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IN VITRO ANTI-DIABETIC AND ANTIOXIDANT ACTIVITIES OF *Azadirachta indica AND Aloe vera* FORMULATION

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Abstract

The many number of herbal plants are used in the cellular and metabolic disease treatment such as diabetes, obesity and cancer etc. There are several theories that the production of free radicals within the body, which could be neutralised by antioxidants from various medicinal plants, causes cellular changes and the growth of cancer, among other things. Plant-derived antioxidant neutraceuticals have been shown in many studies to scavenge free radicals and modulate oxidative stress-related degenerative effects. The *in vitro* anti-oxidant activity was carried out by the inhibitory activity of against the DPPH , H_2O_2 Scavenging assay and reducing power assay. The inhibition of these compounds may increase the anti-oxidant and antidiabetic capability.

Key words: oxidative stress , *Azadirachta indica and Aloe vera.*

I. INTRODUCTION

Traditional medicine based on plant extracts has been shown to be clinically beneficial and less toxic than currently available drugs [1]. The form of solvent used in the extraction process has a big impact on the success of determining biologically active compounds from plant material [2]. Phytochemicals (secondary metabolites) are plant-derived bioactive chemicals. They are produced naturally in all parts of the plant's body, including the bark, leaves, stems, roots, flowers, fruits, and seeds. [3]. They've long been known as the foundation for traditional herbal medicine, both past and present [4]. Phytochemicals are typically screened in all plant sections, and the presence of a phytochemical of interest can contribute to its isolation, purification, and characterization. It can then be used to develop a new pharmaceutical product. Medicines derived from plant extract are being used to treat a wide variety of clinical disease [5]. Traditionally, natural products has established store house of numerous bioactive compounds, which provide an endless source of medicine. Many traditional medicines have been based on crude herbs for a long time. Aloe vera gel is high in antioxidants. These polyphenols, along with a number of other compounds found in Aloe vera, can help to prevent the growth of bacteria that can cause infections in humans. (6). They are Antioxidant and antibacterial properties, helps treat cancer soresNeem leaf is used to treat leprosy, eye problems, bleeding noses, intestinal worms, stomach upset, loss of appetite, skin ulcers, heart and blood vessel diseases (cardiovascular disease), fever, diabetes, gum disease (gingivitis), and liver problems. (7).

II. Material and methods

In Vitro antioxidant activity(8,9 and 10)

Scavenging activity of 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) radical (10)

The method calculated the ability of DPPH to scavenge the stable free radical as a decrease in absorbance at 517 nm. About 0.1 ml of DPPH.-methanol solution (0.135 mM) was mixed with 1.0 ml of different concentrations of various extracts of *Azadirachta indica and Aloe vera* . The reaction mixture was thoroughly vortexed and held at room temperature for 30 minutes. At 517 nm, the mixture's absorbance was measured spectrophotometrically. Standard drugs used are rutin and butylated hydroxyl toluene (BHT). The following equation was used to measure the percentage of free radical scavenging:

%scavenging = 100–(Abs sample–Abs blank)/Abs Control × 100.

Scavenging of hydrogen peroxide

The ability of the *Azadirachta indica and Aloe vera* to scavenge H_2O_2 was determined .

In phosphate buffer, a solution of H2O2 (40 mM) was prepared (pH 7.4). The concentration of H2O2 was measured using a spectrophotometer and absorption at 230 nm (SL 159, UV- Visible Spec, Elico, India). Extracts (200, 400, 600, 800 and 1000 µg) in distilled water were added to a H_2O_2 solution (0.6 mL, 40 mM). Absorbance of H_2O_2 at 230 nm was determined after ten minute against a blank solution containing phosphate buffer without H_2O_2. The percentage of scavenging of H_2O_2 of *P. guajava* and standard was calculated using the following equation:

$$\text{\% of free radical Scavenging activity} = \frac{\text{Control - Test}}{\text{Control}} \times 100$$

Reducing Power assay

The reducing power of ethanolic leaf extract of *P. guajava* was determined by the method of Oyaizu (1986). Substances which have reduction potential react with potassium ferricynaide to form potassium ferrocynaide, which then reacts with ferric chloride to form ferric-ferrous complex that has an absorption maximum at 700 nm. The absorbance increases as the reduction of ferric to ferrous ion increases, suggesting that the ethanolic leaf extract of P. guajava has reducing potential.

Procedure

Varying concentrations of ethanolic leaf extract of plant in double distilled water was mixed with 2.5 mL of phosphate buffer and 2.5 mL of potassium ferricyanide. The mixture was incubated at 50 °C for 20 min, after which, 1.5 mL of TCA was added and centrifuged at 3000xg for 10 min. 0.5 mL of supernatant was combined with 1 mL distilled water and 0.5 mL ferric chloride from each tubes. A spectrophotometer was used to calculate the absorbance at 700 nm. The reaction mixture's increased absorbance meant that the reducing power was rising. The blank was an incubation of water instead of additives.

III. Result and Discussion

Table1: Thin layer chromatography of *Azadirachta indica and Aloe vera* formulation

S.no	Extract	No of spot	Rf value
1	Aqueous extract	1	0.15
2		2	0.30
3		3	0.76
4		4	0.92

