenous variables such photochemical pollution, ozone, pesticides, xenobiotics, and ionising radiation are known to produce free radicals (Halliwell *et al*., 1991). There is compelling evidence that damaging free radical reaction. The majority of problems have their origins in the degenerative illnesses that afflict humanity. Some of these conditions include atherosclerosis, cancer, inflammatory joint disease, asthma, diabetes, senile dementia, and degenerative eye disease. Free radicals may potentially play a role in the biological ageing process. **(**Florence *et al.*, 1995)

Table 2: Some free radical scavengers

Action	**Substance**
Ascorbic acid	Destroys nitrosamines and many free radicals.
Alpha-Tocopherol	Destroys lipid peroxides
Beta-carotene	Destroys singlet oxygen
Catalase	Destroys hydrogen peroxide
Superoxide dismutase	Destroys superoxide radical
Glutathione peroxidase	Destroys hydrogen peroxide and lipid peroxides

2.1: Aims and Objective:

Aims and objectives of the study includes- **Pharmacognostic study of the plant *Mikania micrantha***, Physico-chemical evaluation, **Phytochemical screening of the plant**, TLC studies, HPLC Studies, tincture preparation and characterization, **In vitro anti oxidant activity assay, Evaluation of *in-vitro* ant diabetic activity and *In-vivo* anti diabetic activity evaluation of the leaves extract.**

2.2: Basis of Plant Selection

In Assam and different parts of West Bengal, the *Mikania micrantha* plant has been used to treat diabetes for many years. Ethno medicinal evidences support the use of this plant leaves as anti diabetic agent but its activity has not been fully discovered yet. Therefore, I chose this plant to evaluate whether it has anti-diabetic activity by performing *In-Vitro* and *In-vivo* studies.

2.3: Plan of Work

1. Collection, drying and grinding of leaves
2. Authentication of the plant specimen
3. Pharmacognostic study of the plant *Mikania micrantha*
4. Physico-chemical evaluation of the leaves powder
5. Fluorescence Characterization
6. Extraction of the plant leaves with suitable solvent
7. Phytochemical screening of the leaves extract
8. TLC and HPLC analysis of the extract
9. Preparation and characterization of tincture
10. To carry out *In-vitro* antioxidant study
    i. Determination of DPPH radical scavenging activity
    ii. Determination of Hydrogen peroxide radical scavenging activity
    iii. Determination of Total Phenolic content
    iv. Determination of Total Flavonoid content
11. To carry out *In-vitro* anti-diabetic study
    I.α-amylase inhibition assay
12. To carry out *In-vivo* ant diabetic study

3.1: Synonym:

*Eupatorium denticulatum(*Deori *et al.*,2017*)*

3.1.1: Vernacular Name

English: Chinese creeper, climbing hemp wood

Assamese: Japanilota

Hindi:Titaiyabaur

Telegu: Seemajeelugu

Bengali: Banchhalata , Rabon lota **(**Deori *et al.*,2017**)**

3.2: Taxonomical Classification

Domain: Eukaryota

Kingdom: Plantae

Phylum: Spermatophyta

Sub - phylum: Angiospermae

Class: Dicotyledonae

Sub-class: Asteroideae

Order:Asterales

Family: Asteraceae(Compositae)

Genus: Mikania

Species: micrantha

Botanical Name: *Mikania micrantha* (Ellison *et al.*, 2017)

Figure 1: Plant Photograph

3.3: Morphology

The ribbed stem of *Mikania micrantha* is 6 mm long. The leaves have a heart-shaped base with a pointed tip that is 4 to 13 cm long, and the opposite can be serrated or not**(**Ni *et al*., 2007)When mature, flower clusters form about 4.5 to 6.0 mm long on the bud. The seeds are black, linear, rectangular, approx. 1.5 mm. The seeds have white bristles located on the upper part of the crest **(**Dev *et al.*,2015) Each floret is radially symmetrical and bisexual, with 5 symbiotic petals to flared corolla, 5 stamens, and 5 anthers attached to the stigma, filaments separated, inserted into the bottom of the corolla, and mature stamens protruding outside the corolla. Capital of up to 3-5(Tapsell *et al.*, 2006**;** Li *et al*., 2004 **;** Nath *et al.*, 2011 **)**

3.4: Distribution

The native range off *Mikania micrantha* extends from Mexico to different countries in northern Argentina and the Caribbean. *M. micrantha* was found between latitudes 30 ^{0} N and 30 ^{0} S within its range of introduction. It is all over Asia, from Pakistan to Taiwan, Nepal, the Philippines and Indonesia. It is also found in Sri Lanka and the Indian Ocean. *Mikania micrantha* is implementing an "eradication plan" in Queensland, Australia; Florida is reportedly a noxious weed **(**Ellison *et al.*, 2017).

3.5: Growth Condition

Within its native range, *M. micrantha* was found at sea level at an altitude of 3,000 m. Ramakrishan and Vitousek (1989) reported that *M. micrantha* is found in humid tropical areas where the soil is highly leached in its native and exotic ranges. However, recent studies in China have shown that this plant generally grows in various soils ranging from acidic to alkaline (pH 4.15 - 8.35), where the relative proportion of organic matter is in a wide range **(**Deori *et al.*, 2017**;** Ellison *et al.*, 2017)

3.6: Traditional Uses

Mikania micrantha ia a tropical plant in the Asteraceae family, which is used to heal cuts and stop minor external bleeding in Fiji but its medicinal properties are still yet to be fully discovered. It is also very popular local antiseptic medicine in Bangladesh .*M. micrantha* is traditionally used to treat stomach aches, jaundice, respiratory disease, dysentery and rheumatism. It is also consumed as juice as an alternative medicine for the treatment of diabetes, hypertension and hypercholesterolemia. A

poultice made from the leaves of *M.micranthai*s used to treat venomous biting of insect and juice is used to reduce skin rashes and itches **(**Deori *et al.,* 2017; Dev, *et al.* 2015; Chetia *et al., 2014;* Das *et al.,* 2000; Ibrahim *et al.*,2020)

3.7: Phyto - Chemistry of *Mikania micrantha*

In a comprehensive phyto – chemical analysis, Guo *et. al* (2010) isolated 59 chemical compounds from *M. micrantha* , with the major constituents being steroids (44.58%) , esters (26.22%) ,amides (9.45 %) , heterocyclic compounds (5.34 %) and ethers (4.87 %) . In addition at least 22components of volatile oils have been identified from *M.micrantha* using various extraction techniques with the major compounds being mono and sesqui terpenes, alcohols and ketones and their derivatives **(**Deori *et al.,* 2017).

3.8: Pharmacological Activities of *Mikania micrantha*

Modern pharmacological studies provide scientific evidence that bitter vine possess outstanding therapeutic potencies i.e. anti – microbial , anti – inflammatory , cyto – toxic , anti – cancer , anti – diabetic , anti – oxidant and wound healing activities (Deori *et al.,* 2017; Das *et al.,* 2000; Ibrahim *et al.*,2020).

Literature Review:

The goal of the literature review is to find out different activities reported on *M.micrantha* plant and to check whether it possesses any ant diabetic activity or not.

1. **Jyothilakshmi *et al.*, 2015** investigated the antidermatophytic activity of *Mikania micrantha* from the dried and aerial parts of *M. micrantha*. The dried powdered aerial parts of *M.micrantha* were extracted separately by using three different solvents namely – petroleum ether, ethyl acetate, and methanol. The antidermatophytic activity was determined by the agar tube dilution method against *Epidermophyton floccosumver nigricans, Microsporum canis, Microsporum gypseum, and, Trichophyton rubrum*. Fungicidal efficacy and trypsin inhibiting activity of the whole plant flower, and leaves were also analyzed using the ethyl acetate extracts. The study signifies that *M.micrantha* possesses antidermatophytic activity.

2. **Dev *et al.*, 2015** done the Phytochemical investigation,Antioxidant and Anthelmintic activity of *M.micrantha* leaves. Phytochemical screening of different extracts for the presence of alkaloids, flavanoids, reducing sugars, saponins, phenolic compounds, and tannins, proteins, and amino acids were carried out. The antioxidant properties were investigated using the powered leaves extract extracted using sequential extraction with four different solvents – petroleum ether, chloroform, methanol, and water. The anthelmintic activity was evaluated on adult earthworm *Pheritima posthuma*. The results of the study signify that the plant extract of *M.micrantha* possesses moderate to good antioxidant activity comparable to that of standard drugs BHT, Ascorbic acid, and Gallic acid.

3. **Dou *et al.*, 2014** investigated the antitumor activity of *Mikania micrantha* aqueous extract *in-vitro* and *in-vivo*. In *in-vitro* experiments, two kinds of human cancer cell lines, K562 and Hela were used to test the antitumor activity. The *in-vivo* anti-tumor activity was evaluated by calculating the tumor inhibitory rates, thymus index, and spleen index of S180 bearing mice. This study signifies that *Mikania micrantha* inhibits the activity of K562 and Hela cells *in-vitro* and the growth of S180 sarcoma cells *in-vivo* through multiple mechanisms including inhibition of proliferation, induction of apoptosis, and arrest of the cell cycle.

4. **Biswas *et al.*, 2019** performed the study on biosynthesis, characterization, and antibacterial activity of *Mikania micrantha* leaf extract mediated AgNPS. *In-vitro* antibacterial analysis was carried out by *Bacillus subtilis, E.coli, P.aeruginosa, and Streptococcus pneumonia.* This study signifies that the highest antibacterial activity of AgNPS was observed in *Bacillus subtilis* with a distinct zone of inhibition of 26.17 mm.

5. **Deori *et al.*, 2017** evaluated the anti-inflammatory activity of ethanolic extract of leaves of *Mikania micrantha* on experimental animal models. This study was undertaken to evaluate the anti-inflammatory activity of ethanolic extracts of leaves of *Mikania micrantha* on carrageenan-induced rat paw edema, granuloma pouch method, and adjuvant-induced chronic arthritis on experimental animals. This study signifies that *Mikania micrantha* has significant anti-inflammatory activity.

6. **Yan *et al.*, 2013** evaluated the antimicrobial potential and chemical constituent of *Mikania micrantha* by using leaf extract of this plant. Four standard and two reference bacteria were used in the study. The results of this study demonstrated the broadest range of antimicrobial properties of *M.micrantha.*

7. **Laurella *et al.*, 2012** performed the in vitro evaluation of antiprotozoal, and antiviral activities of extracts from Argentinean Mikania species. In this study antiprotozoal and antiviral activities of four Argentinean Mikania species were investigated. The organic and aqueous extracts of *Mikania micrantha*, *M.parodii,M. periplocifolia,* and, *M.cordifolia*were *tested* on *Trypanosoma cruzi.* epimastigotes,*Leishmaniabraziliensis*promastigotes, and dengue virus type 2.This study signifies that the organic extract of *M.micrantha*was the most active against *T. cruzi and L.braziliensis exhibiting a* growth inhibition of 77.6±4.5 % and 84.9±6.1 % respectively at a concentration of 10 μg/ml.

8. **Matuwali *et al.*, 2016** performed the *in-vitro* evaluation of Antikinase, Antiphosphatase, and cytotoxic activities of *M.micrantha*(Asteraceae) from Malaysia. The leaf sextracts of the plant were used. Both antikinase and antiphosphatase assays targeted protein MKK1, MSG5, and PP1 in mutated yeast strains – MKK1 p386 _ MSG5, PAY 704-1, and PAY 700 – 4 respectively. The result of the study signifies that the crude methanolic extract has been observed as

the only inhibition for PPI screening assay – MTT assay of this plant extract also showed good cytotoxic activity against the HL60 cell line.

9. **Yuhe *et al.,* 2005 *performed*** a study on the immunological activity of the secondary metabolite in *Mikania micrantha.* The influences of the secondary metabolite on the immune function of the mouse were studied by breeding mice with water-soluble and fat-soluble extracts of *M.micranthaa*nd the changes of T lymphocyte subsets of mice were analyzed by flow cytometry. The results showed that both the water-soluble and fat-soluble extract could stimulate the production of CD – (4) ~ (+) and CD – (8) ~ (+) in mice as compared with control, the ratio of CD-(4) ~ (+) / CD – (8) ~ (+) increased from 0.68 to 1.47. The water-soluble extract had a more significant effect on T lymphocyte changes of the mouse than fat-soluble extracts in *Mikania micrantha.* It indicates that some secondary metabolites can stimulate the immunological function of the mouse and enhances the anticancer ability in *Mikania micrantha.*

10. **Ibrahim *et al.,* 2020** performed a study named "*Mikania micrantha* extracts inhibits HMG CoA Reductase and ACAT2 Ameliorates hypercholesterolemia and lipid peroxidation in high cholesterol feed rats." The study was aimed to determine the effect of ethyl acetate extracts of *Mikania micrantha* stems in hypercholesterolemia induced rats. This study signifies that *M.micrantha* stem extracts exhibited anti hypercholesterolemia properties by improving lipid profile, enzyme inhibitory, reducing lipid peroxidation, and lipid accumulation in combating hypercholesterolemia.

11. **Andriani *et al.,* 2019** performed a study on "Mikania *micrantha,* improved memory performs on dementia model". The Dementia model is formed by diabetic test animals. The test group consisted of extract group, three fractions (hexane, ethyl acetate, and butanol fraction at dose 500 mg/kg) and one group of antidiabetic drug Glibenclamide, and the group of normal animal and test animals were tested with radial arm maze (RAM) for seven days before alloxan-induced to obtain dementia model. The results showed that extract and ethyl acetate fraction at dose 500 mg/kg gave a positive effect on memory improvement based on animal performance on RAM tool during a testing time ($P< 0.05$) With LSD statistical analysis.

