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Research Article

Development Of *Bidens Pilosa* Extract Based Emulsion Using Carbopol 950 For Its Anti-Oxidant, Anti-Diabetic, Anti-Microbial and Anti-Cancer Studies

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ABSTRACT

In order to investigate the antioxidant, anti-diabetic, anti-microbial, and anti-cancer activities of *Bidens pilosa* extract with Carbopol 950 as a gelling agent, this study focuses on creating a stable emulsion-based formulation. To improve its stability and bioavailability, *Bidens pilosa*, a medicinal plant high in bioactive chemicals, was isolated and added to an emulsion system. Because of its exceptional emulsifying and gel-forming properties, Carbopol 950 was chosen. The emulsion's stability, biological activity, and physicochemical characteristics were assessed. The emulsion demonstrated significant free radical neutralization comparable to standard antioxidants in antioxidant assays, including TCA, TAC, and H₂O₂ radical scavenging activities. This effectiveness was attributed to the flavonoids, polyphenols, and other phytoconstituents that are naturally present in *Bidens pilosa*; α -amylase and α -glucosidase inhibition assays were used to measure anti-diabetic potential; anti-microbial efficacy was tested against a variety of bacterial and fungal strains; and MTT assays were used to study the anti-cancer effects on specific cancer cell lines.

INTRODUCTION

For thousands of years, people have used aromatic and medicinal plants, particularly those having ethnopharmacological applications, as a natural

source of cures and medical treatment. Powders, tinctures, macerations, teas, infusions, percolation products, poultices, decoctions, tinctures, inhalations, and other herbal preparations were

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among the first forms of these widely used drugs. Oral tradition has been used to pass down the exact dosage of a plant and the method of administration for particular ailments from one generation to the next. Traditional pharmacopeia finally recorded this knowledge of medicinal plants. Drug development from medicinal plants has involved a variety of research techniques and academic fields [1]. Blackjack, or *Bidens pilosa*, is a medicinal plant with a wide range of therapeutic benefits that are ascribed to its various bioactive constituents. The plant's antioxidant, antimicrobial, anti-inflammatory, and antidiabetic properties are attributed to flavonoids, phenolic acids, polyacetylenes, alkaloids, and terpenoids. Because of its capacity to fight infections and encourage tissue regeneration, it has long been used to treat wounds, gastrointestinal issues, fever, and malaria. By causing cancer cells to undergo apoptosis, the plant also demonstrates anticancer activity. Both contemporary pharmacological research and traditional medicine continue to benefit greatly from this adaptable plant. Because of its distinct set of biological characteristics, *Bidens pilosa* holds great promise for accelerating the healing of diabetic wounds. Plant phenolic compounds, including flavonoids and chlorogenic acid, have strong antioxidant properties that aid in lowering oxidative stress, a primary cause of diabetic wound healing delays. Its antibacterial qualities work well against common diabetic wound-healing pathogens, avoiding infections and encouraging a sterile wound-healing environment. Additionally, *Bidens pilosa* has potent anti-inflammatory properties that aid in reducing inflammation at the wound site, promoting quicker tissue regeneration and repair.

MATERIALS:

Collection of Plant Sample:

Bidens pilosa is collected from the unused agricultural land in the village side of Kodaikanal. The plant is been collected during its flowering stage, as bioactive compounds are typically at their peak during this time. Aerial parts are usually harvested for their high therapeutic content. Harvesting is done manually in sterile condition to avoid contamination and preserve the integrity of the material. To confirm the identified plant is *Bidens pilosa*, we consulted a botanist in "Botanical Survey of India, TNAU", with the herbarium sample. Harvested plant is properly rinsed with tap water to remove the dirt and other impurities. Air dry the plant in a shaded well-ventilated area to prevent the loss of bioactive compounds due to heat or direct sunlight. Once the plant is dried properly, store it in a dried air tight container and store the container at room temperature, to preserve phytochemicals until extract.

Preparation of Extract:

Three different solvents (Ethanol, Distilled water and n-Hexane) are been used for the extraction process. From the stored sample 3-5grams of dried plant is taken and grained in chilled motor with 20ml of Ethanol, and the same process is repeated for water and n-Hexane. Once the sample is properly grained filtered the mixture [2]. Filtration is done using Whatman filter paper to separate the liquid extract from these plant residues. Mix the sample with 3 different solvents properly and place the samples in shaker incubator at 40°C at 60-80 rpm for 24-48 hours for proper mixing.

