Phytochemical Screening, Anti-Oxidant and Enzymatic Activity of *Azadirachta Indica*

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ABSTRACT

Introduction: *Azadirachta indica* (Meliaceae) is an evergreen tree. A. indica's various components have wide range of chemicals which includes azadirachtin, nimbin, nimbinin, nimbandiol, nimbolide, and nimbidin. A.indica is utilizedto treat various diseases like rheumatism, syphilitic sores, leucoderma, ophthalmopathy, leprosy, intermittent fevers, burning, pruritus, dyspepsia, tuberculosis, lumbago, psoriasis, acne, eczema, leprosy and blood impurities. Itspharmacologicalproperties includesantioxidant,antimicrobial, antipyretic, anti-inflammatory, antidiabetic, antifungal, antiviral and anxiolytic. **Methodology:** The anti-oxidant activity was evaluated in vitro by DPPH free radical scavenging method and alpha amylase as well as alpha glucosidase enzymes inhibitory activity was performed. **Results:** The Azadirachta indica leaf extract showed significant anti-oxidant and enzyme inhibition activity as compared to the standard drugs. Antioxidant activity showed 83.23 ± 0.1% at the concentration of 5mg/ml with an IC_{50} value of 0.488 mg/ml. Ascorbic acid was used as a standard.A.indica showed 66.7±0.1% and 63.5± 0.01%at 5mg/ml inhibition for Alpha glucosidase enzymes and Alpha amylase with an IC_{50} value of 1.325 mg/ml and1.414 mg/ml respectively. A.indica leaves extract phytochemical screening demonstrated the existence of lipids, carbohydrates, tannins, phenols,alkaloids, glycosides, flavonoids, steroids/terpenes, coumarins, and quinones. **Conclusion:** In accordance with the findings, it can be concluded that A.indica has significant antioxidant and enzymes

inhibitory activities. A.indica can be employed as an antioxidant agent, alpha glucosidae and alpha amylaseinhibitors for treating various diseases.

Key Words: Azadirachta indica, Antioxidant, Alpha glucosidae, Alpha amylase, phytochemical

1. Introduction

In molecular biology, the development of degenerative processes is linked to the existence of an excess of free radicals, which encourages oxidative reactions that are bad for the organism. Interest in using plants in preventative and therapeutic phytotherapy has been sparked by their high concentration of some substances possessing anti-oxidant qualities that can scavenge free radicals.In biological cells, antioxidants have the job of scavenging free radicals, which seem to be bad for living organisms. enzyme known as superoxyde dismutase has a specific function in reducing the consequences induced by the existence of free radicals, oxidative stress (SOD).In molecular biology, the prevalence of degenerative processes is associated this metaloenzyme, which controls oxidation processes in biological cells primarily, has a subunitary structural organisation. The oxygen radicals' recombination reaction is catalysed by this enzyme. In the past thirty years, oxidative stress has become a popular notion in medical sciences. It actively participates in the physiology of many common disorders, comprising elevated blood pressure, Parkinson's disease, Alzheimer's, preeclampsia, atherosclerosis, also acute renal failure. [1]

Direct antioxidants, that can prevent components from oxidation and may manifest their function in a tube or in a living creature, must be distinguished from indirect antioxidants, whereby cannot provide any protection for materials that are oxidizable but can strengthen the antioxidant defense in such a living thing, for eg by exacerbating a metabolic pathways of antioxidant enzymes. [2]

Antioxidants can be produced chemically or can be found naturally in plants, animals, and microbes. Natural antioxidants such as tocopherols as well as polyphenols,which are plentiful in foods including teas, grains, seeds, fruits, veggies, cereals, herbs, spices, and oils,has abundance in higher plants and their constituents.[3]Many bioactive substances with significant antioxidant activity can be found in plants. [4]Natural or manufactured antioxidants, such as tocopherols, inhibit the negative outcomes of oxygen generation as well as halt free radical production causing harm via ensnaring interlocking or neutralising them. However, natural antioxidants seem to be insufficient to fulfill the requirements of the food sector due to extraction challenges and inadequate stability. [5]