12**. Ittiyavirah and Sajid 2014** evaluated the adaptogenic activity of methanolic and aqueous extract of roots of *Mikania micrantha kunth* in wistar albino rats using different models such as Anoxia stress tolerance, determination of organ weight were carried out in immobilization stress model. These activities are tested at oral doses of extract at 250 and 500mg/kg and Diazepam 2mg/kg was used for comparison. There were dose dependent significant reduction in biochemical parameters like serum glucose, cholesterol and BUN levels exhibited by alcoholic extract. The stress induced increase in liver; adrenal gland weight and decrease in weight of spleen were significantly reversed by the methanolic extract at higher dose. The results from the study indicated that methanolic extract of *Mikania micrantha* roots possessed significant antistress activity.

13. **Saikia *et al.*, 2020** studied the applicability of *M.micrantha* flower essential oil against disease causing microorganisms and inhibition of cancerous cells. Sixty-six compounds were characterized from the flower essential oil by gas chromatography. Well-diffusion and resazurin assay were conducted to determine antimicrobial activity and Minimum Inhibitory Concentration (MIC) of essential oil. Flower oil showed best activity against *Mycobacterium smegmatis* and *Candida albicans* with MIC 8 µg/ml. Through MTT (3-(4, 5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, they found that oil has excellent cytotoxicity against HeLa cervical cancer cell lines(IC_{50} 5.44±1.33 µg/ml).

14. **Dong *et al.*, 2023** evaluated four new germacrane sesquiterpene dilactones, 2*β*-hydroxyl-11*β*,13-dihydrodeoxymikanolide(1),3*β*-hydroxyl-11*β*,13-dihydrodeoxymikanolide(2),1*α*,3*β*-dihydroxy-4,9-germacradiene-12,8:15,6-diolide(3) , and (11*β*,13-dihydrodeoxymikanolide-13-yl)-adenine(4) isolated from the aerial parts of *Mikania micrantha* for their in vitro antibacterial activity against four Gram-(+) bacteria of *Staphyloccocus aureus* (*SA*), methicillin-resistant *Staphylococcus aureus* (*MRSA*), *Bacillus cereus* (*BC*) and *Curtobacterium. flaccumfaciens* (*CF*), and three Gram-(–) bacteria of *Escherichia coli* (*EC*), *Salmonella. typhimurium* (*SA*), and *Pseudomonas Solanacearum* (*PS*). Compound **4** was found to show strong in vitro antibacterial activity toward all the tested bacteria with the MIC values ranging from 1.56 to 12.5µg/ml. Notably, compound **4** also showed significant antibacterial activity against the drug-resistant

bacterium of *MRSA* with MIC value 6.25 µg/ml, which was close to reference compound vancomycin (MIC 3.125 µg/ml). Compound **4** further revealed to show in vitro cytotoxic activity toward human tumor A549, HepG2, MCF-7, and HeLa cell lines, with IC_{50} values ranging from 8.97 to 27.39 µM. The present research provided new data to support that *M. micrantha* is rich in structurally diverse bioactive compounds worthy of further development for pharmaceutical applications and for crop protection in agricultural fields.

15. **Lallianchhunga *et al.*, 2015** evaluated the antioxidant activity of the leaves of *M.micrantha*. The The DPPH, FRAPand total Phenolic content of the leaves were 31.97±1.03 mg TE, 18.47±1.15 mg TE and 4.63±0.37 mg GAE/gm dry leaves respectively. This reveals that the antioxidant content of the plant is high and therefore it can scavenge free radicals and reactive oxygen species that are responsible for number of human disorders.

16. **Sumantri and Mustanti *et al.*, 2021** determined the wound healing of nanogels containing *M.micrantha* leaves extract in hyperglycemic rats as a model for diabetic wounds. *Mikania micrantha* leaves were extracted with the maceration method using 96% ethanol in 5 days. Carbopol 940 was used as the gelling agent. Antibacterial activity was tested on *Staphylococcus aureus*, *Staphylococcus epidermis*, and *Escherichia coli*. Moreover, wound healing activity was tested in hyperglycemic rats after observing for 14 days. Diabetic wound healing was treated with 4 groups (P1, P2, K1, K2) where P1was Diabetic experimental rats treated with MMEL gels, P2 was Diabetic experimental rats treated with MMEL nanogels, K1was Diabetic experimental rats treated with MMEL cutimed® gel, and K2 was Diabetic experimental rats treated with gel base. Data were analyzed using IBM SPSS Stastistics 22 software.Nanogel showed homogeneity, dark green color, transparency, pH 6.1± 0.1, and particle size range in 255-456 nm. The inhibition zones of antibacterial testing, *i.e.*, *Staphylococcus aureus*, *Staphylococcus epidermis*, and *Escherichia coli*, were 10.57 ± 0.26 mm, 9.73 ± 0.21 mm, and 8.4 ± 0.1 mm. The percentage of diabetic wound healing was in the range of 92.79±3.81% to 94.08 ± 2.33% for 14 days of observation.It was observed from this study that *M.micranthal*eaves extract nanogels have the potential as a treatment for diabetic wound healing.

17. **Colares *et al.,* 2013** studied the Antispasmodic effects of Mikania micrantha Kunth and dual gastrointestinal effect of *Mikania cordifolia* (L.F.) Willd (asteraceae) on isolated rat thin intestine. The aqueous extract of*M.micrantha* was prepared by decoction at 20% and lyophilized) were added to isolated rat intestines, before evoking contraction by dose-response curves (DRC) of acethylcholine (ACh). The aqueous extract of *M.micrantha* inhibited in a non-competitive way the ACh-DRC (IC50: 0.54±0.05 mg/ml), as well as the Calcium-DRC (Ca-DRC) in high-[K+].The preliminary phytochemical tests showed the presence of flavonoids in aqueous extract of *M.micrantha* .The TLC detected flavonoids (with EthylAcetate/MeOH/H2O 100:13:10 and EthylAcetate/FormicAcid/ AcOH/H2O 100:11:11:26) and alkaloids (with tholuene/AcOEt/diethylamine 70:20:10). Results validate the popular use of *M.micrantha* as eupeptic, with antispasmodic effect associated to a non-competitive Ca2+-influx blockade typical of flavonoids.

18. **But *et al.,* 2009** evaluated the Antiviral Constituents against Respiratory Viruses from *Mikania micrantha*. Phytochemical investigation of the dried aerial parts of *Mikania micrantha* led to the isolation of a new sesquiterpene, 3β-acetoxy-1,10-epoxy-4-germacrene-12,8;15,6-diolide (1), along with six known constituents: 1,10-epoxy-4-germacrene-12,8;15,6-diolide (2), dihydromikanolide (3), potassium mikanin 3-sulfate (4), mikanin (5), alpinetin (6), and ergosta-7,22-dien-3β-ol (7). Compound 2 showed moderate activity against respiratory syncytial virus (IC50 = 37.4 uM) and parainfluenza type 3 virus (IC50 = 37.4 uM) with a therapeutic index (TI) of 16.0 for both compounds. Compound 4, the main component of *M. micrantha*, exhibited inhibitory activity against Para influenza type 3 virus with IC50 (19.7 uM) and TI (24.0) values comparable to those of ribavirin, serving as a positive control. This study showed that *M.micrantha* possesses notable antiviral activity.

19. **Dasgupta *et al.,* 2014** evaluated the Anticancer Activity of *Mikania micrantha* Kunth (Asteraceae) Against Ehrlich Ascites Carcinoma in Swiss Albino Mice. The n-butanolic extract of *M. micrantha* (BEMM) containing flavonoids were selected for anticancer activity against EAC cell line in Swiss albino mice. The BEMM was assessed by evaluating tumor volume, viable and nonviable tumor cell count, tumor weight and hematological parameters of EAC bearing animals. The n-butanolic

extracts were used at the doses of 250, 500 and 1000 mg/kg/day p.o (per oral). Administration of BEMM 500 and 1000 mg/kg/day significantly decreased the tumor volume (3.23 ± 0.20ml and 4.02 ± 0.36ml), increased the life span (58.81% and 54.37%) and significantly decreased tumor mass (1.92 ± 0.067g) as compared with control. The results showed that the extract possessess dose dependent anticancer activity.

20**. Sarimah and Mizaton *et al.*, 2018** investigated the wound healing activity of *Mikania micrantha* ethanolic leaf extract.Cell viability and scratch assay were carried out in BJ fibroblast cells treated with various concentrations of the extract. Trolox (100 ÂµM) was used as positive control. Results on the MTT assay showed low cytotoxic effect after 24, 48 and 72 h incubation (IC50 > 150 Âµg/ml). Wound healing process was significantly (P Â£ 0.005) accelerated after treatment with *Mikania micrantha* ethanolic leaf extract (7 and 15 Âµg/ml). From the study, it can be concluded that *Mikania micrantha* ethanolic leaf extract has great potential in accelerating the wound healing process.

5.1: Collection and authentication of the plant component

The leaves of the plant *Mikania micrantha* kunth (Asteraceae) were collected in the month of November of 2022 from Jadavpur, West Bengal. A specimen of the plant was deposited in the Central National Herbarium at the Botanical Survey of India, Shibpur, Howrah (CNH/Tech.II/2O23/45) for identification and authentication. The leaves were collected, thoroughly washed with water, and then dried for a month under shade. The dried leaves were then mechanically ground into a powder and placed in an airtight container for later usage.

5.2: Morphological and Organoleptic characters

The fresh leaves of the *Mikania micrantha* were collected and subjected for evaluation of morphological characteristics like colour, size, shape, and other macroscopic characteristics. Other organoleptic characteristics including taste, odour and texture were also evaluated.

5.3: Microscopic characteristics

Microscopic characterization of the leaves was carried out by cutting thin transverse sections of the lamina of the fresh leaves. The sections were mounted on a glass slide with the help of glycerin and covered with cover slips. The sections were observed under compound microscope with 10X magnifications to study the internal anatomical characteristics of the leaves. In the next step, dried leaves were converted into coarse powder with the help of a mortar and pestle and passed through a sieve to separate the coarse powder from larger fragments. Then a pinch of the powder was placed on a glass slide and mounted with glycerin. The glass slide was covered with cover slip and observed under compound microscope with 10X magnification for powder characteristics **(**Khan *et al.*,2013;**)**.

5.4: Physicochemical assessment (Kokate *et al.,* 2005; Mukherjee 2002; Mandal *et al.,*1999; Mandal *et al.,* 2015; Khandelwal 2006)

5.4.1: Loss on Drying (LOD)

2g of the powdered leaves were placed in a shallow, dried weighing bottle. The sample bed's height was kept 10mm or less. The sample was then dried in a hot air oven at 105° C for 30 minutes. Then, the shallow weighing bottle was taken out and cooled in a desiccator, and weighed at room temperature. The procedure was repeated until the difference between two successive weights was less than 0.5mg. The LOD was determined as percentage of w/w.

5.4.2: Ash Value

Total Ash Value

2g of the powdered leaf sample was weighed accurately and placed in a silica crucible which had been weighed previously. The crucible was placed in a muffle furnace and incinerated at 450°C until the sample was free from carbon. After the incineration is complete, the crucible is taken out from the muffle furnace and cooled in a desiccator. Once cooled, the weight of the crucible was recorded at room temperature in a careful way, so that the sample does not absorb moisture from the surrounding air. The total ash was calculated as the percentage (w/w) of ash generated from the leaf sample after incineration.

Water Soluble Ash Value

After obtaining the total ash residue from the crucible, it was carefully placed in a beaker with 25ml distilled water. The beaker was then placed on a water bath and the contents were boiled for 25 minutes. After 25 minutes, the mixture was cooled and filtered using an ash less filter paper (Whatman filter paper 42). Upon completion of the filtration, the residue on the filter paper was thoroughly washed with hot water and placed in a silica crucible which had been weighed previously. The crucible was placed in a muffle furnace and then incinerated at 450°C till no carbon residue of the filter paper was left in the crucible. The weight of the residue

in the crucible was carefully recorded and subtracted from the weight of the total ash to calculate the fraction of ash that had been dissolved in water. The value of water soluble ash was calculated as percentage (w/w) of total ash that got dissolved in distilled water upon boiling.

Acid Insoluble Ash

Total ash from the previous study was placed in a beaker and 25ml of 2N hydrochloric acid was added to the beaker. The contents of the beaker were then boiled on a hot plate for 5 minutes. After boiling, the contents of the beaker were cooled and filtered using ash less filter paper. The residue on the filter paper along with the filter paper was carefully taken in a previously weighed silica crucible and incinerated at 450°C for 15 minutes. The incinerated residue was weighed carefully to record the amount of ash that remained insoluble in the acid upon boiling. The calculations for the acid insoluble ash percentage (w/w) were made using dried sample data.

5.4.3: Extractive Value

Value of Water Soluble Extractive

In a closed conical flask, 5g of coarsely powdered leaves were macerated with 100 ml of water for 24 hours. The flask was shaken frequently for the first 6 hours before being left to stand for 18 hours. After 24 hours, the mixture was filtered and 25 ml of the filtrate was then measured, and evaporated in a small porcelain plate. The residue on the porcelain plate was weighed and recorded. With reference to the dried sample, the percentage (w/w) of water soluble extractive value was calculated.

Alcohol (Ethanol) Soluble Extractive Value

In a closed conical flask, 5g of coarsely powdered leaves were macerated with 100 ml of ethanol for 24 hours. Alcohol soluble extractive value was determined in the same way as water soluble extractive value.

5.4.4: Analysis of Crude Fiber

200ml of 1.25% (v/v) sulfuric acid and 2g of the dried leaf powder were heated together with constant stirring. After heating, the mixture was filtered using a filter paper and the filter paper was washed with hot water for few times. After that, 200ml of a solution containing 1.25% (w/v) sodium hydroxide was added to the filtrate, and it was heated for 30 minutes. The mixture was filtered after being rinsed in hot water and the filtrate was then burned at 110° C to a constant weight. With reference to the dried sample, the crude fiber percentage was computed.

5.4.5: Swelling Index

A 100 ml measuring cylinder was filled with 1g of dried powder and 25ml of water. For an hour, the cylinder was shaken every ten minutes. The measuring cylinder was then kept for 3 hours. Change in volume of the mixture was recorded to calculate swelling index of the leaves.

5.4.6: Foaming Index.