Fig 1: TLC

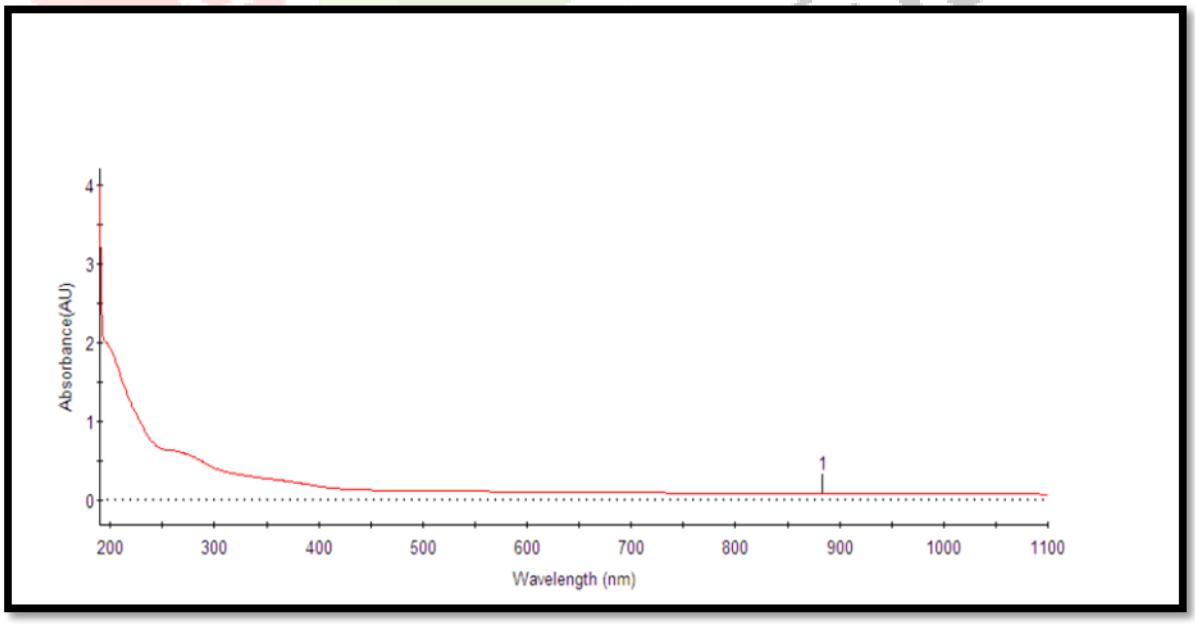


Fig 2 : UV analysis of *Azadirachta indica*

Table 2: UV analysis of *Azadirachta indica*

S.no	Wavelength	Absorbance
1	883.75	0.0913

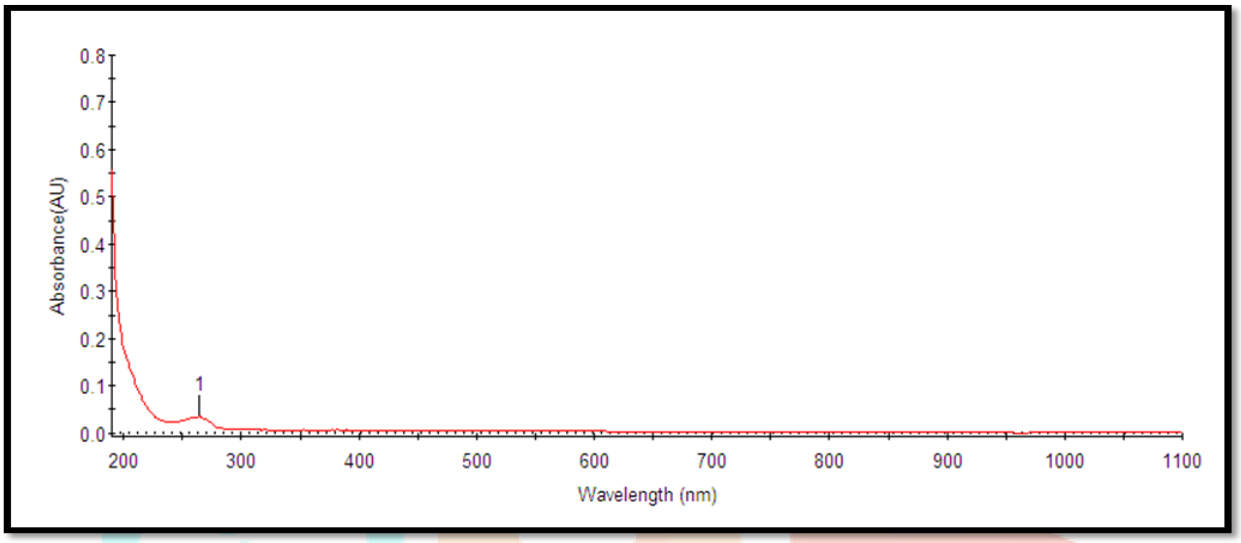


FIG: 3 : UV-VIS ANALYSIS OF *Aloe vera*

TABLE 3 : UV-VIS ANALYSIS OF *Aloe vera*

S.NO	Wave Length	Absorbance
1.	263.75	0.0346

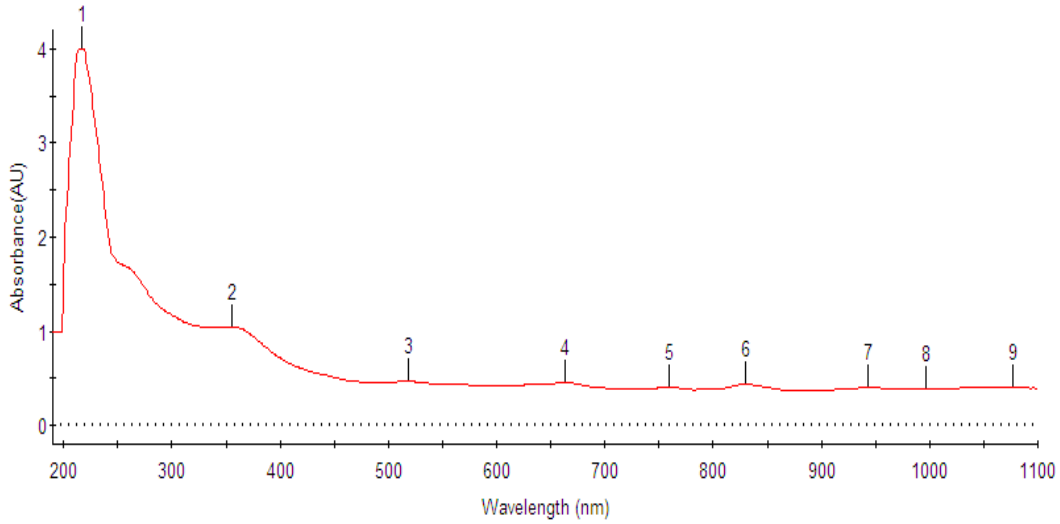


Fig 4: UV-VIS ANALYSIS OF *AZADIRACHTA INDICA And Aloe vera* Formulation

Table :4 UV-VIS ANALYSIS OF *AZADIRACHTA INDICA And Aloe vera* Formulation

S.NO	WAVE LENGTH	ABSORBANCE
1.	216.85	4.0000
2.	663.25	0.4697
3.	759.05	0.4038
4.	830.20	0.4360
5.	943.45	0.4013
6.	997.00	0.3950
7.	1076.60	0.4093

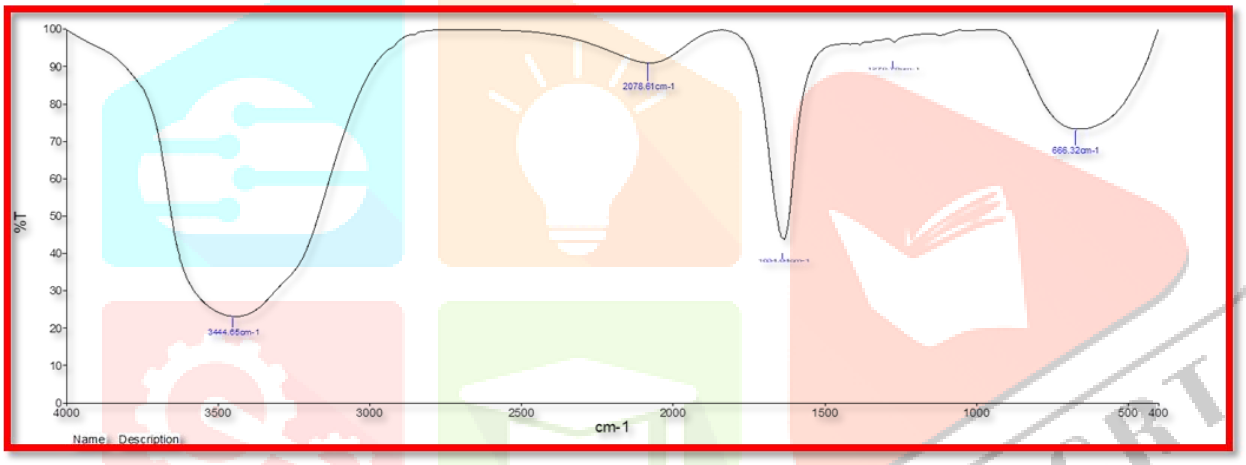


FIGURE: 5 FTIR ANALYSIS OF *Aloe vera*

TABLE: 5 FTIR ANALYSIS OF *Aloe vera*

S.No	Frequency Range	Types of bond	Types And Group
1	3444.65	Hydrogen-bonded O-H stretch	Phenols and alcohols
2	2078.61	N=C=S stretching	Isothiocyanate
3	1634.04	C=N stretching	Imino\oxime
4	1270.70	C-O stretching	Alkyl aryl alcohol
5	666.32	C-Br stretching	Halo compound

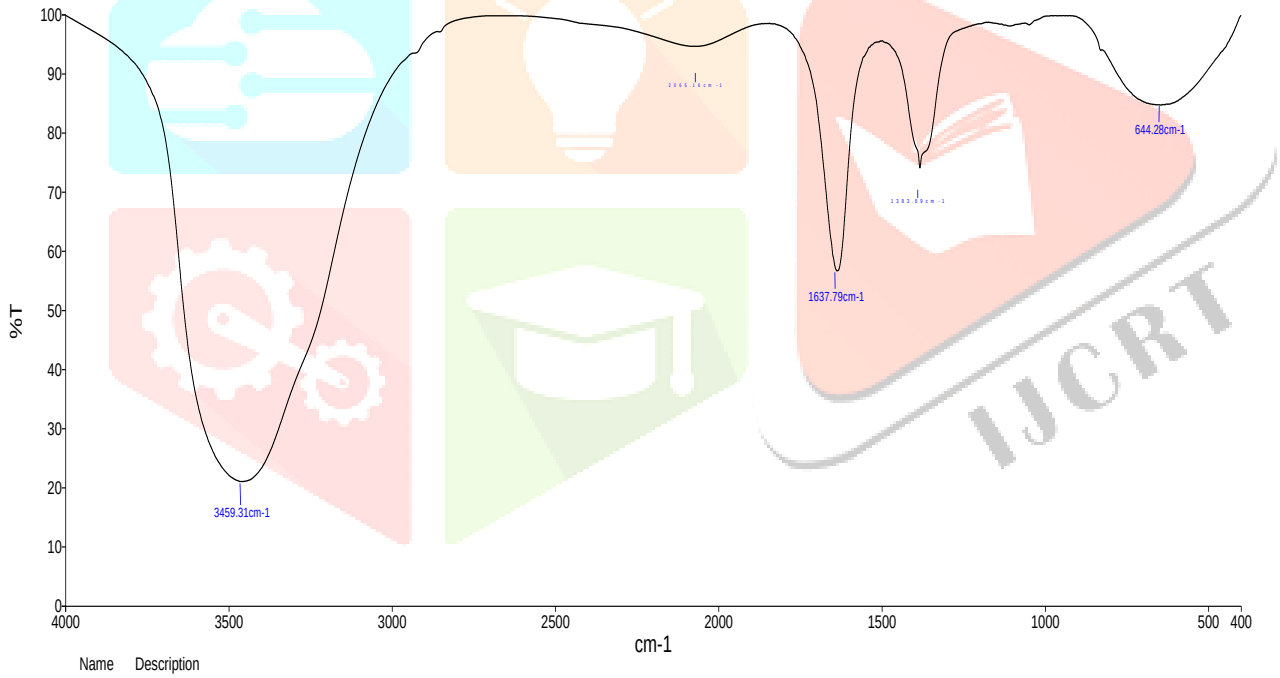


FIGURE: 6 FTIR ANALYSISOF *AZADIRACHTA INDICA*

TABLE: 6 FTIR ANALYSISOF *AZADIRACHTA INDICA*

S.NO	FREQUENCY RANGE	TYPE OF BOND	TYPE AND GROUP
1.	3459.31	O-Hstretching	alcohol
2.	2065.16	N=C=Sstretching	isothiocyanate
3.	1637.79	C=Cstretching	Conjugated alkene
4.	1383.69	C-H bending	alkane
5.	644.28	C-Brstretching	Halo compound

