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Formulation and Evaluation of Antioxident and Antidibetic Activities of Guduchi and Dill Extract

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Abstract: *This project aims to evaluate the antioxidant and anti-diabetic activities of two medicinal plants, Guduchi (Tinospora cordifolia) and Dill (Anethum graveolens). Antioxidants play a crucial role in preventing oxidative stress-induced damage, while anti-diabetic agents help regulate blood sugar levels. By assessing the bioactive compounds present in these plants and conducting in vitro and in vivo assays, this study aims to provide valuable insights into their therapeutic potential for combating oxidative stress and diabetes*

Keywords: Ethanolic, phytochemicals, Tinospora cordifolia, Withania somnifera, flavonoids

I. INTRODUCTION

Diabetic neuropathy is a common and costly complication of both type 1 and type 2 diabetes. Diabetic neuropathy is a heterogenous complication in diabetes, it is late finding in type-1 diabetes, but it can be early finding in type-2 diabetes. The selected animal model of DN should exhibit features present in human pathology. Diabetic animals showed many abnormalities that are seen in the diabetic patients with neuropathy, hyperalgesia, allodynia, slow nerve conduction velocity and progressive sensory and motor deficit. The pathophysiology of neuropathy is very complex & it has been associated with peripheral demyelination, a decrease in peripheral nerve conduction and degeneration of myelinated and unmyelinated sensory fibers.

The DN depends upon various causative factors including persistent hyperglycemia, microvascular insufficiency, oxidative stress, nitrosative stress, defective neurotrophism, & autoimmune-mediated nerve destruction . When we look at the annual costs of diabetic neuropathy and its associated morbidities in the US have been estimated to exceed $10.9 billion. Neurodegeneration is the term relate to progressive loss of structural or functional neurons, including death also. Many neurodegenerative diseases including Parkinson's, Alzheimer's and Huntington's occur as a result of neurodegenerative processes. Diabetes appears to contribute to cognitive impairment during early childhood, when the brain undergoes structural and developmental changes.

Longitudinal studies report lower intelligence quotient, decreased mental efficiency, and worse school performance in children with type-1 diabetes compared to children without diabetes. The reduced insulin production in type-1 diabetes and insulin resistance in type-2 diabetes both can generate AD-like pathology in the CNS. Depression is also associated with both type-1 and type-2 diabetes and this association is bi-directional, with each influencing the presentation of the other. Still information available about the effect of diabetes on neurodegeneration is not sufficient.

Animal models of 'induced diabetes' suggest a direct neurodegenerative effect of diabetes, the majority of studies show results in the hippocampus which is the area associated with learning and memory and the first structure to be affected by the neurodegeneration of Alzheimer's disease. Recent clinical study involving more than 1000 people has shown that those with diabetes have greater cortical atrophy, independent of hypertension, total cholesterol, smoking, BMI, coronary heart disease and socio-demographics than people without the condition.

Conventional therapeutic approaches for DN are Glycemic control, Symptomatic therapies includes antidepressants, SSRIs, anticonvulsants, opiates, NSAIDs, NMDA receptor antagonists and Causal therapies include aldose reductase inhibitors, drugs acts on hexosamine pathways, protein kinase C pathways, AGE receptors, many animals studies shows promise of these pharmacological agents but were with drawed in clinical study either due to lack of efficacy or due to their side effects on major organs , Therefore development of non-pharmacological approaches, alternative medicines for prevention & treatment of diabetes and prediabetes-associated neuropathic changes is highly needed.

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In recent years, progress has been made toward understanding the biochemical and molecular mechanisms leading to diabetic neuropathy. Herbal based approaches are current area of focus in diabetic neuropathy with neurodegeneration.

Antidiabetic drug:

Antidiabetic drug, any drug that works to lower abnormally high glucose (sugar) levels in the blood, which are characteristic of the endocrine system disorder known as diabetes mellitus. Diabetes is caused by the body's inability to produce or respond to the pancreatic hormone insulin

Antioxidants:

Antioxidants are the compounds that inhibit oxidation a chemical reaction that can produce free radicals and chain reaction that may damage cell of organism Antioxidant such as things or ascorbic acid (vitamin C) may act to inhibit these reaction. Free radicals are believed to be one of causes over sixty health problems according to various scientific and medical groups. These problems include cancer, aging and atherosclerosis. Phenolic compounds including flavonoids, Anthovyanins and Tannins are main group of antioxidants play key role in antioxidants mechanism.