1g of the dried sample was added to a conical flask that held 500 ml of boiling water. It was then filtered into a volumetric flask after cooling. Water was added till the content reached 100 ml. Obtained and labeled ten test tubes with stoppers. The drug was added in successive sections of 1, 2ml up to 10 ml into different tubes, and the remaining volume was adjusted with water up to 10ml. The tubes were sealed with stoppers, shaken for 15 seconds, allowed to stand for 15 seconds, and then height of the foam from each tube was measured.

The foaming index is less than 100 if each tub's foam is less than 1cm in height. The dried sample solution decoction volume in this tube (a) is used to calculate the index if the height of foam of 1 cm is measured in the first through tenth tubes. To get a more accurate result, make an intermediate dilution if this tube is the first or second in a series. The foaming index exceeds 1000 if the height of the foam in the first through tenth tubes is greater than 1 cm.

Foaming index =1000/a

Where, a=the amount of decoction in milliliters that was used to prepare the dilution in the tube where foaming to a height of one centimeter is seen.

5.5: Fluorescence analysis of the leaf powder (Mandal *et al.*, 1996)

After being treated with various chemical agents, the color of the leaf powder was observed. Observations of the fluorescence properties of the leaf powders treated with various chemicals and reagents were made under both short-wavelength (254 nm) and long-wavelength (366 nm) ultra violet light.

5.6: Preparation of extracts

The following solvents, in order of increasing polarity, were used to extract the powdered leaves in a soxhlet extractor; petroleum ether (boiling point 60-80°C) and methanol (boiling point 64°C). Using petroleum ether, the powdered plant material (50g) was first extracted. The extraction procedure was carried out until all the contents had been removed, and this was verified by removing a sample from the soxhlet extractor's syphon tube. It was then evaluated by TLC using the appropriate solvent that had been used for the extraction procedure. The absence of a spot on the TLC plate in the iodine chamber served as a sign that the extraction process has been completed. The complete assembly of the soxhlet extractor has been depicted in Figure 2. The extract was then concentrated by vacuum distillation, dried in the open air, and stored in an airtight container. The marc was dried by air before undergoing a second methanol extraction process. The extracts that were produced as a result of each extraction procedure were kept in airtight containers.

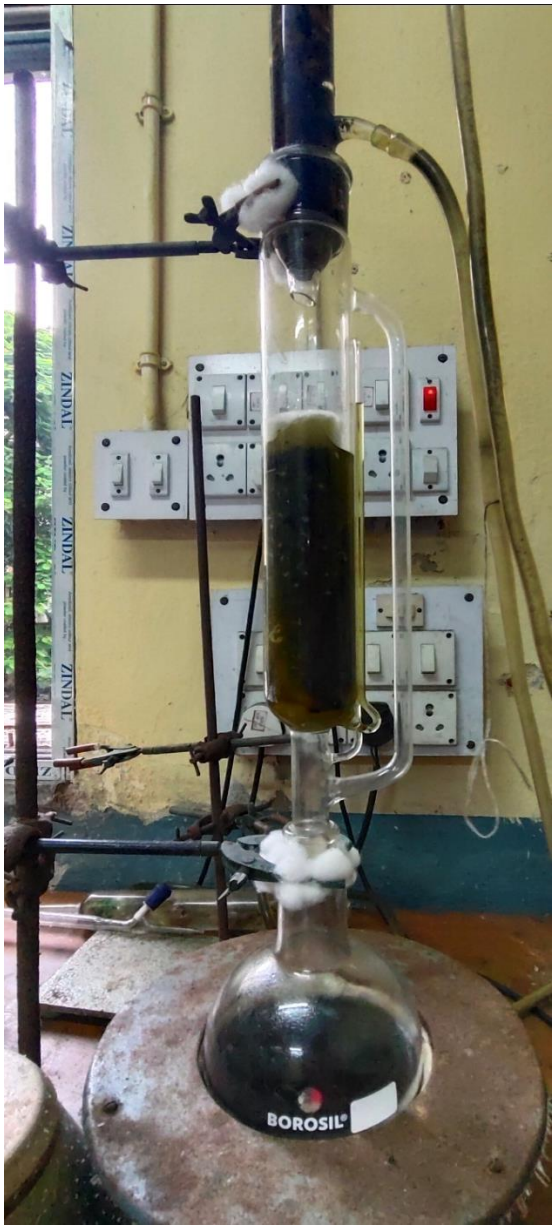


Figure 2: Soxhlet extractor assembly

5.7: Screening for phyto-chemicals in the extract (Mandal *et al.,* 2015; Banerjee *et al.,* 2014)

To identify the presence of various phytocomponents, different chemical analyses were run-on the two drugs extract, petroleum ether extract and methanolic extract.

Test for Carbohydrates

Molisch Test (general)

A few drops of the Molisch reagent were applied to 2–3 ml of the extract.

Sulphuric acid in the amount of two ml was introduced from the test tube's side. Carbohydrates are present when a purple ring forms at the intersection of two liquids.

Test for Reducing Sugars

Fehling's Test

Boiling for one minute after combining 1ml each of Fehling's solutions A and B, equal volume of the test solution was added. Heated for five to ten minutes in a bath of boiling water. When reducing sugars are present, first yellow, then brick red ppt are seen.

Benedict's test

In a test tube, combine Benedict's reagent and test solution in an equal volume. 5 minutes of heating in a pot of boiling water. The colour of the solution depends on how much reducing sugar is present in the test solution; it might be green, yellow, or red.

Test for Monosaccharides

Barfoed's Test

Combine Barfoed's reagent and test solution in an equal volume; heated in a bath of boiling water for one to two minutes. Red ppt is formed if monosaccharide's is present.

For Non-Reducing Sugar

Test solution does not show positive results for Fehling's and Benedict's test.

Test for Proteins and Amino Acids

Biuret Test (general test)

1 ml of biuret reagent was added to 2ml of test solution (extract). Protein is present when the colour is violet.

Ninhydrin Test (general test)

2ml of test solution and 2 drops of 5% Ninhydrin solution were heated for 10 minutes in a boiling water bath. The test solution turns purple or bluish when amino acids are present.

Millon's Test

Combine 5ml of Millon’s reagent with 3ml of test solution. White ppt is formed which turns brick Red after warming if protein is present.

Test for Steroids

Salkowski's Test

Add 2 ml of chloroform and 2 ml of conc. Sulphuric acid to 2 ml of extract and thoroughly shaken. The appearance of a reddish-brown tint in the bottom layer is a sign of steroids.

Liebermann-Burchard Test

Acetic anhydride was used to first treat the extract solution before a few drops of strong sulfuric acid were added along the test tube's side. Green coloration is a sign of the presence of steroids.

Test for terpenoids

Noller's Test

Noller's reagent, which contains0.1% stannic chloride in thionyl chloride, was applied to the extract solution. Terpenoids are present when red hue appears, indicating their existence.

Test for Glycosides

For Cardiac Glycosides

(For cardenolides)Legal's test

1ml pyridine and 1ml sodium nitroprusside were added to the extract. Glycosides are indicated by the colour pink.

Keller-Killiani test (for deoxy-sugars)

Glacial acetic acid, one drop of 5% FeCl3, and sulfuric acid were added to the 2ml of extract. If glycosides are present, a reddish brown colour occurs at the junction of the two liquid layers, while the upper layer appears bluish green.

Test for anthraquinone glycosides

Borntrager's test

Diluted sulfuric acid was added to 3ml of extract. Filtered and boiled. Chloroform

was added in an equal amount to the cold filtrate. Firmly shaken. Make the organic solvent separate. Added ammonia. Anthraquinone glycosides cause the ammonical layer to turn pink or crimson.

Modified Borntrager's test

5% $FeCl_3$ and 5 ml of diluted HCl were added to 5 ml of extract. Heated in a bath of boiling water for five minutes. Chloroform was added after cooling. Firmly shaken. Poured an equal proportion of diluted ammonia to the separated organic layer. The coloration of the ammoniacal layer is pinkish red.

Test for Saponins

Shake the water and powdered extract vigorously.Saponin is detected when there is persistent froth.

Test for Flavonoids

Shinoda test

5 ml 95% ethanol and a few drops of conc. HCl were added to the dried extract. 0.5 gm of magnesium turnings was added to it. Flavonoid's give off an orange, pink, red, or purple tint when they are present.

Ferric Chloride Test

Added a few drops of 10% $FeCl_3$ to the extract. It emits a green tint to show flavonoids are present.

S**odium hydroxide Test**

Solution of sodium hydroxide was added to the extract. The presence of flavonoids is shown by the appearance of yellow hue, which is destroyed by the addition of acid.

Test for Alkaloids

Mayer's test

Mayer's reagent in a few drops with 2-3ml of filtrate results in a cream-colored ppt.

Dragendorff's Test

Add a few drops of Dragendorff's reagent to 2 to 3ml of filtrate. Orange and brown ppt. is created

Hager's test

With Hager's reagent, 2-3ml of filtrate produces yellow ppt.

Wagner's test

With Wagner's reagent, 2–3ml of filtrate yields reddish brown ppt.

Test for Tannins and Phenolics

$FeCl_3$ test

Dark green or deep blue colouring after treatment with 5% Fecl3 solution suggests the presence of tannin and phenolics.

Gelatin Test

10% sodium chloride and 1% gelatin solution were added to the test solution. Precipitate formation suggests the presence of tannins.

10% NaOH test

When 4ml of 10% NaOH solution is added to the extract solution and it is thoroughly agitated, an emulsion forms that shows tannins and phenolics are present.

5.8: Chromatographic analysis of the prepared extracts

Thin layer chromatographic (TLC) analysis was performed on both the extracts prepared from the powdered leaves of the plant.

Preparation of a sample:

Dried petroleum ether and methanol extracts, each weighing 1g, were diluted in an adequate quantity of the extraction solvents, petroleum ether and methanol, and then utilized as samples for TLC analysis.

Stationary phase

Pre-coated Silica gel G was utilized as stationary phase for performing TLC analyses of the extracts.

Mobile phase

For methanol extract, the mobile phase was used as hexane: chloroform: methanol (6:2.5:1.5) while for the petroleum ether extract, petroleum ether: acetone (8.5:1.5), was used as mobile phase.

Observation

Retention factor (R_f) value for each of the spots was determined after the spots were observed in an UV chamber at wavelengths of 254 nm and 366 nm.

5.9: High Performance Liquid Chromatography

This technique employs a liquid mobile phase and a very finely divided stationary phase for separation. In order to obtain satisfactory flow rates, the mobile phase is pressurized to several hundred pounds per square inch or more. The solution of the methanolic extract *M. micrantha* was scanned in the UV region of 260nm, 280nm and 310nm and the UV spectrum was recorded.

Initial Separation Conditions

1. Column for separation : Acclaim C18 (250mm x 4.6mm, 5µm)
2. Instrument: Dionex Ultimate 3000 (Thermo Sci. USA)
3. Detector: Photo Diode Array (PDA)
4. Pump: Quaternary system LPG 3400 SD
5. Injection volume: 20µl (loop 20µl)
6. Detection wavelength: 260nm, 280nm, 310nm
7. Flow rate: 1ml/min
8. Mobile phase: Methanol (Solvent A) , 0.5% aqueous acetic acid in water (Solvent B)
9. Gradient Programme: The gradient elusion was 90 % solvent B and 10% solvent A and flow rate was settled from 1ml/min to 0.7 ml/min in 27 min, from 10 to 40 % solvent A with flow rate 0.7 ml/min for 23 min, 40% solvent A and 60% B with flow rate 0.7 ml/min primarily for 2 min and then flow rate altered from 0.7 to 0.3 ml/min in 65min, from 40 to 44% solvent A with flow rate 0.3 to 0.7ml/min in 70 min, 44% solvent A with flow rate 0.7

to 1ml/min for 10 min duration, solvent A changed from 44% to 58 % with flow rate 1ml/min for 5 min, 58 to 70% solvent A in 98 min at constant flow rate 1 ml/min . Then 10% A in 101min and equilibrated for 4min for next injection.

10. Data acquisition software: Chromeleon 6.8

11. Identification and quantization: External standard method. Reference standards protocatechuic acid, gentisic acid, chlorogenic acid, p-hydroxy benzoic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, sinapic acid, salicylic acid, gallic acid and ellagic acid, catechin, rutin, myricetin, quercetin, naringin, apigenin and kaempferol were used for identification. Standard curves were performed for quantization of each component.

5.10: Preparation and characterization of tincture (Mandal *et al.*, 1996)

A total of 21 amber-colored bottles with 10 ml capacity were used. To these, 10 ml of alcohol with varied strengths—40, 50, 60, 70, 80, and 90 percent (V/V) and absolute alcohol were added. Each of the twenty-one bottles contained 1g of *Mikania micrantha* leaves powder. Each batch was allowed to macerate for a specific number of days: 7, 14, and 21. The materials were filtered through a sintered glass crucible (G4) while under reduced pressure after the required amount of maceration time. Physical characteristics were investigated, including colour, pH, specific gravity, and total solid content.

Figure 3: **Tinctures of different alcohol concentrations after maceration for differing time period**

5.11: *In-vitro* antioxidant studies

The word antioxidant simply means "against oxidation". An antioxidant is any substance that retards or prevents the deterioration, damage, or destruction of cells by oxidation **(**Mandal *et al.,* 2009;Dekkers *et al.,*1996) It is a classification of a number of organic compounds, including selenium, the carotenoids chemical family, vitamin A (which is made from beta carotene), and the vitamins C and E.

5.11.1: Determination of Total Phenolic content

The total phenolic content of the sample was determined by the Folin-Ciocalteau method **(**Miliauskas *et al.,* 2004) with slight modifications. About 1 ml of the extract solution was mixed with 5 ml of Folin-Ciocalteau reagent (FCR). After 3 min, 4 ml of sodium carbonate solution was added and the mixture was allowed to stand for 1 h at room temperature followed by measuring the absorbance at 765 nm. From the calibration curve of Gallic acid prepared in different concentrations (20, 40, 60, 80 and 100µg/ml). The amount of phenolic compounds was determined and expressed as mg Gallic acid equivalent (GAE) of dried or fresh extract exudates. The total phenolic content in the extract expressed in Gallic acid equivalents (GAE) was calculated by the following formula:

T=C X V / M

Where, T- Total phenolic contents in mg/g plant extract, in Gallic Acid Equivalent(GAE)C-Concentration (mg/ml) of Gallic acid obtained from the calibration curve=Volume of extract (ml),

M=Weight (mg) of plant extract.