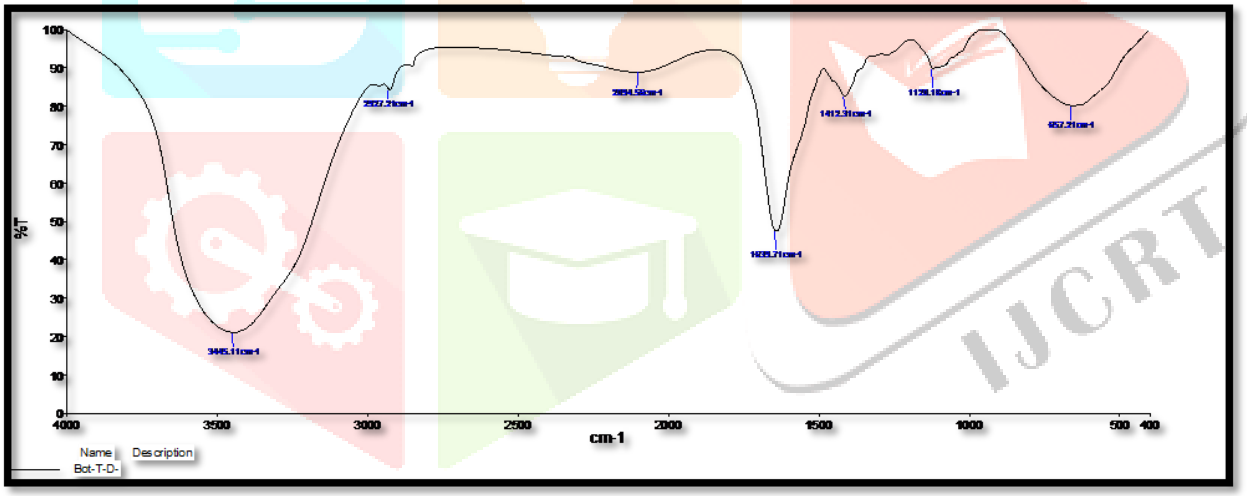


Figure:**7 FTIR analysis of *Azadirachta indica And Aloe vera***

Table 7: FTIR analysis of *Azadirachta indica and Aloe vera* formulation

S. No	Frequency Range	Type Of Bond	Type Of Group
1	3445.11	O-H stretching	Alcohol
2	2927.21	N-H stretching	Amine salt
3	2094.56	N=C=S stretching	Isothiocyanate
4	1639.71	C=N stretching	Imine\oxime
5	1412.31	S=O stretching	Sulfate
6	1120.18	C-O stretching	secondary alcohol
7	657.21	C-Br stretching	Halo compound

Table 8:GC-MS analysis of *Azadirachta indica and Aloe vera* formulation

PEAK	RETENTION	HEIGHT %	NAME
1	6.357	1.43	Cyclotetrasiloxane, octamethyl-
2	9.939	2.87	Cyclopentasiloxane, Decamethyl-
3	26.625	11.26	L-(+)-Ascorbic Acid 2,6-Dihexadecanoate
4	28.682	1.02	2-Oxaspiro[4.5]Decan-3-One
5	28.778	0.93	2-Cyclobutene-1-Carboxamide
6	29.443	63.71	2-Octylcyclopropene-1-heptanol
7	29.685	8.19	Z,E-3,13-Octadecadien-1-ol
8	29.77	7.08	2-Aminoethanethiol Hydrogen Sulfate (Ester)
9	38.388	1.2	(-)-Thujopsen
10	39.086	2.32	Ethyl Iso-Allocholate

Table: 9 *In vitro* anti-oxidant activity of *Azadirachta indica and Aloe vera* formulation by using DPPH assay

Test	Con of *A. indica and A.vera* formulation (mg\mL)	% Of inhibition for *A. indica and A. vera* formulation	%Of inhibition for Ascorbic acid
DPPH assay	20	25	40
	40	42	50
	60	53	60
	80	64	75
IC 50 Value		56	40

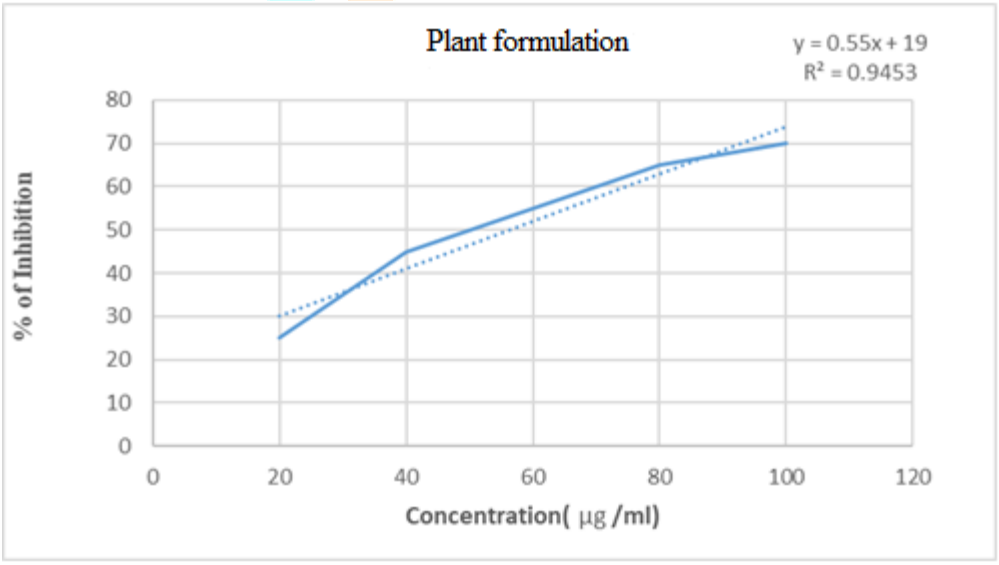


Figure: 8 a - *In vitro* anti-oxidant activity of *Azadirachta indica and Aloe vera* formulation by using DPPH assay

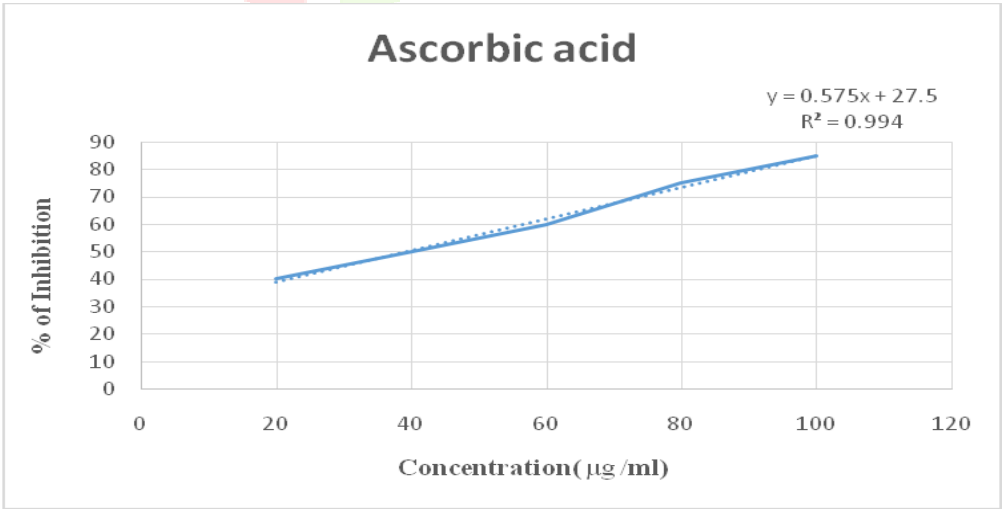


Figure: 8 b - *In vitro* anti-oxidant activity of *Azadirachta indica and Aloe vera* formulation

By using DPPH assay

Table: 10 Anti-oxidant activity of *Azadirachta Indica and Aloe vera* formulation by using H2O2 assay

Test	**Concentration of *Azadirachta Indica and Aloe Vera* formulation/ Ascorbic acid** mg/mL	**% of inhibition for *Azadirachta Indica and Aloe Vera* formulation**	**% of inhibition for Ascorbic acid**
H2O2 assay	20	34	46
	40	55	65
	60	66	76
	80	76	86
IC 50 Value		38	20