Phytochemical Analysis:

Phytochemical test is qualitative or quantitative analysis used to detect the presence of specific bioactive compounds in plants. To identify the different preliminary phytochemical constituents (Hexane, ethanol and water extracts) such as



Terpenoids, Alkaloids, Quinines, Saponin, Steroids, Flavonoids, Sugars, Proteins, Phenols. Chemical tests were carried out on the plant extracts using standard procedure.

Antioxidant Test:

The Antioxidant Test measures the ability of compounds to neutralize free radicals and prevent oxidative stress, protecting cells from damage. It includes three tests

FRAP:

The FRAP assay was performed on the samples in their respective solvents by mixing them with 1 ml of phosphate buffer and 1 ml of 0.1% potassium ferricyanide solution. The mixture was then incubated in a water bath at 50°C for 20 minutes. After cooling, 1 ml of 10% trichloroacetic acid was added, followed by the addition of 1 ml of distilled water and 0.5 ml of freshly prepared 0.1% ferric chloride solution. The absorbance of the resulting solution was measured at 700 nm ^[4]. A control sample was prepared following the same procedure, except without the test samples.

Hydrogen Peroxide Scavenging Assay:

The hydrogen peroxide (H₂O₂) scavenging ability of the extract was evaluated using the H₂O₂ method. A 0.5 mL aliquot of the extract was transferred into test tubes, and the total volume was adjusted to 2.5 mL using a 50 mM phosphate buffer (pH 7.4). Following this, 2 mL of a 2 mM H₂O₂ solution was added to the mixture. The reaction mixture was vortexed and allowed to react for 10 minutes. After the incubation period, the absorbance was measured at 230 nm ^[5].

Total Antioxidant Activity:

Using phospho molybdenum method total antioxidant activity was confirmed by the protocol

of Phatak and Hendre (2014) with slight modification. 0.5ml of the sample was mixed with the 0.5ml of reaction mixture contain 0.6 M H₂SO₄, 28mM sodium phosphate and 4mM ammonium molybdate reagent. The solutions were incubated at 50°C for 90minutes with blank. After incubation the tubes were normalised into room temperature and the absorbance was read at 695nm using spectrophotometer (LT 291 labtronics microprocessor). Ascorbic acid was used a standard to calculate the mg/gm of the total antioxidant activity ^[6,7].

Thin Layer Chromatography (TLC):

Three different solvent samples (Water, Ethanol, n-Hexane) are been taken in 3 Eppendorf tube. Three TLC sheets are taken and with the help of toothpick spots are been placed in the sheets 1-2cm from the bottom until a fine visible circle is obtained in the sheet (approximately 30-40 fine dots). Allow the spot to dry completely. Suitable solvent is prepared in the ratio of 3:2:2:1 (n-Butanol: Ethyl acetate: Acetic acid: Water). Pour the solvent in the TLC chamber and place the TLC sheet in the chamber. Ensure the sample spot remains above the solvent level and cover it to prevent evaporation. Remove the sheet and immediately mark the solvent front and allow the sheets to dry. Measure the distance travelled by each compound and calculate the R_f value for each spot.

$$R_f = \frac{\text{Distance moved by the solute}}{\text{Distance moved by the solvent}}$$

Formulation of Emulsion:

10ml of aqueous extract is taken and to that add 1ml of Carbopol 950 (Gelling agent). Add 0.15g of Carboxyl Methyl Cellulose (CMC) and stir it for 30 minutes in magnetic stirrer for proper mixing. After stirring add 1ml of Poly Ethylene Glycol



(PEG) and stir it for 30 minutes. Pour the substance on a sterile petri plate and keep it overnight in room temperature for the formation of emulsion [8].

Antibacterial and Antifungal Activity:

The agar well diffusion method was used to evaluate the antibacterial and antifungal activities of the extracts. A 70 μ L aliquot of fresh bacterial (*Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Proteus mirabilis*) and fungal (*Fusarium oxysporum* and *Aspergillus flavus*) culture was pipetted onto the centre of sterile petri dishes containing solidified media, sterilized at 121°C for 15 minutes. For bacterial cultures, 39 g of Mueller-Hinton agar was dissolved in 1000 mL of distilled water, while for fungal cultures, 45 g of malt agar was used in the same volume of water. The cultures were then spread evenly using a sterile cotton swab, and wells (6 mm in diameter) were created using a sterile cork borer. Subsequently, 50 μ L of each extract was added to the respective wells, and the plates were incubated at 37°C for 24 hours. The antimicrobial activity was assessed by measuring the zone of inhibition (excluding the well diameter) observed after incubation. DMSO served as the negative control, while a Gatifloxacin disc was used as the positive control [9].