5.11.2: Determination of Total Flavonoid content

Total soluble flavonoid content of the fractions was determined with aluminum chloride using quercetin as a standard **(**Halliwell and Gutteridge 1985) with slight modifications. To 1ml of each different concentration (20, 40, 60, 80,100µg/ml) of quercetin, 2ml of methanol was added. Then it was mixed with 0.2 ml of aluminum chloride and 0.2 ml of potassium acetate and finally 5.6 ml of distilled water was added to each concentration. Extract solution of different concentration was also prepared using the same method. All the samples were incubated for 30 minutes at room temperature and absorbance was measured at 415 nm against

control. The total flavonoid content in the fractions was determined as µg quercetin equivalent by using the standard quercetin graph and using the following formula:

$$T = C \times V / M$$

Where, T-Total flavonoid content in mg/g plant extract, in Quercetin Equivalent (QE), C=Concentration (mg/ml) of Quercetin obtained from calibration curve,
V= Volume of extract (ml),

M= Weight (mg) of plant extract.

5.11.3: Determination of DPPH radical scavenging activity

Minor adjustments were made to an earlier approach (Blois 1958) of DPPH activity while using Ascorbic acid as the Standard for comparison. In order to create a series of sample solutions with various pre-set concentrations (20, 40, 60, 80,100 µg/ml) the extract was dissolved in methanol. 2ml of the extract sample solution were combined with 1ml of a 0.3mM DPPH solution in methanol, and the mixture was then incubated for 30min at37°C. A UV-vis spectrophotometer was used to detect absorbance at 517 nm. The same method was applied to standard solutions with comparable concentrations. The ability of the sample/standard to scavenge the DPPH radical was determined as the free radical inhibition percentage using the following formula:

% Inhibition of DPPH scavenging activity =Ac - At/ Ac X 100

Where Ac is the absorbance of the control reaction and At is the absorbance of the test/standard. The extract's antioxidant activity was quantified as IC_{50}. The IC_{50} values were computed using linear regression of plots, where the ordinate represents the average percent of scavenging capacity and the abscissa indicates the concentration of the studied plant extracts. The IC_{50} value is defined as the concentration (in µg/ml) of extract that inhibits the formation of DPPH radical's by 50%.

5.11.4: Determination of hydrogen peroxide radical scavenging activity:

The ability of both *M. micrantha* extracts to scavenge hydrogen peroxide was determined according to the method of Oktay Munir and others**(**Oktay *et al.*, 2003)In phosphate buffer with a pH of 7.4, hydrogen peroxide (2m mol/L) was produced as a solution. Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm with molar absorbtivity81 mol/L-1 /cm. Extracts samples (100–600µg/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml). After 10minutes, the absorbance of hydrogen peroxide at 230 nm was measured in comparison to a blank solution made up of phosphate buffer and no hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of both *M.micrantha* extract and standard compounds (Ascorbic acid) can be calculated using the following formula-

%H_2O_2 radical Scavenging activity =Ac At/Ac X 100

Where Ac is the absorbance of the control reaction and At is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value is defined as the concentration (µg/ml) of dry extract that inhibits the formation of H_2O_2 radicals by 50%.

5.12: In- vitro anti diabetic activity **(**Telagari and Hullatti 2015; Velmani and Mandal 2016)

5.12.1: Inhibition *of α Amylase*

1ml of phosphate buffer was added in 5 different test tubes. To these, 0.2 ml alpha amylase solution was added. To the above mixture 0.4 ml of standard drug solution was added .The above mixture from each of the test tubes were incubated at 37^0 C for 20 minutes. To this incubated mixture of each test tube, 0.4 ml of starch solution was added and the mixture was then again incubated for 30 minutes at a temperature of 37 ^{0} C in a water bath. After 30 minutes, 2 ml of DNS solution was added to each test tubes and boiled for 10 minutes. After that the absorbance of the sample was measured at 540nm.The measurement of absorbance of control was conducted using similar method and by replacing standard drug solution with vehicle. Same method was used to measure the absorbance of test sample by using extract solution in place of standard drug solution. The concentration of the plant

extract required to scavenge 50% of the radicals (%inhibition) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by-

% Inhibition = Abs Control – Abs Test / Abs Control X 100

The IC_{50} values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha amylase inhibitor. All tests were performed in triplicate .All the measurements were done in triplicate and results are expressed in terms of Mean±standard deviation and IC_{50} values were calculated using MS-Excel software.

5.13: Acute toxicity study:

The acute toxicity study was already performed by Ittiyavirah *et. al*., LD_{50} values of the methanolic extract of *M.micrantha* was found to be safe up to 2000mg/kg body weight by oral route **(**Ittiyavirah and Sajid 2014)

5.14: *In-vivo* anti diabetic studies

5.14.1: Preparation of 0.1M sodium citrate buffer solution (4.5pH):

To prepare 100ml of 0.1M citrate buffer mixed 270.278 Mg of Sodium citrate dehydrate and 207.7 mg of citric acid to 80 ml of distilled water. The solution was adjusted to desired p H using HCl or NaOH. The final volume was made up to 100 ml with distilled water. 44(Gomori 1955)

5.14.2: Preparation of Streptozotocin solution

A solution of STZ was prepared by dissolving the weighed quantity of streptozotocin in 0.1M freshly prepared ice-cold citrate buffer (pH 4.5) solution.

5.14.3: Preparation of 5% Dextrose solution

5gm of dextrose is weighed and dissolved in 100ml of distilled water.

5.14.4: Preparation of Standard (Metformin HCl) solution :(Dose: 50mg/kg)

50 mg of Metformin HCl is dissolved in 1ml of distilled water (Mandal *et al*., 1997; Chakraborty *et al*., 2018)

5.14.5: Preparation of test solution:

Suspension of methanolic extract of *Mikania micrantha* was prepared by using 2% aqueous Tween 80.

5.14.6: Housing of animals

Wistar albino rats weighing 150-200g used for all experiments studied. Animal experiment protocol (**JU/IAEC-22/37**) was duly approved by Institutional Animal Ethics Committee (IAEC), Depertment of Pharmaceutical Technology, Jadavpur University; Kolkata-700032. Animals collected randomly from State centre for Laboratory Animal Breeding West Bengal Livestock Development Corporation Limited, Buddhapark, and Kalyani, Nadia, and PIN-741235 separate cage under controlled conditions of temperature (22 ± 2°C). All animals given standard diet (pellet feed) and water add libitum. Animals kept at light/dark cycle of 12 hrs. Animals divided in five groups with six animals in each group. Institutional Animal Ethics Committee (Regd. No 1805/CPCSEA) approved the research protocol and all experimental procedures followed in the study.

5.14.7: Streptozotocin induced hyperglycemia (Mandal *et al*., 1997; Chakraborty *et al*., 2018)

The acute experimental hyperglycemia produced in rats by the single dose of streptozotocin(STZ) injection (40 mg/kg b.w.i.p) .The selected animals, weighing between 150-200g fasted overnight were administered with Streptozotocin (40 mg/kg b.w) intraperitoneal (1.0 ml/100 g). Fasting blood sugar levels were determined on 5th day after administering STZ to confirm stable hyperglycemia. The diabetic rats after confirmation of stable hyperglycemia divided into different groups of 6 rats each. That day considered as the 0^{th} day. Metformin hydrochloride at a dose of 50mg/kg b.w.used as a standard drug for treatment. After seven days, rats with glycemia ≥ 170 mg/dL were selected for further experimentation. Drug and doses administered accordingly as mentioned below:

1. **Group1** (Normal control): without any drug treatment received only vehicle/distilled water.
2. **Group2**(Positive control):Diabetes(STZ-injected rats)
3. **Group3**(Low dose treted):Diabetes(STZ-injected rats)treated with200mg/kg body weight with *M.micrantha* extract
4. **Group4** (High dose treated): Diabetes (STZ-injected rats) treated with 400 mg/kg body weight with *M.micrantha* extract.

5. **Group5** (Standard treated): Diabetes (STZ-injected rats) treated with standard drug–metformin hydrochloride (50 mg/kg b.w.).

Methanolic extract of *M. micrantha* in 2% Tween 80 suspension and standard drug metformin was orally administered for 21 days. Body weights and blood glucose levels of overnight-fasted rats were measured weekly. At the end of the experimental period, the animals were sacrificed under the influence of anesthetic and pancreas was collected from histopathologic examination.

5.14.8: Calculation of Doses: (Mandal *et al*., 1997; Chakraborty *et al*., 2018)

For test sample

The LD_{50} was found from the acute toxicity studies. LD_{50} values of the Methanolic extract of *M.micrantha* were found to be safe up to 2000 mg. The *in vivo* biological studies on serum and tissues of male wistar rats at the doses of 200 mg and 400mg/kg body weight.

For Streptozotocin*:* 40mg/kgb.w.

For standard (Metformin HCl):50 mg/kg b.w.

5.14.9: Evaluation of hypoglycaemic activity: Mandal *et al*., 1997; Chakraborty *et al*., 2018)

i. Fasting Blood Glucose(FBG)analysis

To quantify the levels of blood glucose, blood samples were aseptically taken from the end of rat's tails and placed on glucose test strips. Blood was collected from the tail of overnight fasted rats with the help of a syringe and fasting blood glucose concentration noted down on days 0,7,14 and 21 using ACCU-CHEK Guide Glucometer.

ii. Statistical analysis

The values expressed as Mean±Standard Deviation (SD) of the indicated number of experiments/animals. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnett's comparison test to determine the level of significance.

iii. Histopathological studies

Histopathological studies were carried out with the parts of pancreas which were isolated from the sacrificed rats. The tissues were washed with normal

saline and immediately fixed in 10% formalin for 24 h, dehydrated with alcohol, embedded in paraffin, and then cut into 4-5-um-thick sections and stained with hematoxylin-eosin dye for photomicroscopic observations. (Madic *et al.*, 2021).

6.1: Authentication of the plant material

A figure of the matured leaves of *Mikania micrantha* has been provided in figure 4. The authentication certificate from the central national herbarium, Botanical Survey of India is presented in figure 5. The certificate from the CNH confirms the identity of the collected species as *Mikania micrantha* of Asteraceae family.

Figure4: *Mikania micrantha* leaves

भारत सरकार
GOVERNMENT OF INDIA
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय
MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
फैक्स/ Fax: (033)26686226
दूरभाष/ Phone: (033)26683235/3364
इमेल/ E-mail: calherbarium@yahoo.co.in

भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA
केंद्रीय राष्ट्रीय पादपालय
CENTRAL NATIONAL HERBARIUM
हावड़ा / HOWRAH – 711 103

संख्या/No.: CNH/Tech.II/2023/45

दिनांक/Date: 02-05-2023

To,
Ms. Sumi Barman
M. Pharm
Department of Pharmaceutical Technology
Jadavpur University
Kolkata –700032
West Bengal

Sub.: Identification of one plant specimen – reg.

Dear Ms. Barman.

Please refer to your letter dated 21st March 2023 along with a plant specimen for identification. It is to inform you that the specimen has been identified by the concerned expert as:

Sl. No.	Specimen No.	Scientific Name	Family
1.	JU/SB–02	**Mikania micrantha** Kunth	Asteraceae

The receipt of ₹ 250/- (Rupees Two hundred fifty only) Receipt No. 1R-5, C-057276 dated 22-03-2023 is enclosed herewith.

Please send someone for collecting the specimen within three months from the date of receipt of this letter failing which it will be discarded.

Yours sincerely

(K. KARTHIGEYAN)
Scientist –'E'

वैज्ञानिक 'ई' Scientist- 'E'
केन्द्रीय राष्ट्रीय पादपालय
Central National Herbarium
भारतीय वनस्पति सर्वेक्षण
Botanical Survey of India
हावड़ा/Howrah-711 103

Figure 5: Authentication certificate from Botanical Survey of India

6.2: Morphological characteristics of the species

The morphological characters from the matured leaves of the plant are represented in Table 3. Upon studying the leaves, the colour was found to be light olive green with faint aromatic odour. The heart shaped bitter, pungent leaves had rough texture with a length of 4-10 cm according to their maturity.

Table 3: Macroscopic characteristics of leaves

Sl No.	Characteristics	Leaves part
1	Color	Light olive Green
2	Odour	Faintly aromatic
3	Taste	Bitter ,pungent
4	Texture	Rough
5	Shape	Heart-shaped
6	Size	4-10cmlong

6.3: Microscopical characteristics of the species

The microscopic characters from the transverse section of the leaves of the plant are represented in Figure 6. The section shows the presence of various anatomical characters of the leaves including epidermal cells, vascular bundle and diagnostic character like multicellular covering trichomes. On Peeling of lower epidermis presence of another diagnostic characteristic in the form anisocytic stomata is represented in Figure 7. Powder characterization of the leaves revealed presence of anisocytic stomata along with large fibers and annular xylem vessels as shown in Figure 8.