1. GUDUCHI

Tinospora cordifolia

Guduchi, scientifically known as ***Tinospora cordifolia***, is a vital remedy in the Indian system of medicine. It belongs to the ***Menispermaceae*** family and has been used as a medicament since ancient times. It is also known as Giloy, Amrita or Indian bitter. It is found in dense and dry forests all over India, growing over small trees and bushes at high altitudes. The stem, roots and leaves of Guduchi all have medicinal properties In the traditional Ayurveda classical textbooks, such as Charaka, Sushruta and other texts, Guduchi is mentioned, under different names, as a medicine to treat a range of ailments .

Nutritional Value of GUDUCHI

The stem and the leaves of Guduchi have different nutritional values.

Nutritional Components	Value
Calcium	102.23 ppm
Phosphorus	24.81 ppm
Iron	26.058 ppm
Copper	3.733 ppm
Zinc	7.342 ppm
Manganese	12.242 ppm
Crude fibre	56.42 %
Crude protein	7.74 %

Table 1: Nutritional value present in stem of Guduchi

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Nutrients	Fresh leaves	Dehydrated leaves
Carbohydrate (g)	3.34	7.53
Protein (g)	2.30	5.23
Fat (g)	0.36	1.05
Fibre (g)	11.321	52.295
Iron (mg)	5.87	22.55
Calcium (mg)	85.247	210
Vitamin C (mg)	56	16
Beta-carotene (µg)	303.7	428.5
Energy (kcal)	88.64	240
Polyphenols (mg)	4.8	12.2
Flavonoids (mg)	6.76	18.28

Table 2: Nutrients present in 100 grams of Guduchi leaves

Therapeutic Uses of Guduchi :

The dried, fully developed stem of *Tinospora cordifolia* constitutes the drug 'Guduchi'. The therapeutic properties of Guduchi are:

- Memory enhancer
- Anti-cancer
- Anti-inflammatory[1]
- Immunity enhancer
- Digestion enhancer
- Anti-oxidant
- Anti-diabetic (reduces the blood sugar level)
- Anti-arthritic (helps to mitigate the joint pains associated with arthritis)
- Liver-protective
- Anti-allergic (helps to fight allergic reactions)

Benefits of Guduchi :

1. Benefits of Guduchi for digestion Guduchi is useful for managing various bowel issues. One study has specifically shown that it is effective against amoebic infection of the digestive system. The consumption of powdered Guduchi mixed with amla or jaggery is an effective remedy for constipation. 'Guduchi Satva', the starch obtained from the stem of Guduchi, is especially beneficial for the digestive system.

2. Benefits of Guduchi for diabetes

Guduchi has been shown to reduce blood sugar levels, especially during fasting levels. The anti-diabetic effect is attributed to the various phytochemicals present in it. It regulates the blood sugar level by reducing oxidative stress, enhancing insulin release, and reducing the production and breakdown of glucose in the body. Guduchi is especially useful for type 2 diabetes.

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3. Benefits of Guduchi for arthritis

The stem of Guduchi helps in inflammation and arthritis. It also helps in alleviating joint pain and many other symptoms associated with arthritis. Rheumatoid arthritis can be managed by consuming powdered Guduchi stem mixed with ginger.

2.DILL

Dill is just a tool in the belt of the chefs and cooks of the world! Dill is one such herb that is used for flavouring and seasoning food. However, it acts as more than just seasoning. It might have certain medicinal properties as well, that lend it as a useful ingredient in alternative medicine. Dill has been used in Ayurveda for managing various ailments and in the preparation of concoctions and medicines. It has also been used in the Unani system of medicine, in the preparation of gripe water used for sudden stomach pain in children (colic).