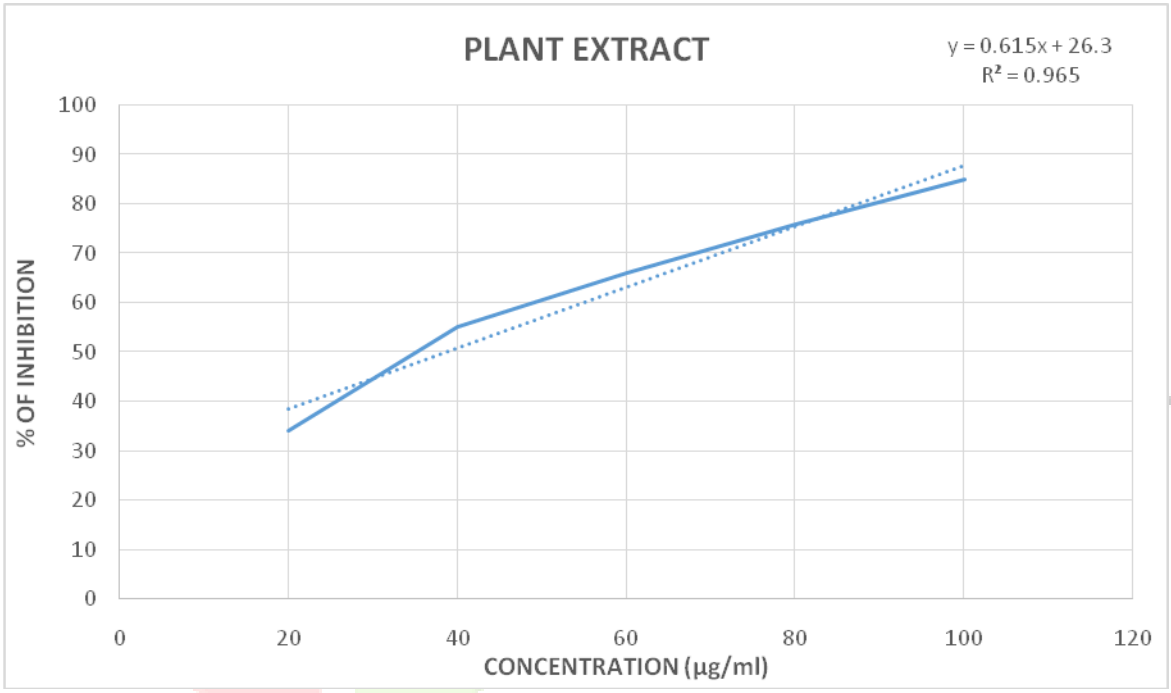


Figure: 9 a - Anti-oxidant activity of *Azadirachta Indica and Aloe Vera* formulation by using H2O2 assay

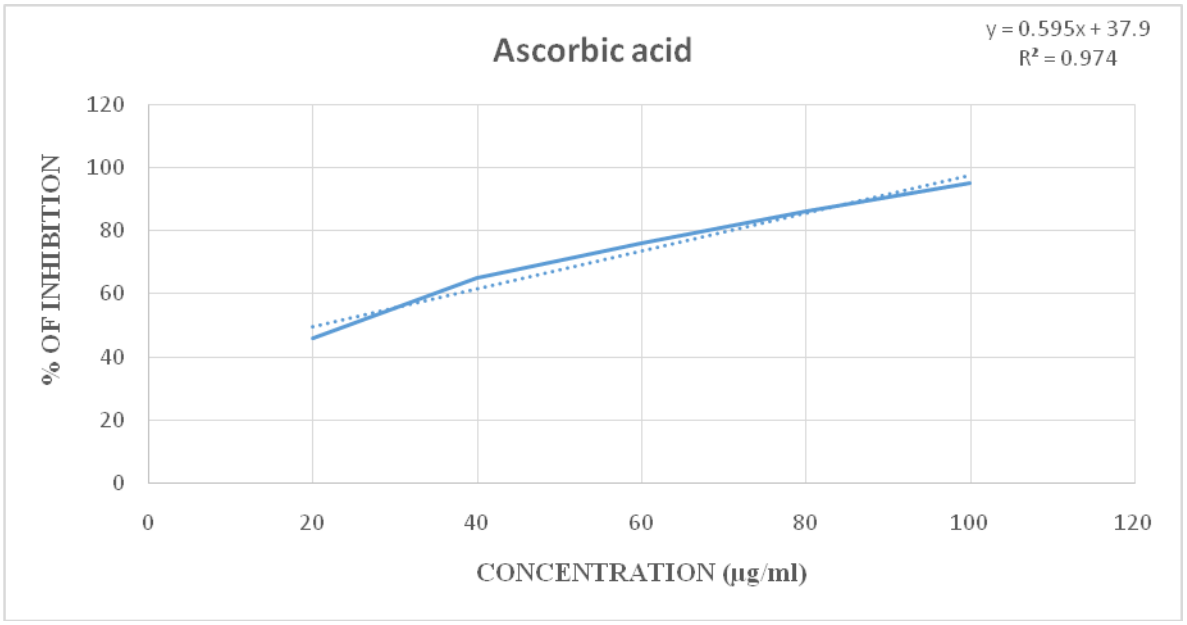


Figure: 9 b - Anti-oxidant activity of *Azadirachta Indica and Aloe Vera* formulation by using H2O2 assay

Table: 11 Anti-oxidant activity of *Azadirachta Indica and Aloe Vera* formulation by using Reducing power assay

Test	**Concentration of plant extract** mg/mL	**% of inhibition**	**Ascorbic acid** mg/mL
ing power assay	20	24.0	26
	40	40.0	52
	60	64.0	75
	80	70.5	87
IC 50 Value		50.30	40

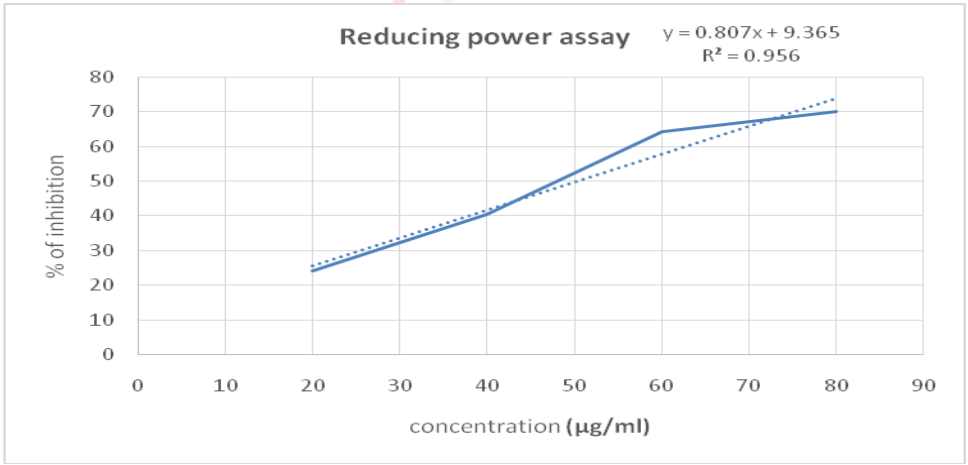


Figure: 10 a - Anti-oxidant activity of *Azadirachta Indica and Aloe Vera* formulation by using Reducing power assay