Anti-Cancer Test:

The Anti-Cancer test evaluates an extract's ability to inhibit cancer cell growth or induce cell death. Cell line preparation involves mixing 19.5g DMEM (Dulbecco's Modified Eagle Medium), 3.7g Na₂CO₃, 4.5g Glucose, 5-10% BSA (Bovine Serum Albumin) and 1mg/ml Antibiotic. The mixture is incubated in a T-flask under 5% CO₂, at 37°C, pH7.5 and 70-80% humidity for 24-72hours. For testing, a sterile 96 well plate is prepared by

adding 100 μ L DMSO to the blank well, different concentrations of the extract (2,4,6,8,10 μ L) to sample wells, and add 100 μ L of the cell line to each well, including a control. After incubating in a CO₂ incubator for 24-48hours, 50 μ L DMSO is added to each sample for washing, followed by 50 μ L Trypsin-EDTA. Next, 20 μ L MTT dye is added to all wells, and 100 μ L is discarded from each well. The plate is incubated again for 24hours, and optical density (OD) values are measured at 570nm and 630nm [10]. The percentage of cell death were calculated using following equation: % of cell death= ((Positive control OD – Sample OD)/Control OD) x100

Anti-Diabetic Test:

The Anti-Diabetic test evaluates a compound's ability to reduce or prevent diabetes and its complications by assessing its effect on blood glucose levels, insulin sensitivity and metabolic activities. It involves two in-vitro tests: Alpha-Amylase and Alpha-Glucosidase inhibition. Alpha-Amylase essential for carbohydrate digestion is tested by mixing 1ml of the sample with 0.5ml of 0.1% starch solution in 16mM sodium acetate buffer, adding 0.2ml alpha-Amylase solution, 0.5ml sodium potassium tartrate and 3,5-dinitrosalicylic acid (96mM). After incubating at 25°C for 10minutes in alkaline conditions, OD is measured at 540nm. Alpha-Glucosidase, which breaks down disaccharides into glucose, is tested by mixing 1ml of the sample with 2% starch solution and 1ml of 0.2M tris buffer. After incubation at 37°C for 15-30minutes, 0.2ml of alpha-Glucosidase is added and incubated at 35°C for 45minutes. Finally, 2ml of 6N HCl is added, and OD is measured at 540nm [11]. The percentage of inhibition were calculated using following equation: % of inhibition= ((Positive control OD – Sample OD)/Control OD) x100

RESULTS AND DISCUSSION



The plant was collected and identified as *Bidens pilosa* L. - **Asteraceae**, we consulted a botanist in “Botanical Survey of India, TNAU”, Coimbatore-641003.

Preparation of Extracts:

For the extraction procedure, three distinct solvents ethanol, distilled water, and n-hexane are utilized. Three to five grams of dried plant material are removed from the stored sample and ground in a cooled motor with 20 millilitres of ethanol. The procedure is then repeated with water and n-hexane. Filter the mixture when the sample has been appropriately grained. The liquid extract is filtered out of these plant remnants using Whatman filter paper. To ensure adequate mixing, thoroughly mix the sample with three distinct solvents and place it in a shaker incubator set at 40°C and 60–80 rpm for 24–48 hours.



Figure1: Sample Extract

Phytochemical Test:

We examined the phytochemical test from the study "Phytochemical Screening and Antibacterial Activities of *Bidens pilosa* L. and *Tridax*

procumbens L. on Skin Pathogens." The results of the phytochemical analysis in that study indicated that both plants (*B. pilosa* and *T. procumbens*) contained tannins, alkaloids, flavonoids, saponins, and cardiac glycosides, while terpenoids, phlobatannin, and anthraquinone were not present in either plant *B. pilosa* and *T. procumbens* which serve as an inexhaustible source of secondary metabolites with considerable medicinal value [3]. In our current study, we conducted a phytochemical analysis using n-Hexane, Ethanol, and water to *Bidens pilosa*. The results indicate that various compounds, including Alkaloids, Terpenoids, Phenol, Sugar, Saponins, Flavonoids, Quinines, Protein, and Steroids, are predominantly found in the water extract

Table1: Phytochemical Test

Test	n-Hexane	Ethanol	Water
Alkaloids	-	+	+
Terpenoids	+	+	+
Phenol	+	+	+
Sugar	-	-	-
Saponins	-	-	+
Flavonoids-T1	-	+	+
T2	-	+	+
Quinines	-	+	+
Protein	-	+	+
Steroids	+	-	+

TLC Analysis:

Water, ethanol, and n-hexane were used as solvents in the Thin Layer Chromatography (TLC) examination, which showed clear component separation.