Nutrional profile : One cup (9 grams) of fresh dill sprigs provides approximately

Calories: 4

Vitamin C: 8% of the Daily Value (DV)

Manganese: 5% of the DV

Vitamin A: 4% of the DV

Folate: 3% of the DV

Iron: 3% of the DV

Benefits of Dill

Rich in antioxidants Antioxidants are naturally occurring compounds that help protect cells against damage caused by unstable molecules known as free radicals.As a result, research suggests that consuming foods rich in antioxidants may help reduce chronic inflammation and prevent or even treat certain conditions, including heart disease, Alzheimer's, rheumatoid arthritis, and certain forms of cancer.Both the seeds and leaves of the dill plant have been found to be rich in several plant compounds with antioxidant properties, including.

Terpenoids. These compounds are found in essential oils and may protect against liver, heart, kidney, and brain diseases.

Tannins. Responsible for bitterness in many plant foods, tannins have been shown to have potent antioxidant properties, as well as antimicrobial effects.

May help lower blood sugar levels

Having chronically high blood sugar levels is concerning as they can increase your risk of conditions like insulin resistance, metabolic syndrome, and type 2 diabetes.Dill has been suggested to have blood-sugar-lowering effects.In fact, several studies in animals with diabetes have shown a significant improvement in fasting blood sugar levels with daily doses of dill extract. Still, research in humans is limited.

II. MATERIALS AND METHODS

Extract preparation of *Tinospora cordifolia* :

Guduchi satva was prepared to process fresh T. cordifolia stems that were thoroughly washed with potable water, chopped into small pieces of 1–2 inches, pounded completely into course slimy mass, and soaked in water overnight. The material was thoroughly macerated the next day and filtered through four folded muslin cloths.The filtrate or extracted liquid was kept for sedimentation up to 5 hours, then supernatant liquid decanted carefully, and the starchy material left into the bottom was scrapped into the tray. This starchy material was then air-dried using a hot air oven with blowing air at 40^0c , which took 4–5 hour and finally obtained white color crystalline powder.

Extract preparation of *Anethum graveolens L*:

Anethum graveolens was prepared from Chikhli(Buldhana) and identified by our colleague.

For preparation of methanolic extract, dill leaves powder was dried and crushed. Dried dill powder (100 g) was mixed with 300 mL of methanol at room temperature for 48 hours. The prepared solution was filtered and subsequently concentrated and evaporated to dryness in vacuum. The extract was kept in dark vials at -20°C until analysis

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Phytochemical test

Phytochemical analysis The qualitative phytochemical analysis was carried out to detect the presence of different phytochemicals in Guduchi and Dill . The procedures for the tests are as follows:

Phytochemical tests for Guduchi Extract

Test for tannins

0.5 g of dried guduchi were taken in test tubes. 20ml of distilled water was added and boiled in water bath at about 100°C. The solution was filtered through Whatman No. 1 filter paper. After that add few drop of 0.1% ferric chloride (FeCl3). Development of brownish green or blue black coloration was indication of positive result.

Test for Flavonoids

2 g of dried guduchi powder were taken in test tubes, then add 20ml of distilled water and boil it for 2 min in water bath at 100 °C. The solution was filtered through Whatman No. 1 filter paper and 10ml of filtrate was taken in another test tube. Add 5 ml of distilled water and shake vigorously. The presence of persistent froth was taken as positive result. Test for Flavonoids 0.2 g of dried guduchi and ashwagandha powder separately in different test tubes were dissolved in 1% sodium hydroxide (NaOH). 10% HCl was added and change in the color of solution to yellow indicated the presence of flavonoids.

Test for Cardiac Glycosides [Kellar-Kiliani test]

2 g of dried powder of guduchi were taken in test tubes. 5 ml of distilled water was added in each test tube and then it was boiled for 2 min in water bath at 100°C. The solution was filtered through Whatman No.1 filter paper. The 1ml of extract and 0.5 ml of glacial acetic acid was taken in another test tube. Few drops of 5% Ferric Chloride and few drops of conc. H2SO4 were added. The appearance of greenish blue color was indicated as the presence of cardiac glycosides.

Test for Steroids

2 g of dried powder of guduchi were taken in test tubes and then boiled with 2 ml of distilled water in water bath at 100 °C for 2 min. The solution was filtered through Whatman No.1 filter paper. The 200 µl of extract and 10 volumes of chloroform and conc. H2SO4 were added carefully along the sides of test tubes. The change in color of lower layer to yellowish with green fluorescence and reddish upper layer indicated the presence of steroids.