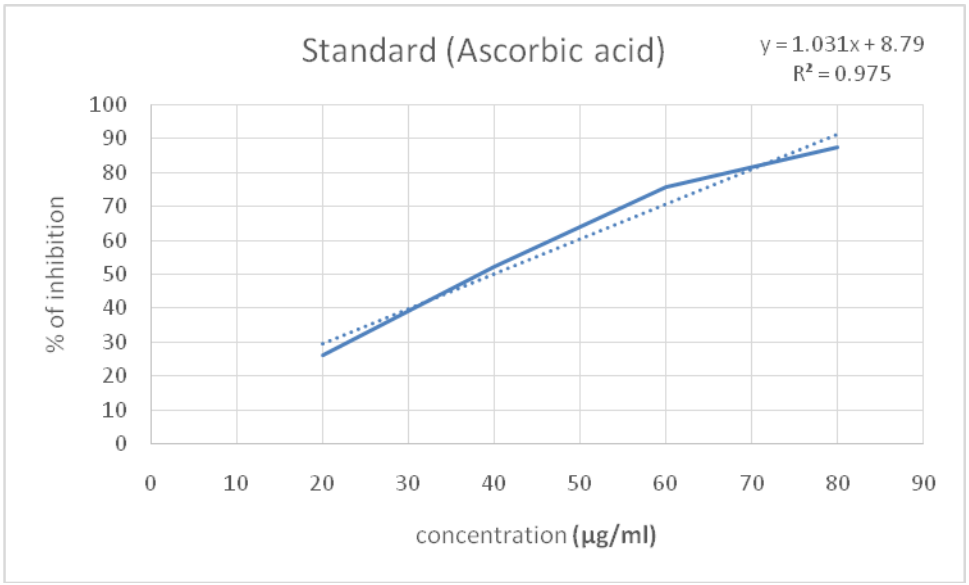
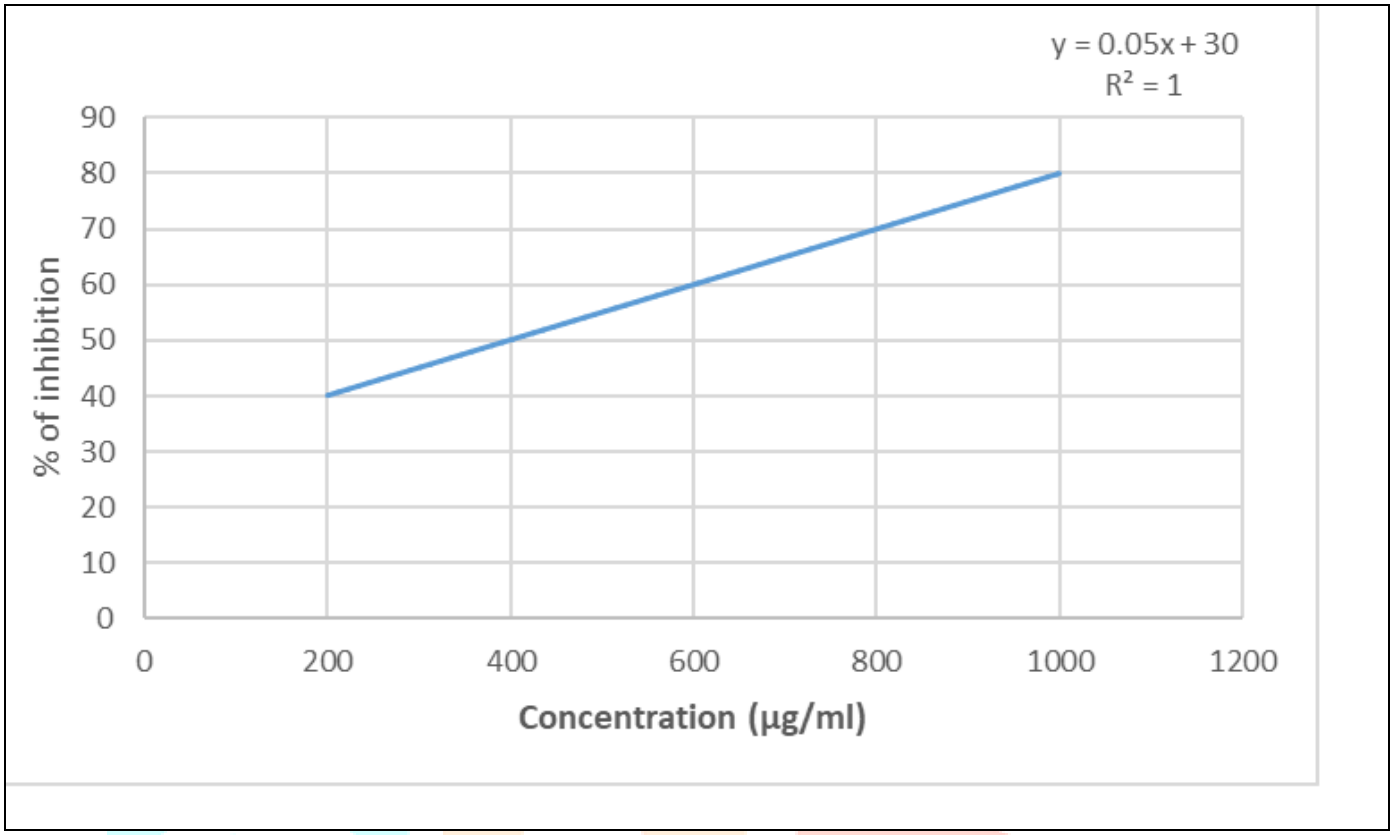


Figure: 10 b- Anti-oxidant activity of *Azadirachta Indica and Aloe Vera* formulation by using Reducing power assay

TABLE:12 Alpha amylase assay of ***Azadirachta Indica and Aloe Vera*** extracts

TYPE	**CONCENTRATION OF PLANT EXTRACT(µg/ml)**	**% OF INHIBITION**	**Acarbose**
Alpha Amylase Enzyme	200	40	45
	400	50	55
	600	60	65
	800	70	75
IC $_{50}$ Value		400	300



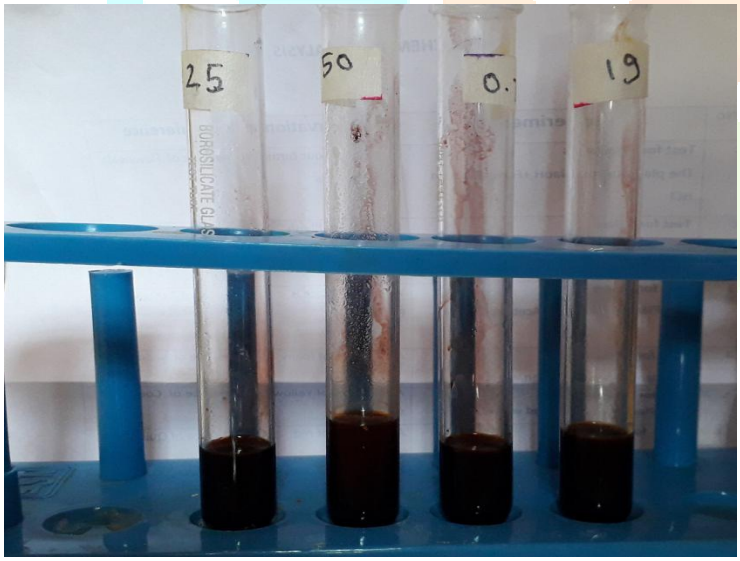


Fig:11 Alpha amylase assay of *Azadirachta Indica and Aloe Vera* formulation

Table: 13 - *In vitro* Anti-diabetic activity of *Azadirachta Indica and Aloe Vera* formulation by using Alpha- Glucosidase enzyme

Type	**entration of plant extract** mg/mL	**% of inhibition**	**Acarbose** mg/mL
α-Glucosidase enyme	200	56	45
	400	66	55
	600	76	65
	800	86	75
IC 50 Value		36.3	300

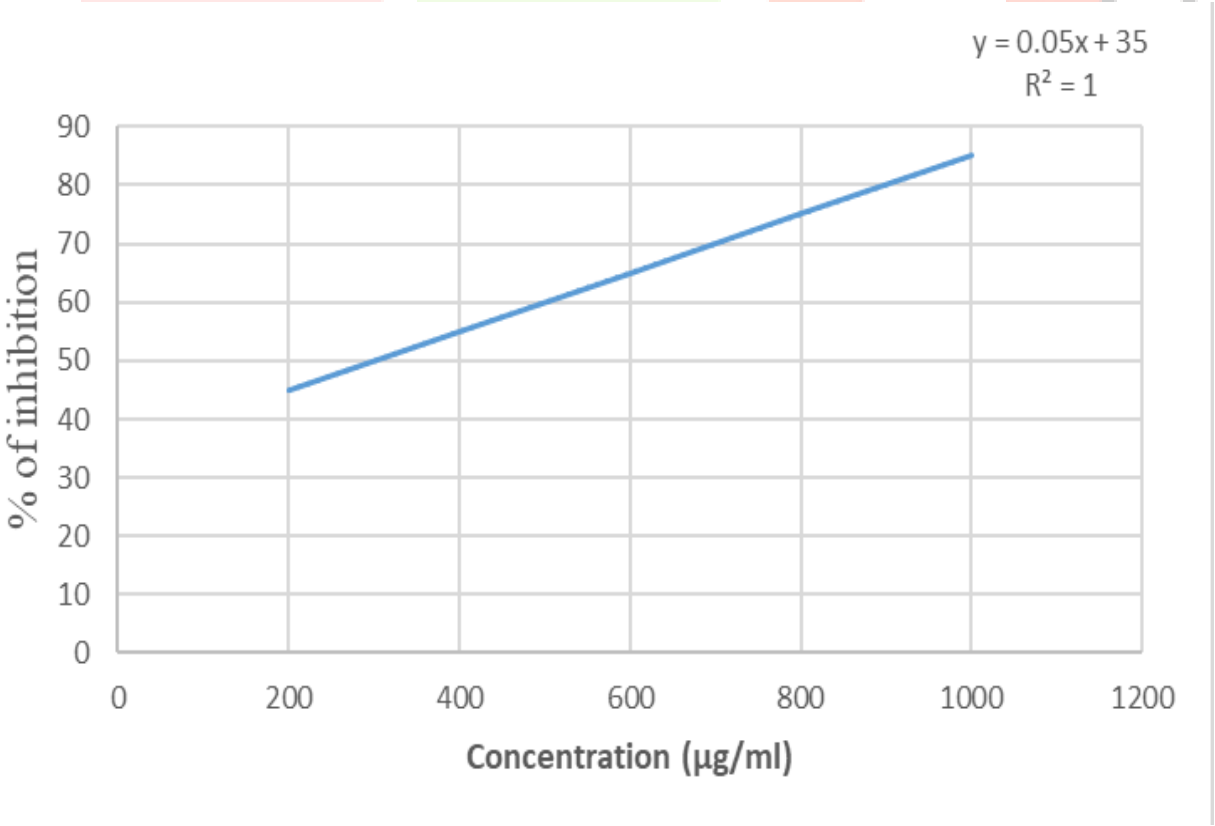


Fig 12 a : Anti-diabetic activity of *Azadirachta Indica and Aloe Vera* formulation by using Alpha-Glucosidase enzyme