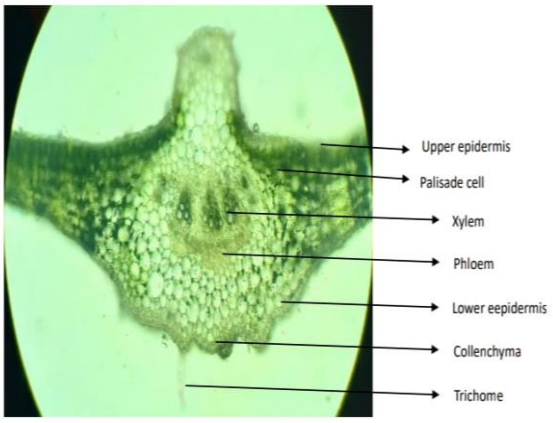


Figure 6: Transverse section of leaves

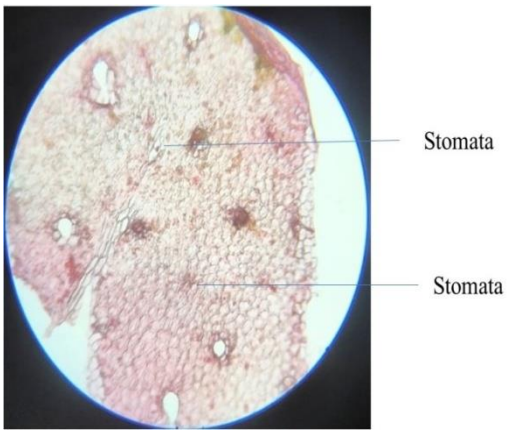
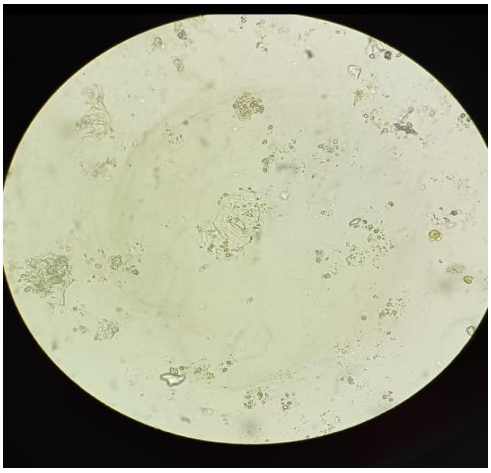
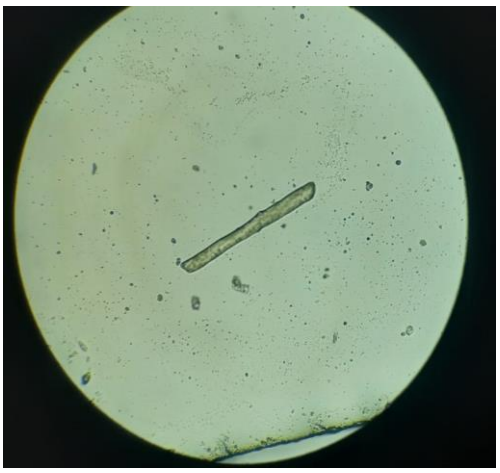


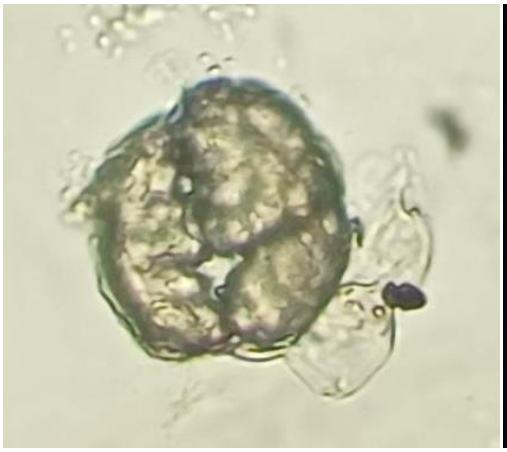
Figure 7: Lower epidermis of the leaf Depicting stomata;



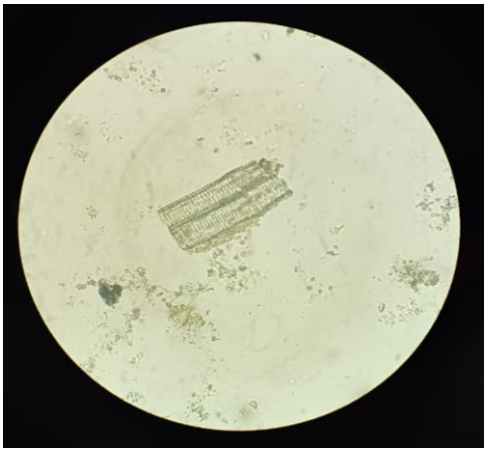
Stomata of leaves powder



larger elongated simple fiber



Stomata



Vessel element

Figure8: Powder characteristic of *Mikania micrantha* leaves under 10X45 magnifications

6.4: Physicochemical Characteristics of Leaves Powder

The leaves powder was used for the determination of different physicochemical parameters which helps in identification of the plants have been evaluated and they are represented in Table 4.

Table 4: Physico-Chemical Characteristics of Leaves Powder

Sl No.	Physico-Chemical Parameters	Results, Mean±SD(n=3)
1	Loss On Drying	9.33±0.036
2	Total Ash	5.23±0.035
3	Acid Insoluble Ash	4.77±0.064
4	Water Soluble Ash	5.11±0.050
5	Water Soluble Extractive	25±0.090
6	Alcohol(Ethanol)soluble extractive	15±0.082
7	Crude fiber Content	7.5±0.07
8	Swelling Index	2.5ml/g
9	Foaming Index	100

6.5: Fluorescence analysis of the leaf powder

The fluorescence characteristics of leaves powder on treatment with various chemicals and reagents on normal light is shown in Table 5 and under both short (254nm) and long (366nm) wavelength of UV light are shown in table 5.

Table 5: Colour of the powdered leaves on treatment with various chemical reagents

Sl No.	Reagents	Colour of powder
1	Powder+ Picric acid	Sacramento Green
2	Powder+ HNO3	Amber Orange
3	Powder+ HCl	Forest Green
4	Powder+ H2SO4	Lime Green
5	Powder+ Glacial acetic acid	Olive Green
6	Powder+ NaOH(5Naq.solution)	Olive Green
7	Powder+ Iodine(aq.Solution)	Leguna Yellow
8	Powder+ FeCl3(5%aq.Solution)	Olive Green
9	Powder+ antimony trichloride (alcoholic solution)	Moss Green
10	Powder+ methanol	Hunter Green
11	Powder+70%ethanol	Army Green
12	Powder+ Petroleum ether	Sacramento Green
13	Powder+50%H2SO4	Lime Green
14	Powder+1N NaOH(aq.solution)	Corn Yellow
15	Powder+1N NaOH(alcoholic solution)	Olive Green
16	Powder+50% HNO3	Fire Orange
17	Powder+5% KOH	Lime Green
18	Powder+ Ammonia	Olive Green

Table 6: **Fluorescence analyses of powdered leaves on treatment with different chemical reagents under UV light**

Sl No.	Reagents	Fluorescence under UV	
		Short wavelength(254nm)	**Long wavelength(366nm)**
1	Powder+ Picric acid	Army green	Hunter green
2	Powder+ HNO3	Chocolate brown	Mahogany red
3	Powder+ HCl	Sacramento green	Fern green
4	Powder+ H_2SO_4	No change	Kalley green
5	Powder+ Glacial acetic acid	Crimson red	Kalley green
6	Powder+ NaOH (aq.solution)	Forest green	Kalley green
7	Powder+ Iodine(aq. solution)	Sacramento green	Hunter green
8	Powder+ $FeCl_3$(5%aq.Solution)	Shadow green	Hunter green
9	Powder + antimony trichloride (alcoholic solution)	Bronze orange	Forest green
10	Powder+ methanol	Charcoal grey	Sacramento green
11	Powder+70% ethanol	Shadow grey	Forest green

12	Powder+ Petroleum ether	Mauve violet	Fern green
13	Powder+50% H_2SO_4	Byzantine violet	Kalley green
14	Powder+1N NaOH(aq.solution)	Sacramento green	Fern green
15	Powder+1N NaOH(alcoholic solution)	Hunter green	Kalley orange
16	Powder+ 50% HNO_3	Kalley green	Kalley green
17	Powder+ 5% KOH	Forest green	Kalley green
18	Powder+ Ammonia	No change	Kalley green

6.6: Phytochemical screening of the extracts

The results of phytochemical screening of both petroleum ether and methanol extracts of leaves were represented in table 7. The phytochemical studies have revealed the presence of carbohydrates, monosaccharides, steroids, terpenoids, saponins and phenolics in the petroleum ether extract. Methanol extract on the other hand has revealed the occurrence of carbohydrates, reducing sugar, steroids, terpenoids, glycosides, and flavonoids through phytochemical analysis.

Table 7: Phytochemical Screening of the petroleum ether and methanol extracts of the leaves

Chemical Constituents	Chemical Test	Petroleum ether extract	Methanol extract
Carbohydrates	Molisch Test	+	+
Reducing Sugars	Fehling's Test	-	+
	Benedict's Test	_	+
Monosaccharides	Barfoed's Test	+	-
Proteins and Amino Acids	Biuret Test	-	-
	Ninhydrin Test	_	-
	Millon's Test	_	-
Steroids	Salkowski reaction	+	+
	Liebermann-Burchard Test	+	+
Terpenoids	Noller's Test	+	+
Glycosides(Cardiac Glycosides)	Legal's test	-	+
	Keller–killianitest	-	+
Anthraquinone glycosides	Borntrager's test	-	+
	Modified Borntrager's test	-	+
Saponins	Foam Test	+	-

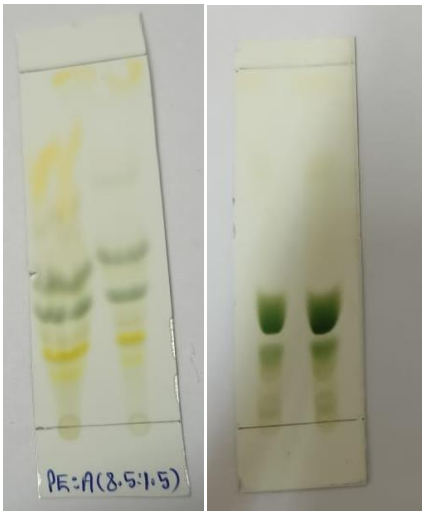
Flavonoids	Shinoda Test	-	+
	Ferric Chloride Test	-	+
	Sodium hydroxide Test	-	+
Alkaloids	Mayer's test	-	+
	Dragendorff'stest	-	+
	Hager's test	-	+
	Wagner's test	-	+
Tannins and Phenolics	Ferric Chloride Test	+	+
	Gelatin Test	+	+
	10% NaOH test	+	+

6.7: TLC Profiling

For the methanol extract (a), hexane: chloroform: methanol (6:2.5:1.5) was used as mobile phase while for the petroleum ether extract (b), petroleum ether: acetone (8.5:1.5) was used as mobile phase. The petroleum ether extract showed 6 distinct spots while the methanolic extract showed 5 spots as presented in Figure 9. The TLC characteristics in the form of retention factor (R_f) of both petroleum ether and methanol extract of *Mikania micrantha* leaves powder were shown in table 8.

Table 8: TLC Profiling of extracts

Sl. No.	R_f Value	
	Petroleum Ether Extract	**Methanol extract**
1	0.2	0.16
2	0.25	0.31
3	0.2	0.59
4	0.38	0.65
5	0.45	0.71
6	0.7	
7	0.95	



A: TLC of Pet. Ether exreact

B: TLC of Methanolic Extract

A **B**

Figure 9: TLC plates for petroleum ether and methanol extracts

6.8: HPLC Analysis of extract

The methanolic extract of leaves was subjected to HPLC analysis and it showed the presence of different phenolics and flavonoids as the major phytoconstituent. Figure 10 depicts the chromatogram of the HPLC analysis performed. Table no. 9 represents different compounds visualized on HPLC analysis under UV light at a wavelength of 260 nm with their Retention time, Peak height and % Relative area.