Figure2: TLC Formulation

Non-polar molecules went farther, but polar compounds had a greater affinity for the stationary phase. While n-hexane promoted migration, ethanol promoted moderate mobility. Compound polarity and solvent efficacy in separation were shown by the Rf values.

Table2: TLC Analysis

TLC Test	1 st Spot	2 nd Spot	3 rd Spot
Ethanol	0.78		
N- HEXANE	0.568	0.689	
Water	0.46	0.48	0.56

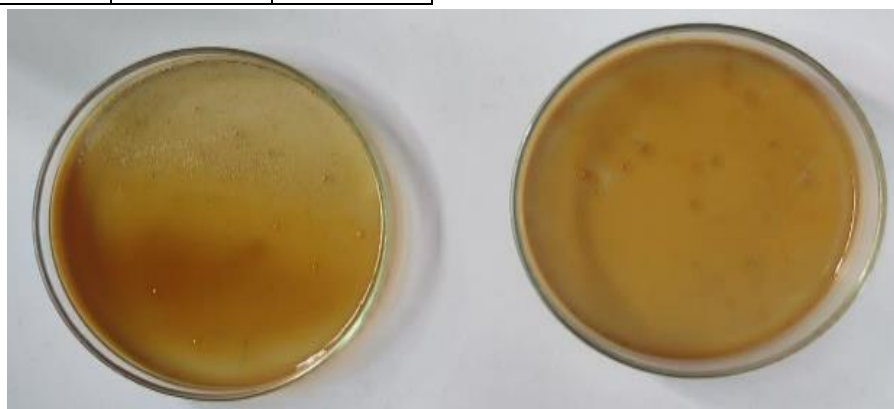


Figure3: Emulsion Formulation

Anti-Bacterial Test:

We examined the results of the study "In vitro Antibacterial Activity of Aqueous Extracts of *Bidens pilosa* L. (Asteraceae) from Nigeria," which included information on the extracts' antibacterial activity against the tested bacteria in Tables 2 and 3 ^[12]. These results included zones of inhibition, minimal inhibitory, and bactericidal concentrations.

Formulation of Emulsion:

Ten millilitres of aqueous extract are obtained, and one millilitre of Carbopol 950 (gelling agent) is added. To ensure adequate mixing, add 0.15g of carboxyl methyl methylcellulose (CMC) and stir for 30 minutes using a magnetic stirrer. Stir for 30 minutes, then add 1 ml of Poly Ethylene Glycol (PEG). To allow the emulsion to develop, pour the material onto a sterile petri plate and let it at room temperature for the whole night.

Staphylococcus aureus, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Proteus mirabilis* were among the microorganisms we used in our current study's antibacterial test. The outcomes obtained were