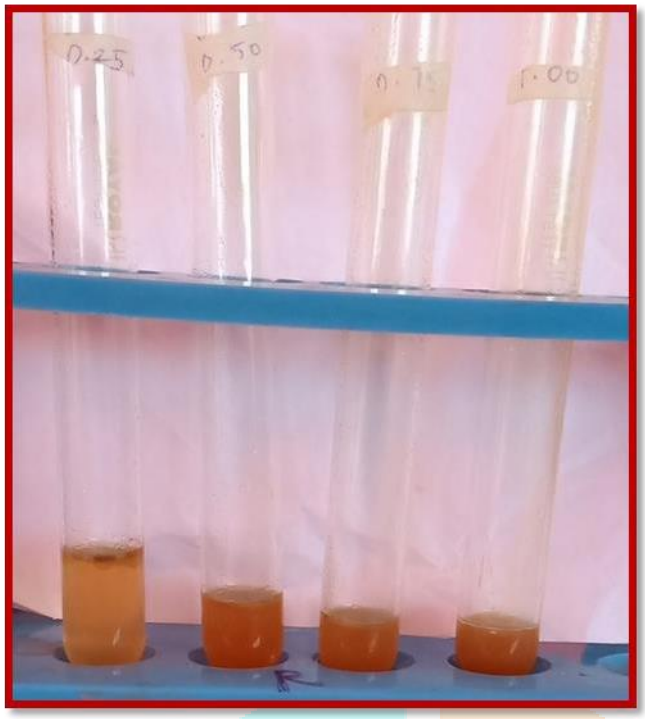
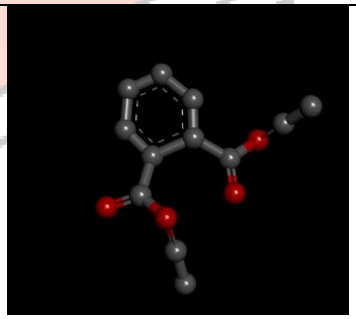


Figure: 12 b - *In vitro* Anti-diabetic activity of *Azadirachta Indica and Aloe Vera* formulation by using Alpha-Glucosidase enzyme

Table: 14 Structure Phytocompound (Diethyl Phthalate)

Table:

S.No	Compound Names	Canonical SMILES	Compounds structure
1.	ethyl Phthalate	O=C(OCC)C1=CC=CC=C1C(OCC)=O	

15 :Molecular docking analysis of compound with binding pocket of Alpha Glucosidase

S.No	Compounds	Binding energy	H-bond interaction	H-bond distance Aº
1.	ethyl Phthalate	**-11.83**	**ARG 337…O1** **GLN 41…O2** **ARG 195…O3** **ASP 300…O4**	**3.4** **3.1** **2.4** **3.2**

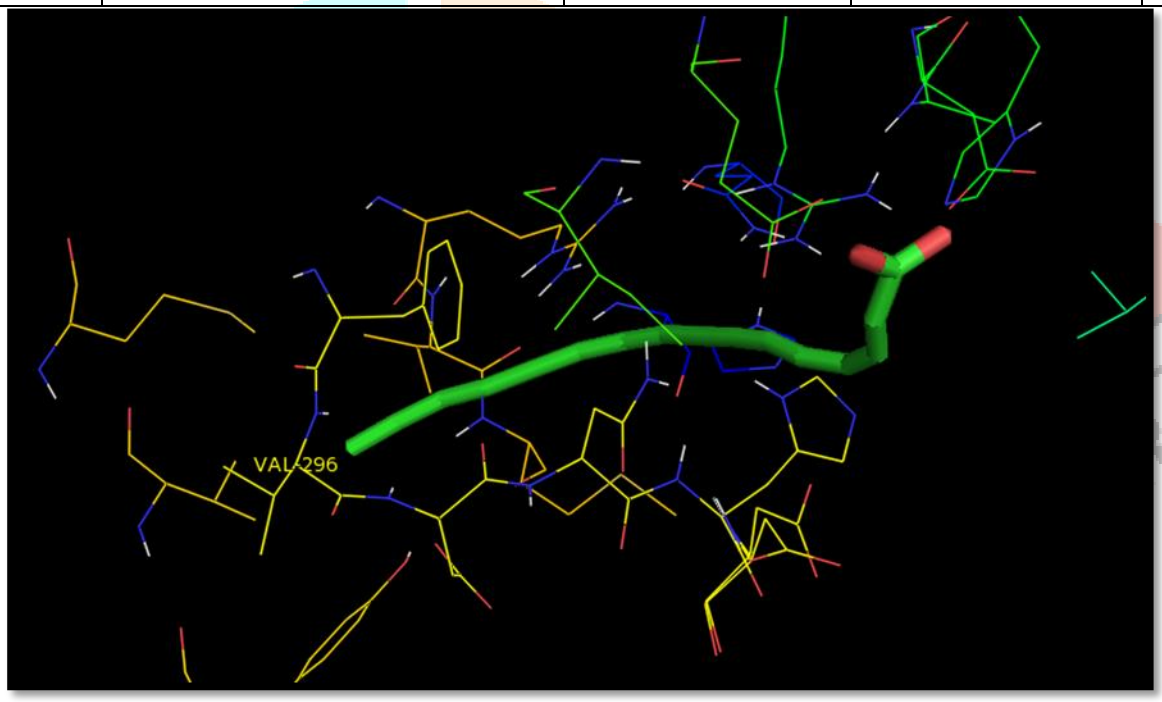


FIG.13 Docking pose of compound on binding pocket of alpha amylase. (Yellow line represents Hydrogen bond interactions).

Table: 16 - *In vitro* Anti-diabetic activity of *Azadirachta Indica and Aloe Vera* formulation by using Alpha- Glucosidase enzyme

Type	**entration of plant extract** mg/mL	**% of inhibition**	**Acarbose** mg/mL
pha glucosidase enzyme	200	56	45
	400	66	55
	600	76	65
	800	86	75
IC 50 Value		36.3	300

Figure: 14 a –*In vitro* Anti-diabetic activity of *Azadirachta Indica and Aloe Vera*

formulation by using Alpha-Glucosidase enzyme

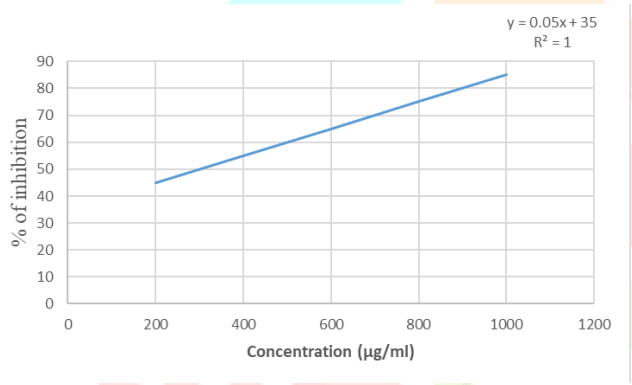


Figure: 14 b - *In vitro* Anti-diabetic activity of *Azadirachta Indica and Aloe Vera* formulation by using 5.8 Alpha-Glucosidase enzyme

Table: 17 Structure Phytocompound (Diethyl Phthalate)

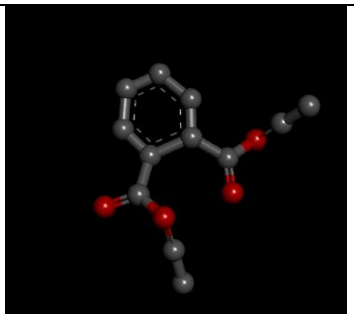
S.No	Compound Names	Canonical SMILES	Compounds structure
1.	ethyl Phthalate	O=C(OCC)C1=CC=CC=C1C(OCC)=O	

Table: 18 Molecular docking analysis of compound with binding pocket of Alpha Glucosidase

S.No	Compounds	Binding energy	H-bond interaction	H-bond distance Aº
1.	ethyl Phthalate	**-11.83**	**ARG 337…O1** **GLN 41…O2** **ARG 195…O3** **ASP 300…O4**	**3.4** **3.1** **2.4** **3.2**