Several biological processes, like the breakdown of carbohydrates, the breakdown of glycoconjugates in the lysosomes, and the post-translational alteration of cellular glycoproteins, glycosidases, which catalyse a hydrolysis of glycosidic linkage in polysaccharides as well as glycoconjugates, play crucial roles. Mammalian -glucosidase (AG) particular catalyses the final stage the breakdown of starch and disaccharides, which is prevalent thus in nutrition of people. The small intestine's mucosal brushes edge is just where AG is located. The identification and creation of innovative treatment modalities for conditions such as diabetes, obesity, metastatic cancer, and viral infections are made possible by the considerable effects that glycosidases inhibition has had on polysaccharide metabolism, glycoprotein synthesis, and cellular contact. Inhibitors of AG slow down the small intestine's digestion of carbohydrates and lessen the postprandial blood sugar surge. [6]

The pseudo-carbohydrates acarbose, voglibose, or miglitol competitively block alpha-glucosidases with in small intestine's brush-border. This inhibitor 'blunts' postprandial hyperglycemia by delaying the upper small bowel's role in the breakdown of complex carbohydrates,which in turn slows glucose absorption. Acarbose that is still intact is not well absorbed and is eliminated in the faeces, where it can be up to 30% fermented by the colonic flora. Similar to how voglibose is swiftly expelled in stools, it is absorbed slowly and weakly. Miglitol, nevertheless, it was entirely ingested in the intestines and then was eliminated unchanged by the kidneys. Alpha-glucosidase inhibitor therapy may require several weeks for the lower smaller intestine to undergo adaptable modifications. These changes ensure complete carbohydrate absorption, reduce the occurrence of the first digestive complaints such stomach bloating, flatulence, or diarrhoea also seem to be connected to a higher endogenous activation of glucagon-like peptide-1. It's suggested to follow the start low, go slow dose philosophy in light of its adaptability & intermittent gastrointestinal side effects. Acarbose has undergone the most rigorous testing in terms of glucose-lowering effectiveness. [7]

Oral anti-hyperglycemic medications known as alpha-glucosidase inhibitorswork by competitively inhibiting intestinal alpha-glucosidase in a reversible manner. [8]Additionally, natural AGIs have been proposed as a first-line treatment by the International Diabetes Federation and the American Association of Clinical Endocrinologists. The development of quick, efficient, and user-friendly methods to screen for AGIs in natural goods is crucial. [9]Acarbose, voglibose, and miglitol are alpha glucosidase inhibitors that can be used for treatment. Comparable doses of voglibose are equally effective for these medications. [10]

Diabetic neuropathy, retinopathy, and cardiovascular disorders are just a few of the serious complications that can result from diabetes, a common metabolic condition defined by unusually high plasma glucose levels. Reducing the glucose absorption through inhibiting enzymes which break down carbs, such as -glucosidase, is one of the most effective strategies to treat diabetes mellitus, notably in particular non-insulin-dependent diabetes mellitus. The essential enzyme -glucosidase is responsible for catalysing the last stage of carbohydrate digestion. As a result, -glucosidase inhibitors can slow down the excretion of d-glucose from dietary complex carbs and postpone the absorption of glucose, both of whom reduce postprandial plasma glucose levels and reduce postprandial hyperglycemia. [11]

The utilization of organic a-glucosidase inhibitors obtained thru food sources as treatment for post-prandial hyperglycemia has gained popularity. Type II diabetes is frequently prevented or medically treated using a-glucosidase inhibitors. [12]

When type 2 diabetes is first discovered, alpha glucosidase inhibitors can be utilised as a first-line treatment. Due to their distinct method of action in regulating the release of glucose from complex carbs and disaccharides, they are especially helpful as a first-line treatment in newly diagnosed type 2 diabetes with high\\ PPG. [13]The early metabolic aberration that manifests in NIDDM, postprandial hyperglycemia, a medicinal objective being discovered for a-glucosidase. Dietary carbohydrates like starch, which are digested by a-glucosidases, are the main source of blood sugar. [14]

The hydrolase class of enzymes, which includes -amylase (-1, 4 glucan-4-glucanohydrolase), is present in microorganisms, plants, and animals. This enzyme is dependent on one Ca2+ ion per enzyme molecule to function and remain stable. Porcine pancreatic -amylase has 496 amino acid residues and is 83% identical to human pancreatic amylase. Amylase is present in saliva and

pancreatic juice. This enzyme's catalytic activity can be controlled to lower glucose synthesis in the postprandial phase, which may be therapeutic for diabetics. [15]