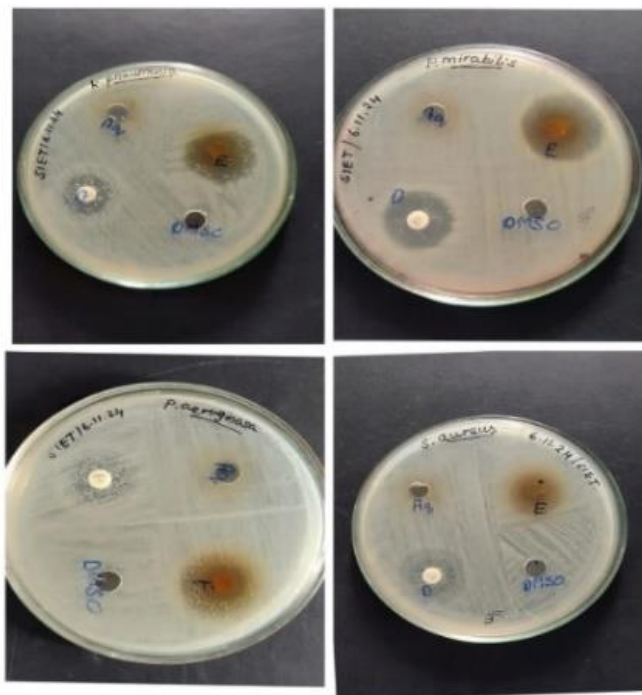


Figure4: Anti-Bacterial Test

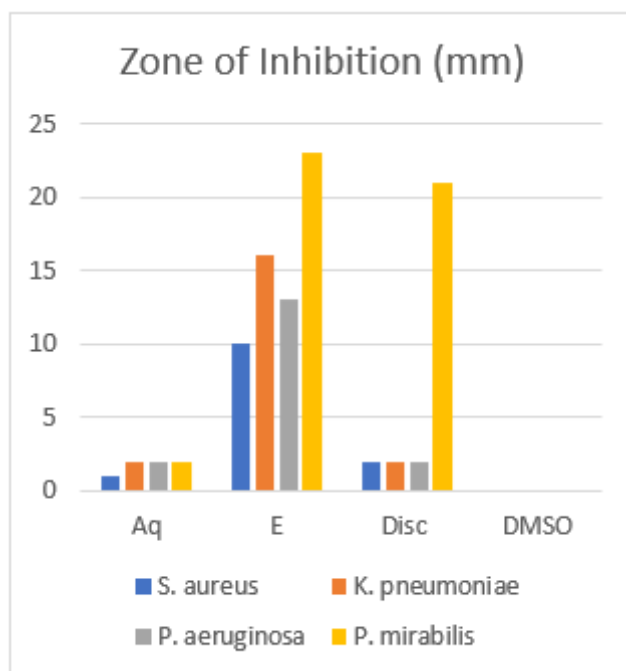


Figure5: Anti-Bacterial Test (Zone of Inhibition)

Anti-Fungal Test:

In the study "Chemical composition and antioxidant, antibacterial, and antifungal activities of the essential oils from *Bidens pilosa* Linn. Var.

Radiata," we looked at the antifungal activity results. The aqueous extract and essential oils of *B. pilosa* reduced fungal growth, according to anti-fungal activity testing (Table 3). Since all tested dosages reduced its growth, *F. solani* exhibited the

most suppression, followed by *F. oxysporum* and *C. rolfii* [13]. We used fungi such as *Fusarium oxysporum* and *Aspergillus flavus* to perform the

Anti-Fungal Test in our present investigation, and the outcomes were

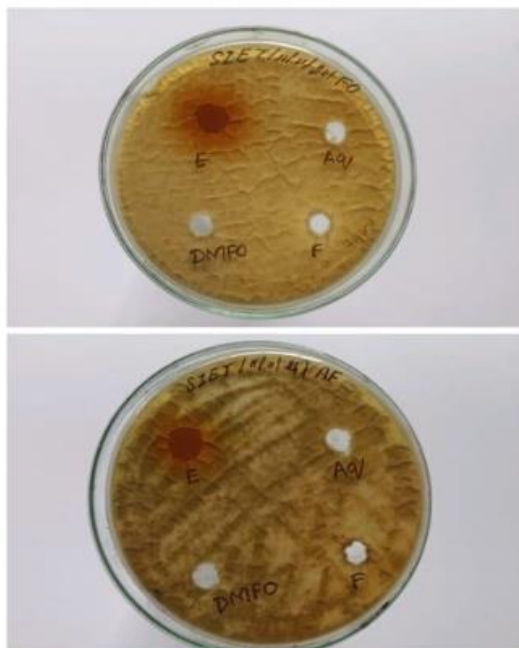


Figure6: Anti-Fungal Test

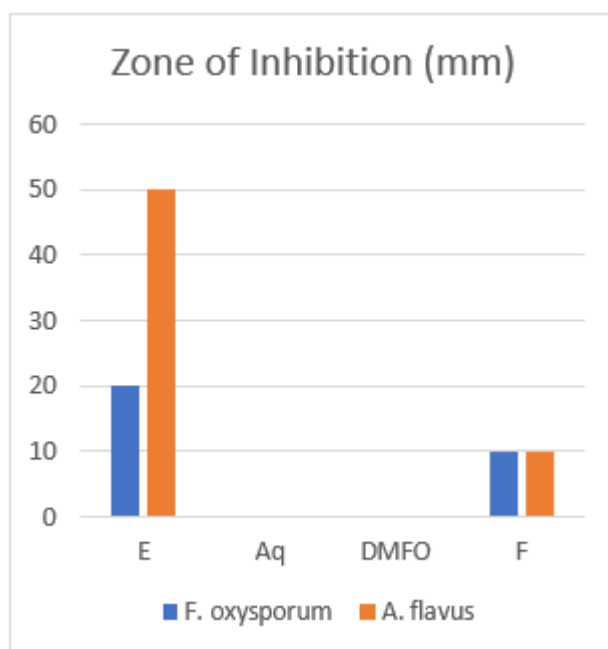


Fig. 7: Anti-Fungal Test (Zone of Inhibition)