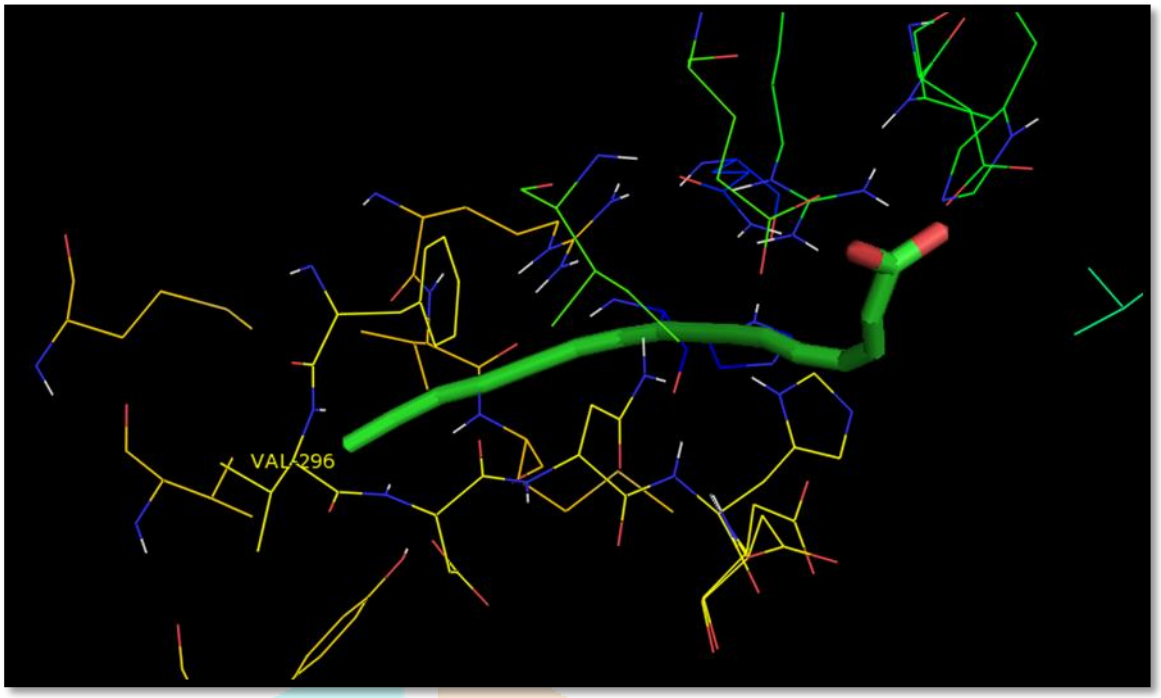


FIG.15 Docking pose of compound on binding pocket of alpha amylase. (Yellow line represents Hydrogen bond interactions).

TLC of plant extract in aqueous reports four spots for various phytochemicals. The reported spots are separated with enough space and having various R_f values showing the presence of atleast three phytochemicals in aqueous extracts. In our study, the most suitable TLC system for analysis was shown to be Water (5:4:1) with the largest discriminating power. Four bands found in this method and its R_f values were 0.15, 0.30, 0.76 and 0.92.This values indicate the presence of phenolic compound, flavonoid ,alkaloid and tannin (Mehta Sonam *et al*., 2017).

Due to the sharpness of the peaks and proper baseline, the UV-VIS profile of Azadirachta indica aqueous extract was taken at wavelengths ranging from 200 nm to 1100 nm. Peaks were visible at 883nm, with an absorption of 0.0913. The absorption spectrum of Azadirachta indica extract is shown in Figure 2, and it is almost transparent in the wavelength range of 200-900 nm. **(Table:2 and Fig 2).**

Due to the sharpness of the peaks and proper baseline, the qualitative UV-VIS profile of Aloe vera water extract was taken at wavelengths of 200 nm to 300 nm. Peaks were seen at 263.75nm, with an absorption of 0.0346. The absorption spectrum of Aloe vera extract is shown in Figure 1, and it is almost transparent in the wavelength range of 200-300 nm. **(Fig 3).**

Due to the sharpness of the peaks and proper baseline, the qualitative UVVIS profile of ethanolic extract of Azadirachta indica Aloe vera formulation was taken at wavelengths of 200 nm to 1100 nm. Peaks were found at 216.85, 355.40, 663.25, 759.05, 830.20, 943.45, 997.00, 1076.60nm, with absorption values of 4.0000, 1.0493, 0.4697, 0.4038, 0.4360, 0.4013, 0.3950, 0.4093. The absorption spectrum of Azadirachta indica Aloe vera formulation is shown in Figure 3, and it is nearly transparent in the wavelength range of 200-1100 nm.

Table 3 shows the absorption bands found in the Azadirachta indica Aloe vera formulation. The existence of one or more peaks in the UV-VIS spectra between 200 and 400 nm indicates the presence of unsaturated groups and heteroatoms such as S, N, and O. Two peaks at 278 nm and 457 nm are visible in the spectrum of Azadirachta indica Aloe vera formulation. This demonstrates that the plant extract contains organic chromophores. However, the use of UV-visible spectrophotometery in the study of complex media is constrained by the inherent difficulties in assigning absorption peaks to specific constituents in the system. To allow proper extract characterization and constituent identification, UV–VIS findings must be supplemented with other analytical techniques such as GC/MS.

The absorption maxima of flavonoids are usually in the ranges of 230-285 nm (band I) and 300-350 nm (band II) (band II). The exact location and relative intensities of these maxima provide important details about the flavonoids' composition. This is consistent with previous research on Acorus calamus. (Nandha Kumar *et al.*, 2015)

Based on the peaks values in the IR radiation region, the FTIR spectrum was used to classify the functional groups of the active components present in the extract. The functional groups of the components were divided based on the peaks ratio when the extract was passed through the FTIR. The existence of alcohol, phenol, alkanes, aldehyde, aromatic compound, secondary alcohol, aromatic amines, and halogen compound was confirmed by FTIR analysis. (**fig-5-7 and Table-5-7)**

FTIR measurements were used to classify the potential biomolecules responsible for Aloe vera's antimicrobial activity. The presence of active functional groups in Aloe vera is shown by the abundance of absorption bands in this spectrum. Some intensity peaks, such as 3444, 2078, and 1634 cm-1, have increased significantly, whereas others, such as 1270 and 666 cm-1, have decreased. The band at 3444 corresponds to hydrogen-bonded O-H Stretching vibrations in phenols and alcohols, as shown in Figure 6. Iminooxime is represented by the peak at 2078, which corresponds to N=C=S stretching, and isothiocyanate is represented by the peak at 1634, which corresponds to C=N stretching.

To Nitriles, stretch in a plane curve. Alkyl aryl alcohol is represented by the peak at 1270, which corresponds to C-O stretching. The weak band at 666 represents the presence of Halo compound in the plant extract and corresponds to C-Br stretching.

GC-MS analysis was used to identify the compounds present in the aqueous extracts of Azadirachta indica and Aloe vera (Figure 8). Table 8 lists the active concepts, along with their retention time (RT), molecular formula, molecular weight (MW), and concentration (percentage). GC-MS found seventeen compounds in methanolic extract. The key components in the CAA formulation, such as. Cyclotetrasiloxane, octamethyl-, Cyclopentasiloxane, Decamethyl-, L-(+)-Ascorbic Acid 2,6-Dihexadecanoate 2-Oxaspiro[4.5]Decan-3-One 2-Cyclobutene-1-Carboxamide 2-Octylcyclopropene-1-heptanol, Z,E-3,13-Octadecadien-1-ol 2-Aminoethanethiol Hydrogen Sulfate (Ester) (-)-Thujopsen, Ethyl Iso-Allocholate. These phytochemicals have antimicrobial and anti-oxidant properties, as well as anti-inflammation, anti-cancer, hepatoprotective, diuretic, and anti-asthma properties. **(Table 8) .**

The percentage of scavenging effect on the DPPH• radical increased in tandem with the increase in normal and Azadirachta indica and Aloe vera formulation concentrations from 20 to 80 mg/mL in the current sample. For Azadirachta indica and Aloe vera formulations, the percentage of inhibition ranged from 25,42,53,64 at 20 mg/mL to 80 mg/mL, and the IC50 value was 56 mg/ml for Azadirachta indica and Aloe vera formulations, while it was 0.60 mg/ml for normal. (Table 9, Fig 8 a &b).

DPPH• is a popular free radical used to test the seed extract's preliminary radical scavenging capacity. The inhibition of lipid peroxidation is linked to the scavenging of the DPPH• radical **(Rekka and Kourounakis, 1991).** DPPH• is a material that is commonly used to evaluate anti-oxidant activity **(Tara Chand et al., 2012).**

The percentage of inhibition in the H2O2 assay was 34, 55, 66, and 76 at 20, 40, 60, and 80 mg/mL concentrations, respectively. The IC50 value for Azadirachta Indica and Aloe Vera formulation was 38 mg/ml, while it was found to be 20 mg/ml for the standard drug. The findings of this study's H2O2 scavenging activity are close to those of Cinnamomum verum's in vitro anti-oxidant activity. **(Mathew *et al.*, 2006)**.

The present study shows that H2O2 inhibits the formation of hydroxyl radicals in a dose-dependent manner. H2O2 is able to quickly pass across cell membranes. These molecules will be transformed into hydroxyl radicals, which will cause cell damage. Anti-oxidants are the compounds that helped H2O2 by donating electrons. By converting them into water, the donating electron interacts with H2O2 and neutralizes it.