Test for Alkaloids

1. Wagner's test

200 µl of crude extract was taken in test tube. The few drops of Wagner's reagent were added to the inner side of test tube. A reddish brown precipitate was formed which confirmed the presence of alkaloids

2. Mayer's and Wagner's test

Equal amount of extract and 1% HCl were added and heated gently. Mayer's and Wagner's reagent were added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

3. Dragendorff test

0.2 g of dried guduchi powder were taken in test tubes. Add 10 ml of methanol individually and after few minutes, it was filtered with Whatman filterpaper no 1. The 2 ml of filtrate in 1 ml of 1% HCl was taken and steam heated the solution for 2 min Again the solution was filtered and 1 ml of filtrate was taken. Six drops of Mayer's reagent/ Wagner's reagent/ Dragendorff reagent were added. The change in color of precipitate to orange red/ brownish red/ creamish showed the presence of alkaloids respectively.

Phytochemical test for Dill Extract

Test for Alkaloids

20 g of powder were moistened in 150 ml of distilled water for 24 hours in a tube. After filtration, the filtrate was left in 2 test tubes to be tested by two reagents. The Dragendorff reagent test appears as an orange-red precipitate proving the presence of alkaloids.

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Test for Tannins

Infused in 5% is prepared from 5g of powder in 100ml of boiling water for 15 minutes afterwards the suspension is filtered and rinsed to produce 100ml. Hydrolysable Gallic tannins are highlighted by adding 15ml of reagent stiansy to 30ml of 5% infused. After 15 minutes of heating in a water bath at 90° C, the mixture is filtered and saturated with 5g of sodium acetate; then, 1ml of a 1% solution of FeCl3 is added. The appearance of a blue - black color indicates the presence of Gallic tannins. The non-hydrolysable catechol tannins are characterized by the addition of 1ml of concentrated HCl in 5ml of previously prepared infusion. The mixture is boiled for 15 minutes. The formation of a red precipitate insoluble in isoamylalcohol affirms the presence of catechin tannins.

Test for Flavonoids

The compounds belonging to the group of flavonoids have been highlighted by the reaction to cyanidin : In a test tube 5ml of infused , add 5ml of hydrochloric acid; 1ml of isoamyl alcohol, then some magnesium shavings. There is a precipitation reaction for several the appearance of an orange pink color indicates the presence of flavones. Pink –purple indicates flavanones and red indicates the presence of flavanones and flavanonols.

In vitro Inhibition of α-amylase Activity

The α-amylase inhibition assay was determined by preparing 1 U/mL of α-amylasemixedinthebufferof 20 mM sodium phosphate with 6.7 mM sodium chloride (pH 6.9) and boiling in a water bath for 15 minutes. The same amount of 96 mM 3, 5-dinitro salicylic acid (DNS), and sodium-potassium tartrate tetrahydrate solution were used to prepare the colorimetric solution. 1000 μL starch solution (0.5% w/v) was mixed with incremental concentration of standard Acarbose (10–100 μg/mL), aqueous extract of T. cordifolia stem (50–300 μg/mL) and Guduchi satva (25–250 μg/mL). The α-amylase solution 1000 μL was added to each tube and incubated at 25oC for 3 minutes. The incubated enzyme mixture was added with 1000 μLofDNSreagentandfollowedtoheatfor15minutes on a boiling water bath. Make up the volume with double distilled water and absorbance was determined at 540 nm using a UV-Visible spectrophotometer. The inhibitory concentration at 50% (IC50) was calculated using the linear curve equation obtained by the calibration graph of concentration versus percentage inhibition. The percentage inhibition was calculated using the below formula.[14] % Inhibition (at each concentration) = (Control absorbance – Test absorbance) × 100 / Control absorbance

In vitro Inhibition of α-glucosidase Activity

In vitro Inhibition of α-glucosidase Activity The enzyme inhibition assay was measured by using a mixture of α-glucosidaseenzyme(1U/mL)100μLof solution with 100 μL of phosphate buffer (pH 7.0). Above solution was added with incremental concentration, 100 μL of Acarbose (0.5–8 μg/mL), aqueous extract of T. cordifolia (30–80 μg/mL) and Guduchisatva (20–60 μg/mL). The mixture was dissolved and incubated at 37°C for 60 minutes in maltose solution. The α-glucosidase action on maltose was stopped by kept the reaction mixture in boiling water for 2 minutes and cooled.