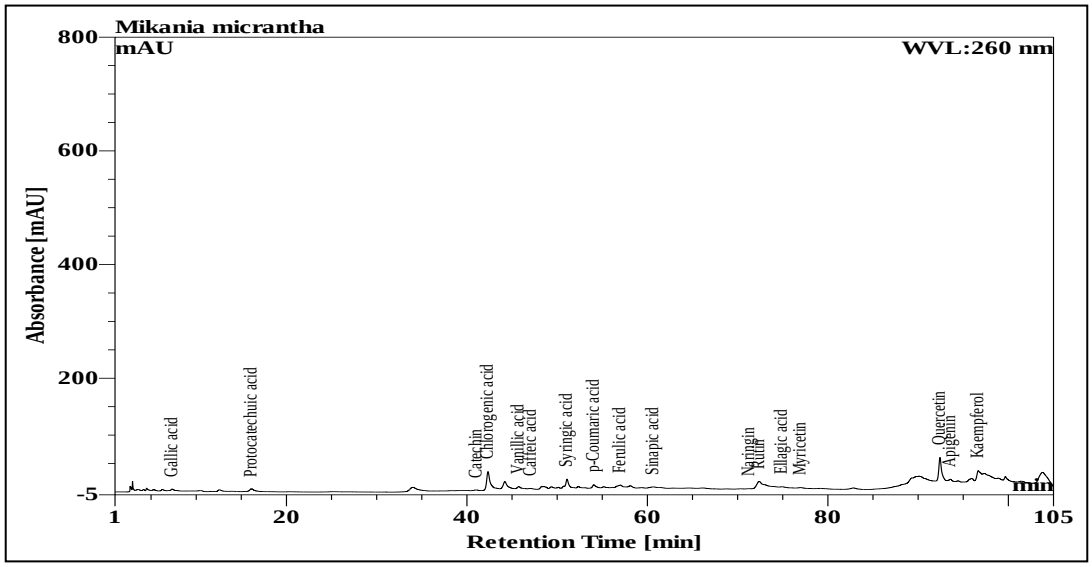
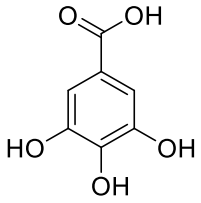
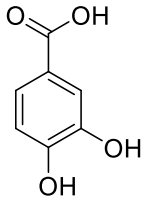
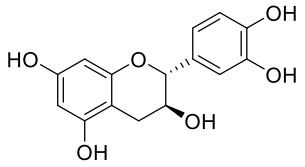
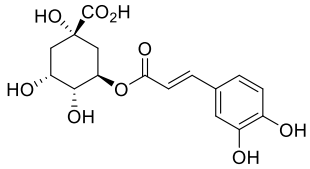
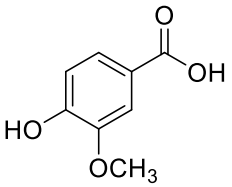
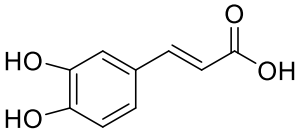
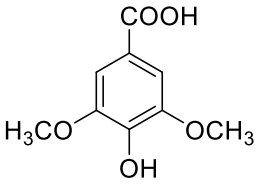
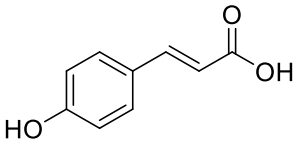
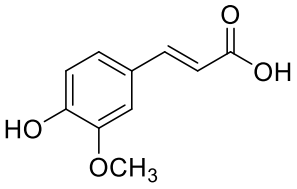
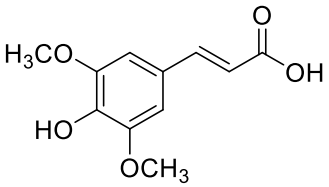
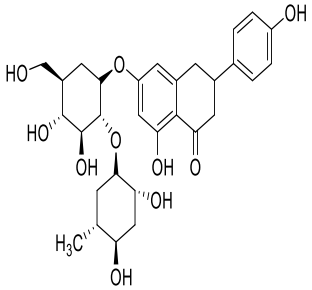
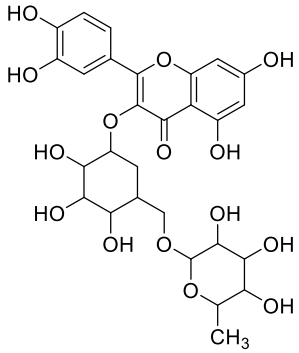
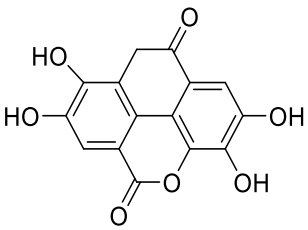
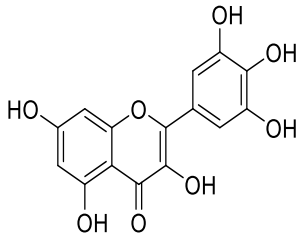
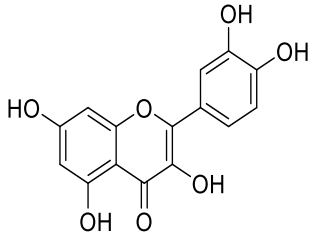
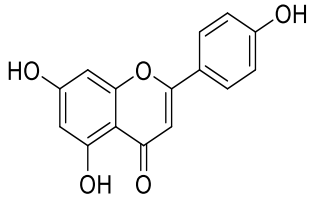


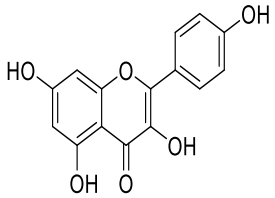
Figure 10: Chromatogram of methanolic extract of Mikania micrantha

Table 9: Different compounds of *Mikania micrantha* visualized on HPLC analysis under UV light at wavelengths of 260 nm

Sl No.	Compounds	Structure	Retention time (Minutes)	Peak height (mAU)	% Relative area
1	Gallic Acid		7.34	3.086	0.37
2	Protocatechuic acid		16.13	5.003	0.85

3	Catechin		41.08	1.114	0.14
4	Chlorogenic acid		42.34	33.078	4.47
5	Vanillic acid		45.74	5.496	0.96
6	Caffeic acid		47.08	1.891	0.30
7	Syringic acid		51.10	16.041	1.73
8	p-Coumaric acid		54.07	6.081	0.77
9	Ferulic acid		57.01	5.666	1.39
10	Sinapic acid		60.65	2.388	0.77

11	Naringin		71.27	0.500	0.06
12	Rutin		72.38	13.302	6.90
13	Ellagic acid		74.92	0.935	0.13
14	Myricetin		76.97	0.982	0.15
15	Quercetin		92.42	54.365	13.24
16	Apigenin		93.58	3.145	0.27

17	Kaempferol		96.66	29.987	20.08

6.9: Characterization of tincture

The different characteristics of the prepared tincture were shown in table 10, 11 and 12. The pH of the prepared tincture at different strengths of alcohol is represented in table 10. The study shows that greater alcohol concentration in the tincture increases acidity of the tincture while storage for longer period of time also slightly increases acidic property. Solid content of the tincture also decreases with time as seen from table 11, while greater alcohol concentration also imparts diminished solid content. Finally, as seen from table 12, specific gravity the prepared tinctures do not vary substantially with time.

Table 10: pH of the tincture

Alcohol Strength	**p H**		
	7 Days	**14 Days**	**21 Days**
40%	6.05	5.85	5.73
50%	5.93	5.81	5.64
60%	5.84	5.64	5.60
70%	5.73	5.59	5.54
80%	5.71	5.63	5.59

90%	5.39	5.56	5.58
Absolute (100%)	5.34	5.24	5.07

Table 11: Total Solid Content of Tincture

Alcohol Strength	**7 Days**	**14 Days**	**21 Days**
40%	19.09	17.2	14
50%	17.18	15.47	12.83
60%	16.95	15.38	12.5
70%	16.69	14.81	10.33
80%	15.96	13.57	10.71
90%	12.13	13.28	10.16
Absolute (100%)	9.93	7.17	5.53

Table 12: Specific Gravity of Tincture

Alcohol Strength	7 Days	14 Days	21 Days
40%	0.952	0.950	0.954
50%	0.932	0.941	0.946
60%	0.900	0.921	0.949
70%	0.887	0.895	0.902
80%	0.865	0.899	0.912
90%	0.841	0.900	0.929
Absolute (100%)	0.797	0.799	0.801

6.10: Antioxidant Assay

6.10.1: Total Phenolic Content Assay

The absorbance of the Standard and test solution were represented in table no.13 The total phenolic content in terms of mg Gallic Acid Equivalent(GAE)of Petroleum ether extract was found to be 6.585 mg/g and methanolic extract was found to be 15.202 mg/g . These results suggest that higher the presence of phenolic components was responsible for the levels of antioxidant activity. The Standard curve of Gallic acid was shown in figure 11.

Table 13.Observation of absorbance in Total phenolic estimation

Sl No.		Concentration(µg/ml)	Absorbance
1	Standard(Gallic acid)	20	0.238
2		40	0.458
3		60	0.686
4		80	0.815
5		100	0.995
1	Sample (Petroleum ether extract)	100	0.139
	Methanolic extract	100	0.220

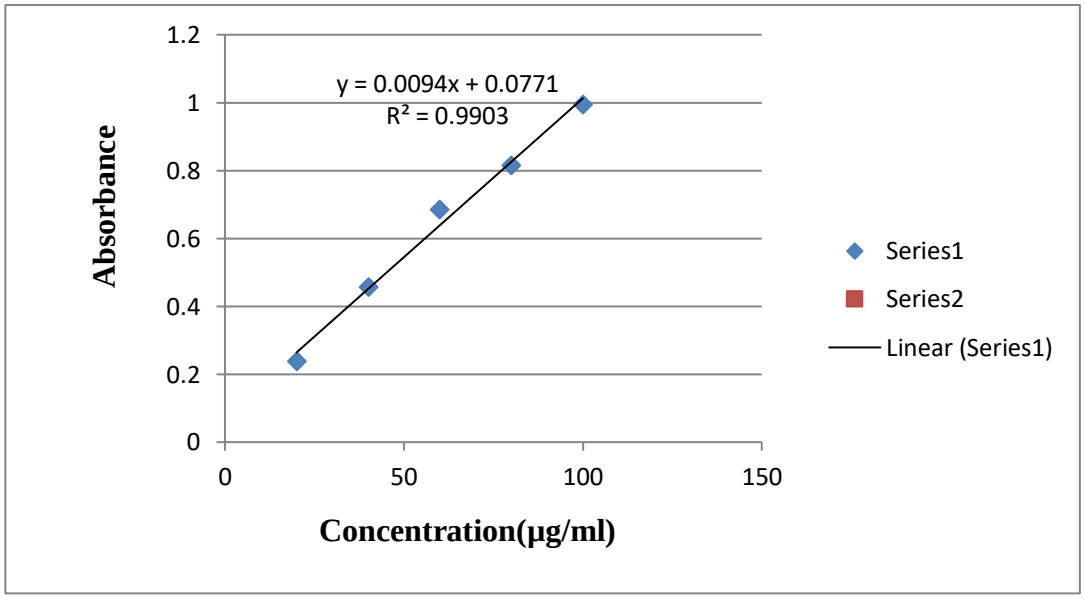


Figure 11: Standard curve of Gallic acid

6.10.2: Total Flavonoid Content Assay

The content of flavonoid compound in both Petroleum ether and methanolic extract of *Milania micrantha* leaves was measured by aluminum chloride reagent in terms of quercetin equivalent and was found to be 9.0588 mg/g and 12.980 mg/g .The absorbance of the Standard and test solution were represented in table no. 14. It is well known that flavonoids have significant antioxidant activity and have a positive impact on human nutrition and health. The

methanolic extract of *Milania micrantha* leaves contains a substantial quantity of flavonoids which may contribute considerable function to the antioxidant activity of the plant. The Standard curve of Quercetin is shown in figure 12.

Table 14: Observation of absorbance in total flavonoid estimation

Sl No.		Concentration (µg/ml)	Absorbance
1	Standard(Quercetin)	20	0.138
2		40	0.250
3		60	0.358
4		80	0.454
5		100	0.547
1	Sample (Petroleum ether extract)	100	0.089
	Methanolic extract	100	0.109

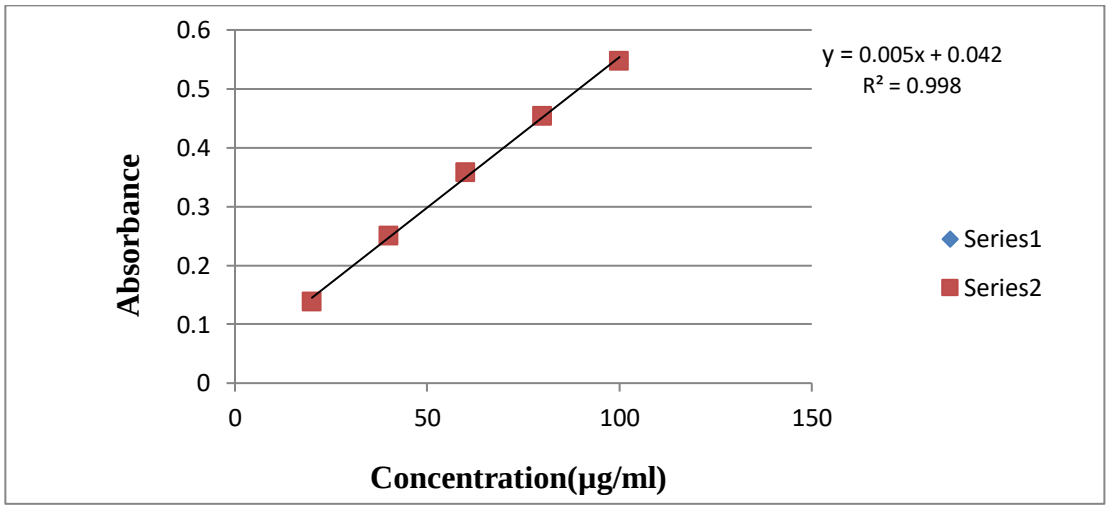


Figure 12: Standard curve of Quercetin

6.10.3: Inhibition of DPPH radicals:

The drop in DPPH's absorbance at 517 nm, which is brought on by antioxidants, was used to assess its capacity for reduction. The extract showed maximum hydrogen donating ability in the presence of DPPH free radicals at high concentrations. The extract showed antioxidant activity with an IC_{50} value of 202.94 µg/ml.

However, the known antioxidant Ascorbic acid exhibited an IC_{50} value of 139.66 µg/ml on DPPH radical. *Mikania micrantha*had significant scavenging effects with increasing concentration when compared with that of Ascorbic acid. The results of the DPPH scavenging activity was shown below in the tableFigure13 represents DPPH radical scavenging assay of standard and test sample.