Anti-Cancer Test:

The absorbance at 570 nm was used to determine the anti-cancerous activity of plant extracts at

varying doses (2µg/ml to 10µg/ml). The extract causes a small rise in the percentage of cell death from 5.6% to 64.2%.



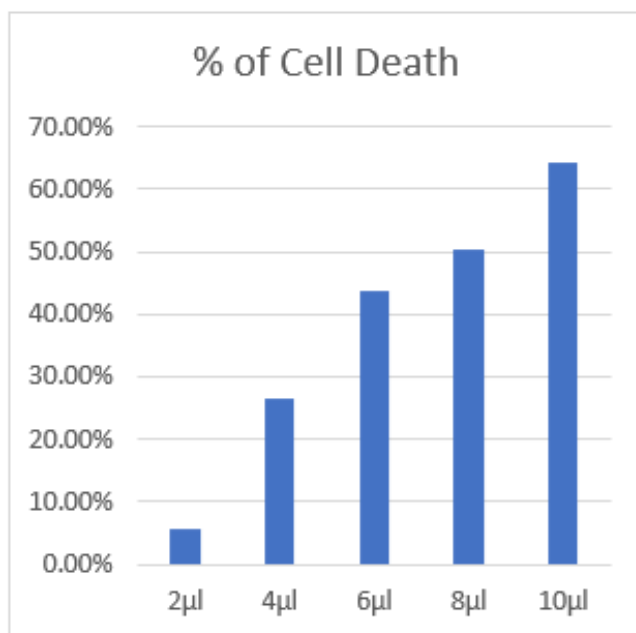


Figure8: Anti-Cancer Test

Anti-Diabetic Test:

The anti-diabetic efficacy of plant extracts for both test alpha-amylase and alpha-glucosidase was assessed using the absorbance at 540 nm. In all

assays, the extract's control range demonstrates its potential anti-diabetic effects.

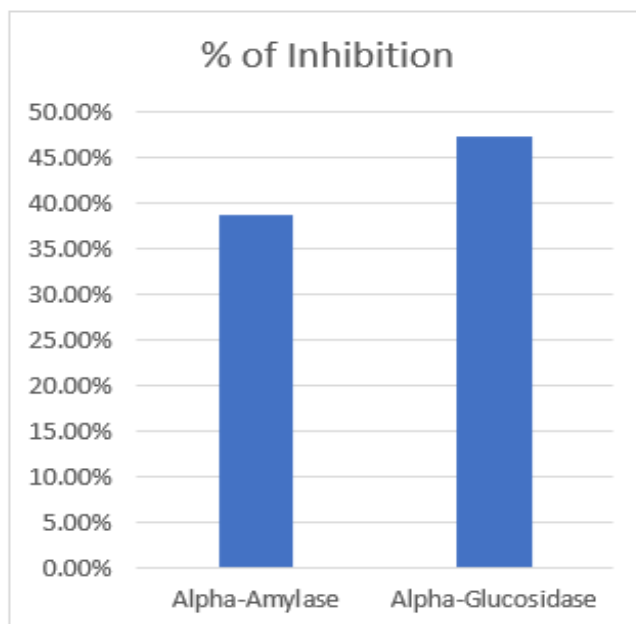


Figure9: Anti-Diabetic Test

CONCLUSION:

To sum up, the creation of an emulsion based on *Bidens pilosa* extract utilizing Carbopol 950 showed encouraging antioxidant, anti-



diabetic, anti-microbial, and anti-cancer qualities. Because of the formulation's stability and improved extract bioavailability, it may find use in therapeutic settings. Its antibacterial and anti-cancer properties also suggest a wide range of therapeutic advantages. To confirm its effectiveness and safety for use in pharmaceutical, more research is required, including in vivo studies and clinical trials.

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