The amount of glucose released was measured by the addition of 2 mL of glucose reagent in the reaction mixture and absorbance was measured at 540 nm using a UV-visible spectrophotometer. The inhibitory concentration at 50% (IC50) was calculated using the linear curve equation obtained by the calibration graph of concentration versus percentage inhibition. The percentage inhibition was calculated using the below formula.

% Inhibition (at each concentration)

= (Control absorbance – Test absorbance) x 100 / Control absorbance

Analytical Study

Fresh Guduchi stem and Guduchi Satva were analysed by employing various analytical parameters. Out of 15 batches, physicochemical analysis was carried out on five randomly selected batches. Organoleptic characteristics (colour, odour, taste, touch) and physicochemical analysis like loss on drying at 110°C, ash value, acid insoluble ash, pH value, specific gravity at 40°C, total solid content, andvarious extractive values like water soluble, methanol soluble, chloroform soluble, benzene soluble, diethyl ether soluble extracts were carried out.GuduchiSatvawas further subjected

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to qualitative tests for various functional groups and quantitative estimations of total alkaloids. Tests for presence of certain heavy metals (mercury, arsenic, lead, cadmium, tin, iron, copper and zinc) and microbial contamination were also carried out. Fluorescence analysis was also done for test sample and observation were done under ordinary light, short wave (254 nm) and long wave (366 nm). Powder microscopy of Guduchi Satva was also carried out through a light microscope. Both unstained and iodine stained images were visualised .Guduchi Satva obtained from batch 1-15 was mixed thoroughly and this sample was subjected to heavy metal analysis, microbial contamination, fluorescence analysis and high performance thin layer chromatography study

HPTLC Profile

Initially sample solutions were prepared. Accurately Weighed 500 mg Guduchi Satva was taken in methanol And was filtered through Whatman I filter paper The filtrate was further subjected to Chromatographic separation. The solvent system used was Chloroform: Methanol (9:1% v/v). Five microliter of sample Solution was applied on pre-coated silica gel 60 F254 TLC Plate (E. Merck) of uniform thickness of 0.2 mm and the Prepared GuduchiSatva Powder microscopy of Guduchi Satva (stained with iodine) Plate was developed in the solvent system up to a distance Of 8 cm. The plate was visualised under short and long Ultraviolet (UV) radiation and density of the separated spots Was recorded using scanner III. The plate was sprayed with Vaniline–sulphuric acid reagent and observed in daylight. The RfValues were recorded . Peak display Densitogram of Guduchi Satva at 254 and 366 nm is placed Chromatographic conditions for HPTLC profile.

III. RESULT

Observation table: for Guduchi Extract

Test	Observation	Inference
Tannins test	Brownish green	Tannins present
Flavonoids test	Yellow colour	Flavonoids Present
Cardiac glycosides	Greenish blue	Cardiac glycosides present
Alkaloids	Reddish Brown	Alkaloids Present

Observation table : for Dill Extract

Test	Observation	Inference
Dragendorff test	Orange red	Alkaloids present
Tannins	Red precipitate	Tannins present
Flavonoids	Red ppt	Flavonoids present

HPTLC OBSERVATION

Guduchi Satva

Under 254 nm		Under 366 nm	
No. of peaks (spots)	R_f values	No. of peaks (spots)	R_f values
4	0.04	3	0.04
	0.50		0.50
	0.58		0.56
	0.73		

HPTLC – High performance thin layer chromatography

Table A: α-Amylase inhibitory study of aqueous extract of T. cordifolia and Guduchi satva

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Sample	Conc (ug/ml)	Average ± SEM	IC50 (µg/mL)
Aqueous extract of T.cordifolia	200	32.23±0.55	275.39
	150	23.17±0.85	
	100	16.63±0.79	
	50	6.99±0.11	
	250	67.00±1.28	
	200	52.25±0.50	
Guduchi satva	150	43.19±1.32	183.26
	100	29.27±0.58	
	50	18.41±0.45	
	25	11.33±0.78	