Table15: DPPH Radical Scavenging Assay of Standard sample and Test Sample

Sl No.	**Concentration(µg/ml)**	**Percentage Inhibition (Mean±SEM)**	
		Test Sample	**Standard(Ascorbic acid)**
1	20	3.11±0.28	16.92±0.33
2	40	8.24±0.13	18.01±0.08
3	60	12.48±0	28.46±0.5
4	80	17.86±0.1	30.54±0.8
5	100	24.16±0.21	39.90±0.09

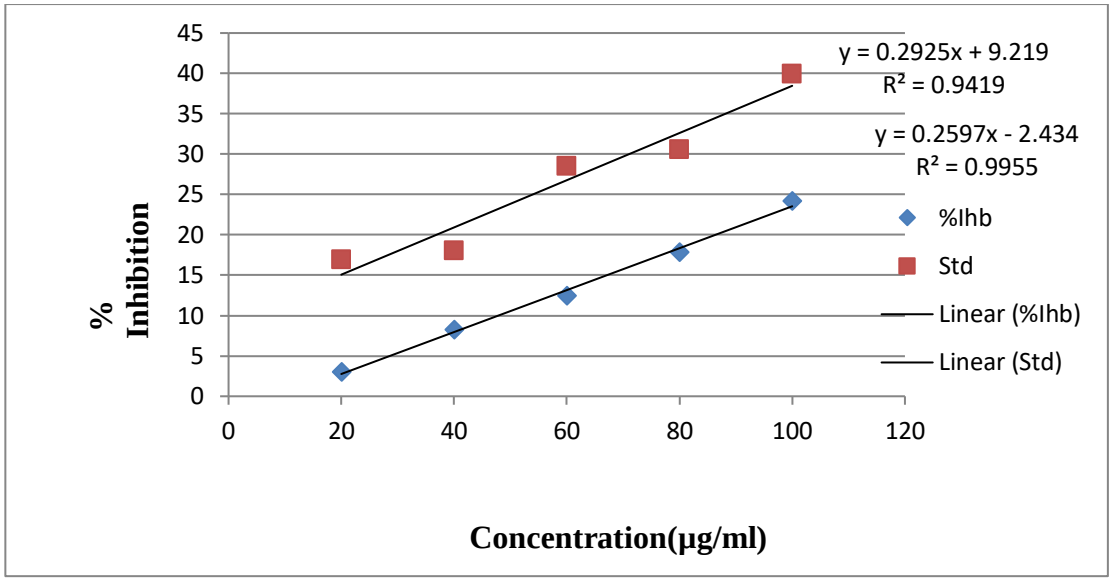


Figure 13: DPPH Radical Scavenging Assay

6.10.4: Inhibition of hydrogen peroxide radicals:

In addition to being highly reactive, hydrogen peroxide itself can occasionally be hazardous to cells due to the hydroxyl radical it produces inside them. Thus removing H_2O_2, as well as O_2 is very important for the protection of food systems. Here hydrogen peroxide scavenging activity of the extract is compared with ascorbic acid. The table below displays the outcomes. The IC_{50} value of extract and ascorbic acid were 561µg/ml and 481µg/ml respectively. The results showed that the test sample was effective in scavenging hydrogen peroxide in a dose- dependent manner. The outcome is comparable to that of the ascorbic acid reference standard. Figure 14represents hydrogen peroxide radical scavenging assay of standard and test sample.

Table 16: H_2O_2 radical scavenging activity of extract and standard

Sl. No.	Concentration (µg/ml)	Percentage inhibition (Mean ± S.E.M)	
		Standard(Ascorbic acid)	Extract
1	100	4.21±0.11	2.12±0.10
2	200	14±1.23	10.39±0.62
3	300	27.32±1.15	22.31±1.14
4	400	45.85±0.67	35.25±0.72
5	500	53.12±.011	45.02±.02
6	600	61.04±2.58	52.47±0.61

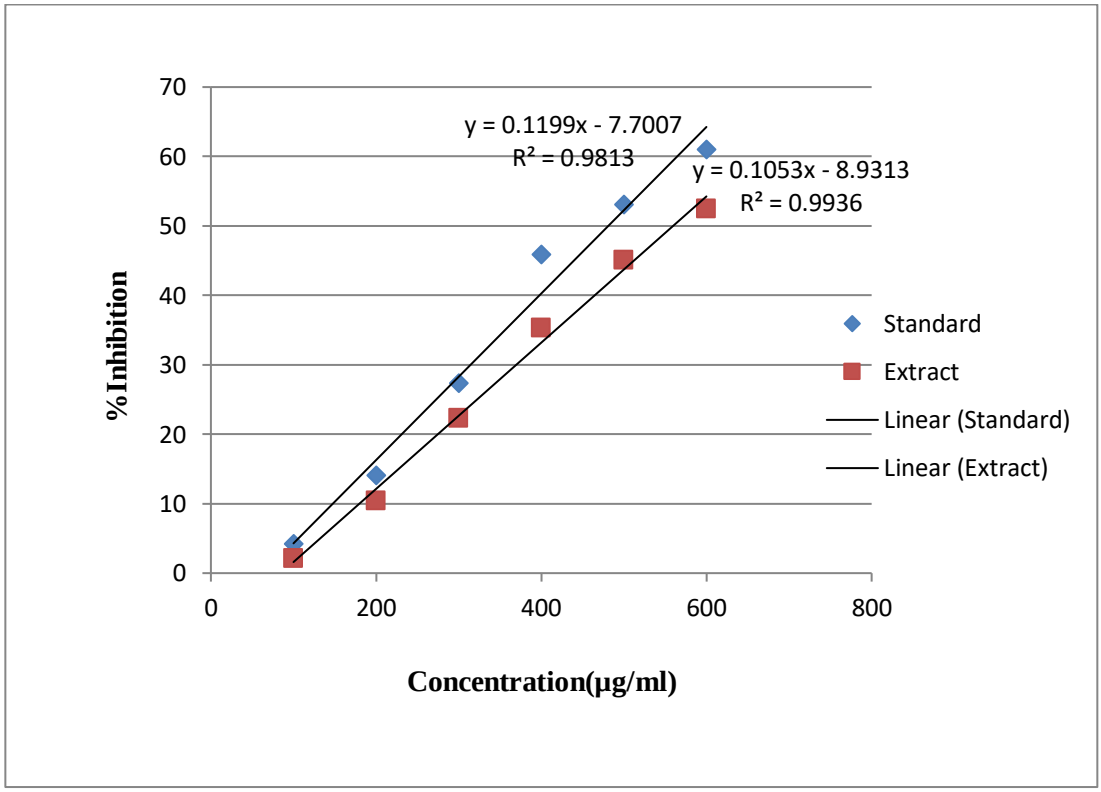


Figure 14: % Hydrogen peroxide radical scavenging activity

6.11: Determination of *in-vitro* Anti diabetic activity:

α-amylase inhibition assay:

α-amylase is one of the enzymes that hydrolyses the α-1,4 glycosidic linkages in starch to produce glucose and maltose. α-amylase hydrolyses complex polysaccharides into oligosaccharides and disaccharides and then hydrolyses by α-glucosidase to monosaccharides. The in vitro antidiabetic activity was evaluated by using α amylase inhibitory assay. The methanolic extract of *Mikania micrantha* produces some inhibitory effects on this enzyme. Acarbose was used as a standard which shows marked inhibitory effect of the enzyme (my thesis ref). The IC_{50} of Acarbose and Methanolic extract was calculated and found to be94.66 µg/ml 105.269µg/ml respectively. Table 17 shows the α-amylase inhibitory activity of Standard and test sample. Figure 15represents the graph of α-amylase inhibition assay of Acarbose and test sample.

Table 17: α -amylase inhibition activity of extract and standard

Sl. No.	**Concentration(µg/ml)**	**% Inhibition of Acarbose, mean±SD(n=3)**	**% Inhibition of methanolic extract of *M.micrantha*, mean±SD**
1	20	13.2±0.447	21.05±0.615
2	40	25.12±0.510	31.58±0.520
3	60	30.75±0.585	36.84±0.605
4	80	39.38±0.708	42.105±0.796
5	100	55.64±0.517	47.368±0.818

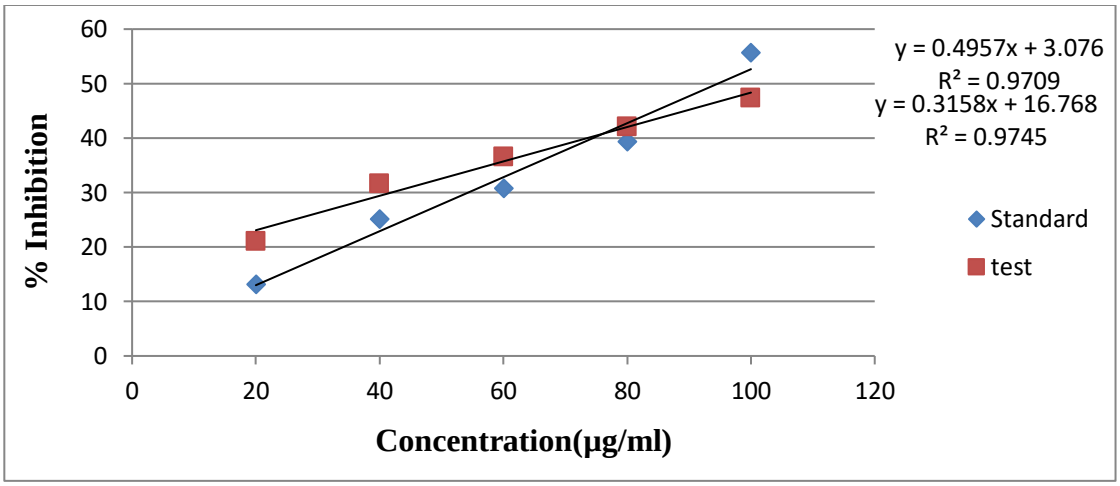


Figure 15: Graph of α-amylase inhibition assay of Acarbose and test sample.

6.12: Determination of *in-vivo* Anti diabetic Activity

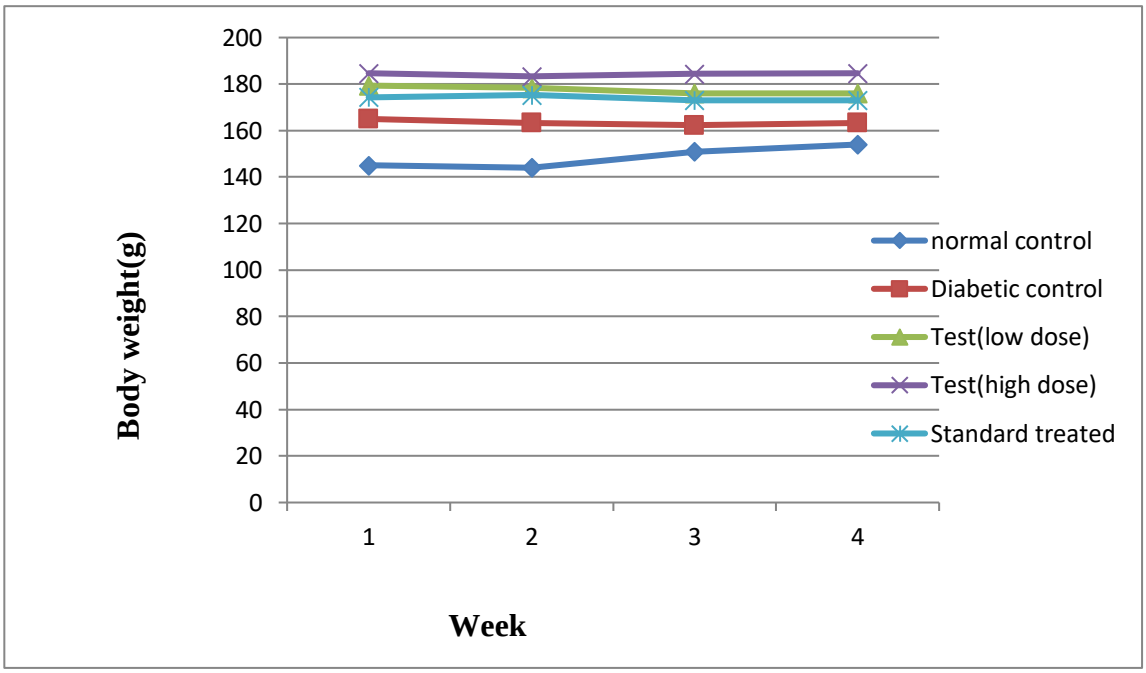
6.12.1: In-vivo blood glucose determination

Table18: Effects of *Mikania micrantha* extract on the changes of body weight in normal control and experimental rats observed at weekly interval

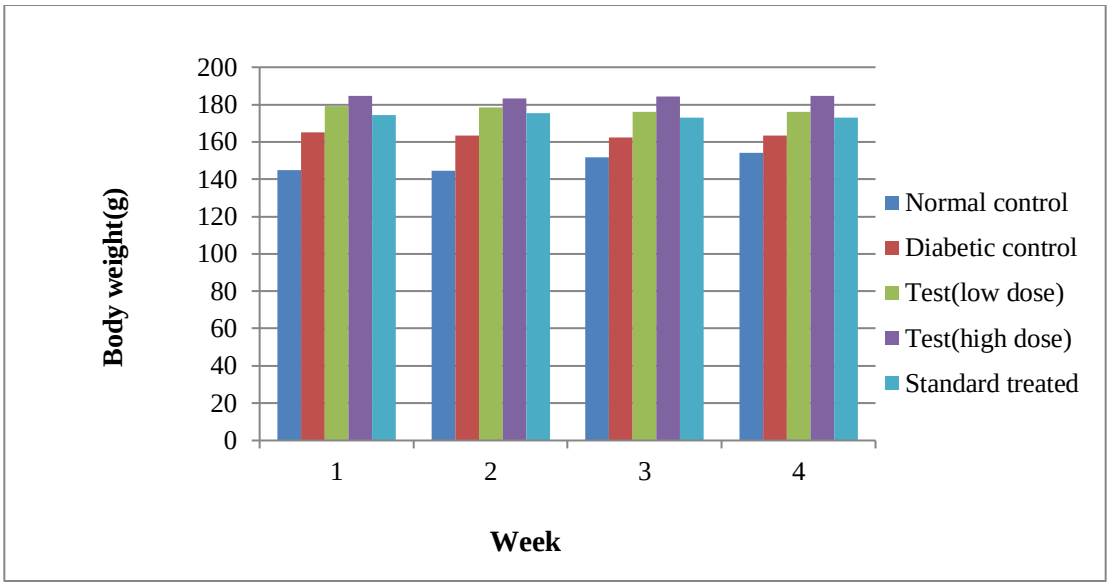
Groups	Body weight (g)			
	Day 0	Day 7	Day 14	Day 21
1	145±5.0	144.66 ±5.033	151.66±0.5774	154±1.00
2	165±1.0	163.33±1.528	162..33±1.952	163.33±1.528
3	179.33 ±0.577	178.33±1.09	176±1.00	176±1.00
4	184..66±0.54	183.33±0.5774	184.33±0.5775	184.66±1.85
5	174.33± 0.594	175.33±0.968	173±1.08	173±1.00

All values are expressed as Mean±SD, n=6, ANOVA followed by Dunnett's test was performed for significance data, comparing with group 1. *p<0.05 05considered significant;

**p<0.01 considered more significant; ***p <0.001 considered extremely significant as compared to different groups; ns is non-significant