Antioxidants play an important role in disease prevention in humans. Antioxidant compounds can serve as free radical scavengers, pro-oxidant metal complexes, reducing agents, and quenchers of single-oxygen production or reactive oxygen molecules, protecting the body from degenerative diseases like cancer. Reactive oxygen species (ROS) may be harmful as a result of products produced during normal cellular metabolism or as a result of a toxic insult. They cause oxidative stress, which damages lipids, proteins, and DNA, contributing to the pathogenesis of a variety of human diseases. **(Steenkamp et al., 2005).**

In Reducing assay the percentage of inhibiton of 34.1, 42.5, 45.8, 66.5 at 0.25, 0.50, 0.75, 1.00 g/mL concentration respectively and the IC_{50} value was for *Azadirachta Indica and Aloe Vera* formulation was found to be 0.69 mg/ml while for standard drug it was found to be 1 mg/ml. The results of reducing assay scavenging activity of this study were similar to the results of the *in vitro* anti-oxidant activity of *Acacia fistula* **(Luximon-Ramma *et al.*, 2005).**

Some of the phytochemical constituents of the extract may be responsible for the anti- oxidant activity as demonstrated in the present study. Flavonoids, also known as bioflavonoids, are a class of polyphenolic compounds found in most plants and abundant in seeds, fruit skin or peel, bark, and flowers.Numerous studies have shown that flavonoids possess potent anti- oxidant activities capable of scavenging hydroxyl radicals, superoxide anions, and lipid peroxy radicals **[Alan *et al.*, (1996)**]. **Shahidi *et al.*, (1992)** documented the pharmacological activities (anti-inflammatory, anti-viral, anti-bacterial, anti-ulcer, anti-osteoporotic, anti-allergic, and anti-hepatotoxic actions) of flavonoids for their potent anti-oxidant activity.

Table 12 and Fig 11 showed 76.9% inhibitory effect on the α- amylase activity at a concentration of 1.00mg/mL. The plant showed higher α-amylase inhibitory activity compare to acarbose. α-amylase is an enzyme that hydrolysis α bonds of large α linked polysaccharide like starch and glycogen to yield disaccharides like maltose which will further hydrolyze by α-glucosidase to yield monosaccharides like glucose[sudha2011]. The inhibitors of α amylases bind to α bond of polysachharide and stop the breakdown of polysaccharide-in mono and disaccharide.

The CCT formulation had an important inhibitory effect on the enzyme -glucosidase. The percentage inhibition of Azadirachta Indica and Aloe Vera formulations at 0.25-1.0 mg/mL concentrations showed a concentration-dependent increase in percentage inhibition. For the lowest concentration to the maximum concentration, the percentage inhibition ranged from 53.3 to 85.0. The inhibitory activity of positive control Acarbose yielded percentages of 50 for 0.25 mg/mL and 90 for 1.0 mg/mL, whereas the concentration needed for 50% inhibition (IC50) was found to be 0.15 mg/mL. The standard drug Acarbose has an IC50 value of 0.13 mg/mL against -glucosidase. (Table 13, fig 12 a & b).

Intestinal -glucosidase is a central enzyme in carbohydrate digestion that has been identified as a therapeutic target for postprandial hyperglycemia modulation. Mammalian species, on the other hand, alpha-glucosidase crude extract from rat intestinal mucosa comprises a mixture of sucrase, maltase, isomaltase, and glucoamylase enzyme activities. **(Jones *et al.*, 2011; Dhital *et al.*, 2013)**.

The complex carbohydrates in food are rapidly absorbed in the intestine, assisted by the alpha -glucosidase enzyme, which splits disaccharides into absorbable mono saccharides. The alpha-glucosidase inhibitor inhibits disaccharide digestion and postprandial glucose excursion, resulting in a smooth glucose profile overall. Previous research studies on α-amylase and α-glucosidase inhibitors identified from medicinal herbs recommend that a number of capable inhibitors belong to terpenes, tripenes, flavonoids that has features of inhibiting α-amylase and α-glucosidase activities.

In the current study, the *Azadirachta Indica and Aloe Vera* formulation also contains triterpenes and flavonoids that have features of inhibiting α-amylase and α-glucosidase activities.

AutoDock4 was used to dock in the ATP-binding bag. The interaction of protein and ligands in the binding pocket was described using a grid map in Autodock. The grid map was used, with 60 points evenly spaced at 0.375A° in each x, y, and z direction. The Lamarckian genetic algorithm was used for docking. 100 docking experiments were carried out, producing 100 docked conformations. The following parameters were used for docking: 150 individuals in the population, with a random starting place and conformation. The results of the molecular docking analysis indicate that the compound from *Caesalpinia Bonducella* were more selective towards the ATP-binding pocket of alpha amylase.

The docked poses with Lowest Binding Energy (LBE), Hydrogen bond interaction results were recorded (table 14) and validated . The expected binding energy was found between -9.38 and -5.29 kcal/mol. These binding energy values indicate that the newly synthesized compounds had shown a fortunate selectivity towards ATP-binding pocket of alpha amylase. Figure 7 shows a 2D view of protein–ligand interactions produced by the 1AJ6 routines that were studied. All the top docked poses generated (table 3) by each docking routine exhibited well-established bonds with one or more amino acids in the binding pocket of 3L2M and 2VTK. Compound **(Diethyl Phthalate)** from CCT formulation shows hydrogen bonds with less distance was observed **with binding pocket of Alpha amylase** (table 7 and 8). From the results of the docking analysis, it was concluded that the compound 9, 12-Octadecadienoic acid (Z,Z) in the CCT formulation accommodated in ATP-binding pocket of alpha amylase which might be a reason for good activity against diabetes.

The CCT formulation had an important inhibitory effect on the enzyme alpha -glucosidase. The percentage inhibition at 0.25-1.0 mg/mL concentrations of *Azadirachta Indica and Aloe Vera* formulation showed a concentration dependent increase in percentage inhibition. The percentage inhibition varied from

53.3 to 85.0 for lowest concentration to the highest concentration. The concentration required for 50% inhibition (IC50) was found to be 0.15 mg/mL whereas the α-glucosidase inhibitory activity of positive control Acarbose produced percentage of 50 for 0.25mg/mL and 90 for 1.0 mg/mL. The IC50 value of standard drug Acarbose against α-glucosidase

was found to be 0.13mg/mL (Table 16, fig 14 a & b).

Intestinal α-glucosidase is a key enzyme for carbohydrate digestion; it has been recognized as a therapeutic target for the modulation of postprandial hyperglycemia. On the other hand, the Mammalian species -glucosidase crude extract from rat intestinal mucosa comprises a mixture of sucrase, maltase, isomaltase, and glucoamylase enzyme activities. **(Jones *et al.*, 2011; Dhital *et al.*, 2013)**.

Since the complex carbohydrates in food are quickly consumed in the intestine with the aid of the α -glucosidase enzyme, a high-carbohydrate diet triggers a sharp increase in blood glucose levels, the alpha -glucosidase inhibitor, which converts disaccharides into absorbable mono saccharides, prevents disaccharide digestion and postprandial glucose excursion, resulting in a more consistent glucose profile. Previous research studies on α-amylase and α-glucosidase inhibitors identified from medicinal herbs recommend that a number of capable inhibitors belong to terpenes, tripenes, flavonoids that has features of inhibiting α-amylase and α-glucosidase activities.

In the current study, the *Azadirachta Indica and Aloe Vera* formulation also contains triterpenes and flavonoids that have features of inhibiting α-amylase and α-glucosidase activities.

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The Hydrogen bond interaction results from docked poses with Lowest Binding Energy (LBE) were reported (table 17) and validated. The expected binding energy was found between -9.38 and -5.29 kcal/mol. These binding energy values indicate that the newly synthesized compounds had shown a fortunate selectivity towards ATP-binding pocket of alpha amylase. Figure 7 shows a 2D view of protein–ligand interactions produced by the 1AJ6 routines that were studied. All the top docked poses generated (table 14) by each docking routine exhibited well-established bonds with one or more amino acids in the binding pocket of 3L2M and 2VTK. Compound **(Diethyl Phthalate)** from CCT formulation shows hydrogen bonds with less distance was observed **with binding pocket of Alpha amylase** (table 14). From the results of the docking analysis, it was concluded that the compound 9, 12-Octadecadienoic acid (Z,Z) in the CCT formulation accommo dated in ATP-binding pocket of alpha amylase which might be a reason for good activity against diabetes.

Conclusion

The present study reveals that extracts are a good source of antioxidant property containing phytoconstituents. The presence of flavanoids, tannins, steroids and saponins present in extracts may be responsible for antioxidant activity. According to the findings, all of the plants have a significant antioxidant impact. The findings revealed that this plant is very significant from a medicinal standpoint, and that further phytochemical research is needed to isolate phytochemical constituents with antioxidant activity.

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