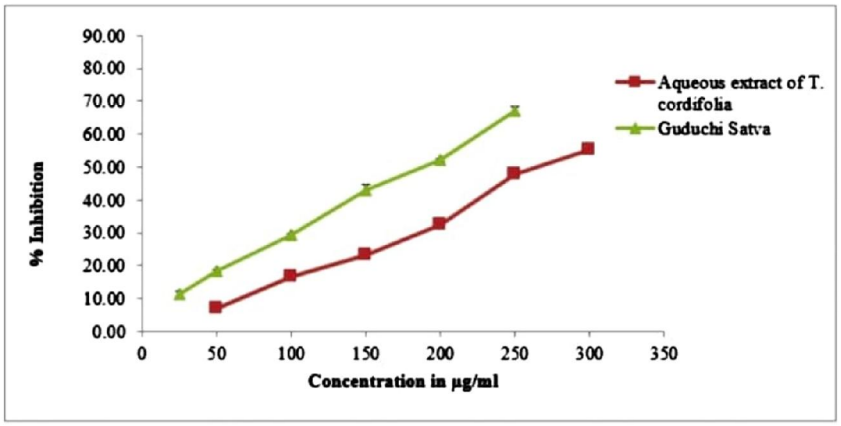

Fig. Graphical presentation of alpha amylase inhibitory activity of standard, aqueous extract of T. cordifolia stem and Guduchi satva.

Table B : Alpha-glucosidase inhibitory study of aqueous extract of T. cordifolia stem and Guduchisatva

Sample	Conc (ug/ml)	Average ± SEM	IC50 (µg/mL)
Aqueous extract of T.cordifolia	60	36.41±0.69	73.65
	50	23.17±0.85	
	40	17.32±0.96	
	30	5.79±0.18	
	60	75.18±0.81	
	50	61.33±0.53	
Guduchisatva	40	47.46±0.71	
	30	35.43±0.55	
	20	25.71±0.50	
	10	14.17±0.50	

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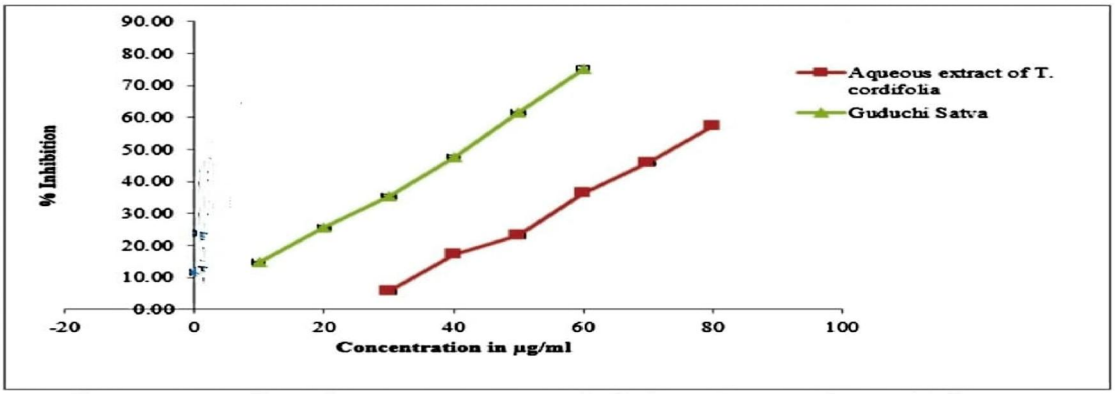

Fig. Graphical presentation of alpha glucosidase inhibitory activity of standard, T. Cordifolia and Guduchisatva.

IV. SUMMARY AND CONCLUSION

SUMMARY:

The project aims to investigate the antioxidant and anti-diabetes properties of extracts from Guduchi and dill plants. It involves formulating the extracts and evaluating their effectiveness in combating oxidative stress and managing diabetes. The study likely includes various laboratory tests and experiments to assess the bioactive components and their impact on antioxidant and anti-diabetic activities.

CONCULSION:

This Aim studies about antioxidant and antidiabetic activity in guduchi extracts and dill extract by Alkaloids and flavonoids test. This concluded presence of antioxidants and antidiabetic activities in both (guduchi and dill extract).

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