A



B

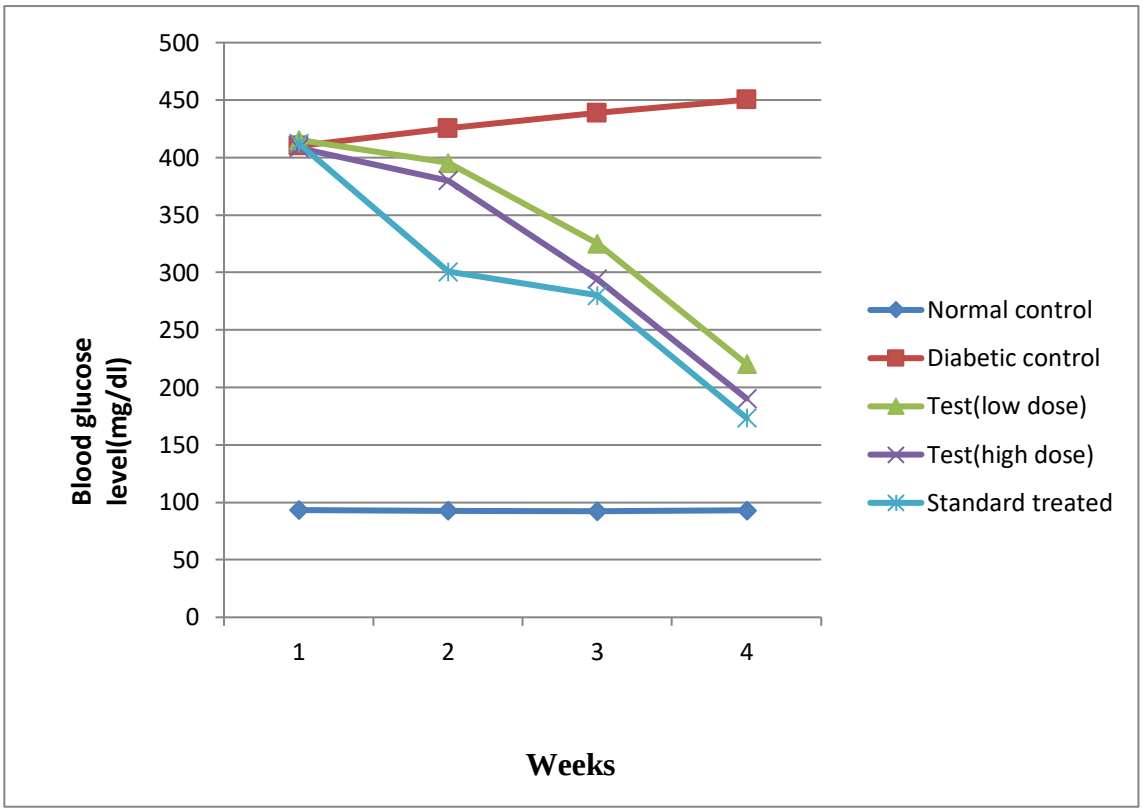
Figure16: A & B -Changes in body weight (g) between the five different groups of rats

Table 19: Blood Glucose Level (mg/dl)

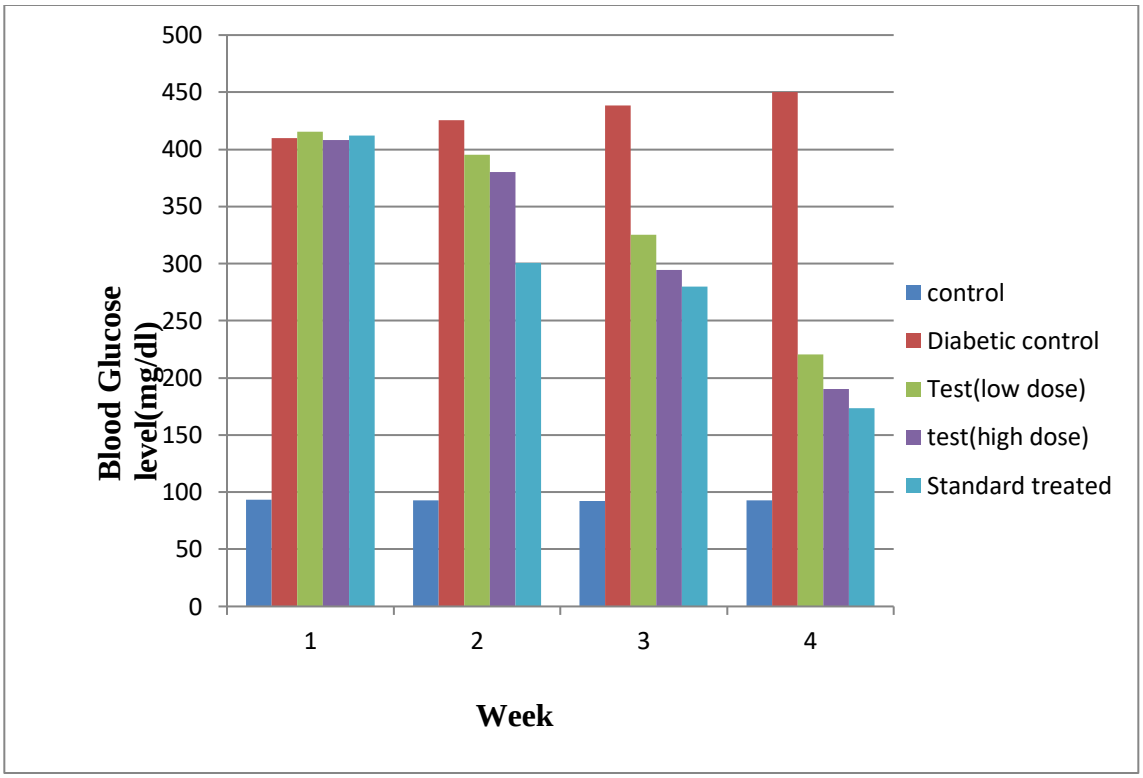
Sl. No.	Treatment	Fasting Blood Glucose level (mg/dl)			
		0 week	1st week	2nd week	3rd week
1	Group 1	93.33±5.686	92.66±2.989	92.33±2.517	93.00±2.00
2	Group 2	410±10**	425.33±10.908**	438.66±5.396**	450.33±5.760**
3	Group 3	415.33±15.275**	395.33±5.330**	325.33±5.380**	220.33±7.980**
4	Group 4	408.33±30**	380±8.909**	294.33±5.987**	190.33±10.876**
5	Group 5	412.33±25.166**	300.66±12.768**	280.00±10.594**	173.33±13.096**

All values are expressed as Mean±SD, n=6, ANOVA followed by Dunnett's test was performed for significance data, comparing with group 1. *p<0.05 05considered significant;

**p<0.01 considered more significant; ***p <0.001 considered extremely significant as compared to different groups; ns is non-significant.



A



B

Figure 17: A & B- Changes in blood glucose level (mg/dl) between the five different groups of rats

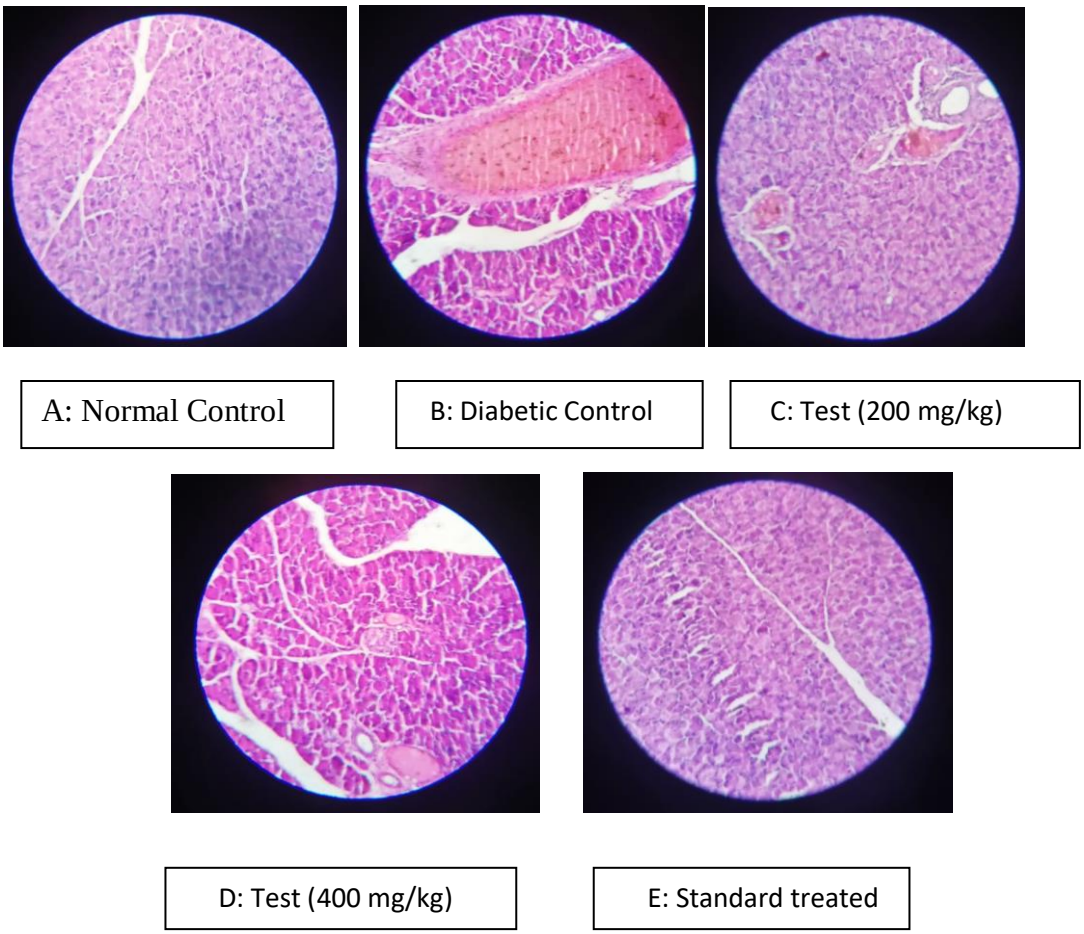


Figure 18: Histopathological sections of the pancreas in rat with H &E (Hematoxylin & Eosin) Stains

Histopathological sections of the pancreas in rat with H&E stain. A. Normal control (presence of normal pancreatic islet cells). B. Diabetic control (lymphocytic infiltration of pancreatic islets and vacuolation of the acinar) (10x magnification); C. Test group received 200 mg/kg showing reduction in dilated islet and the steatose (10x magnification). D. Diabetic group received 400 mg/kg (10 x magnification); E. Diabetic group received metformin (normal histology of pancreas) (10X magnification).

Discussion & Conclusion

In the area, *Mikania micrantha* is one of the most prevalent plants. During the characterization of powders, pharmacognostic research has discovered microscopic diagnostic features such as multicellular covering trichomes and anisocytic stomata. The findings indicate that the main phytoconstituents of *Mikania micrantha* include carbohydrates, steroids, flavonoids, glycosides, and tannins. According to TLC profiling, petroleum ether extract had a maximum of 7 spots while methanol extract had 5.The HPLC analysis shows presence of some flavonoids such as Gallic acid, Protocatechuic acid, Catechin, Chlorogenic acid, Vanillic acid, Caffeic acid, etc . The tincture's pH gradually drops with time, and on the seventh day, the tincture with a 40% (w/v) alcohol concentration showed the highest pH of 6.05. The highest solid content was found in the tincture with a 40% (w/v) alcoholic strength on the 7^{th}, 14^{th}, and 21^{st} days. Since each drug has a different fluorescence and colour, the leaf powder's features can be used to identify the original medication. The primary authentication of the genuine medicine from the contaminated one is made easier by these discoveries. HPLC analysis of methanolic extract of the leaves also reveals the presence of many phenolic and flavonoids as major Phytoconstituents such as Gallic acid, Quercetin, Rutin, Ellagic acid, Kaempferol,etc. These flavonoids are considered to be responsible for the antioxidant and antidiabetic potential of the leaves extract.

The in vitro antioxidant investigations of the methanolic extract of *Mikania micrantha* leaves were carried out with great care. When compared to other common antioxidants, the methanolic extract of *Mikania micrantha* leaves shown potent anti-oxidant activity by suppressing DPPH radical scavenging activities. As a result, it may be inferred that the plant *Mikania micrantha* can be employed as a significant and accessible source of natural antioxidants with subsequent health advantages. These *in vitro* studies demonstrate that this plant extract is a significant natural source of antioxidants, which may be helpful in reversing the effects of a variety of oxidative stresses. It was seen that there was a dose-dependent increase in percentage

inhibitory activity against alpha-amylase enzyme while performing the *in vitro* ant diabetic evaluation.

The acute toxicity study of *Mikania micrantha* leaf extract was already performed. LD_{50} values of the *Mikania micrantha* extract were found to be safe up to 2000mg.

Since diabetes caused a significant lessening in body weight due to increased muscle wasting and loss of tissue proteins which attributed to gluconeogenesis i.e., catabolism of proteins and fats. An increase in body weight in rats treated with optimized *Mikania micrantha* extract was observed as compared to the STZ-induced diabetic control rats (Group 2) which might relate to the rise in insulin levels and fall in glucose levels, which prevent muscle atrophy. The hypoglycemic activity of *Mikania micrantha* extract treated (Group 3&4) were comparatively significant with that of standard metformin-treated (Group 5). The ability of the extract to augment insulin secretion from pancreatic beta cells or sensitizing insulin receptors may be attributed to the potential mechanism.

Thus it can be concluded that the methanolic extract of *Mikania micrantha* leaf exhibited significant *in vitro* antioxidant and anti-diabetic activity. The protective activity of this plant could be attributed due to the presence of tannins, flavonoids, phenols, etc. as confirmed by the preliminary phytochemical screening. Further studies are required in knowing the exact mechanism of action, particularly in terms of reducing blood glucose levels.

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