The tree Azadirachta indica belongs to Meliaceae family. Trees and shrubs comprise the majority of the Meliaceae family, commonly referred to as the Mahogany family. [16] Neem is evergreen, fast growing tree which has ability to grow at heights of 50 to 65 feet and indeed the its various components including fruit, leaves, flowers, seeds as well as the bark have been extensively utilized for various reasons. [17] Various Chemicals such asquercetin, azadirachtin, liminoids, nimbin, nimbinin, and nimbidin, have been derived from the various plant sections. [18]The condensed tannins frombark also contain (+) gallocatechin,(-) epicatechin, (+) catechin, and epigallocatechin. Margolone, margolonone, and isomargolonone are three tricyclic diterpenoids that were found in the stem bark of neem are effective against Serratia, Staphylococcus, and Klebsiella species. [19]Azadirachta indica is employed in traditional medicine for different illnesses[20] including leucoderma, leprosy, fever, burning, pruritus, intestinal worms, dyspepsia, tuberculosis, biliousness, lumbago, earache, rheumatism and blood impurities. [21] Warm oil is effective for treating gum, tooth, and ear problems. [22] Neem oil was proven to have potent antimicrobial properties. [23]

2. Material and Methods

96 wells micro plate, Micropipette 10-100µl, Micropipette 100-1000µl, ELIZA micro plate reader (Synergy HT BioTek® USA), Microtips (yellow/ blue color), Eppendroph tubes, Digital electronic weight balance, Beakers, Sticking and marker, Aluminium foil, Refrigerator Freezer, Spatula.

2.1 Chemicals

Methanol, Ascorbic acid, DPPH, Deionized water, Alpha glucosidase, KOH, KH_2PO_4, DNS reagent (dinitrosalisylic acid), P- Nitro phenyl alpha-D- glucopyranoside, Acarbose, 70 Methanol, NaH_2PO_4, NaOH, Iodine reagent, Alpha amylase, HCL, Starch.

2.2 Plant Collection and extraction

The Neem leaves plucked from the Islamia University of Bahawalpur's Baghdad ul Jadeed Campus. The leaves were cleaned to remove dirt and dust, and they were then left to dry for 15 days in the shade. The leaves were gathered and arranged after 15 days. The plant was verified by Dr. Ghulam Sarwar from the Botany Department at the Islamia University of Bahawalpur and the voucher number was168.

Using the grinder the leaves of the dry plant was crushed into a powder. The finest powder of the azadirachta indica 1kg leaves were soaked in 2 litres of 30/70 percent Ethanol in an airtight container. The plant was soaked for 72 hours at room temperature with vigorous stirring every day. The extract was filtered after soaking for 72 hours with intermittent stirring. The plant's filtration process involved two phases. Whattman's filter paper grade # 01 wereemployed to filter extract after it had first been filtered off using a muslin cloth. There were two revisions of this filtration procedure. For ethanol evaporation, the filtrate was put in a sterile tray and covered with aluminium foil. A fan was used to dry the extract's water content while it were placed inside a room with out any heat. And liquid inside container evaporated over the course of roughly 3 weeks. After the above process, the resulted semi solid mass was collected as crude ethanolic extract.

2.3 Phytochemical screening

According to established methods, phytochemical screening of the etanolic fraction of the Azadirachta indica was done to detect the existence of various chemicals constituents, like as phenols, flavonoids, alkaloids, tannins, saponins, terpenoids, steroids, alkaloids, etc. Results were recorded based on observations of colour changes and/or precipitate formation after adding certain detecting reagents to test solutions of different fractions. For confirmation, each qualitative phytochemical test was performed three times.

Test for Primary metabolites:

2.3.1 Evaluation of Lipids: (Saponification Test) 10% NaOH solution: Add 10g of NaOH in 100ml of water to form 10% solution of NaOH.Take 10ml of sample solution in a beaker and add 20ml of 10% NaOH solution in it. Boil the mixture for 1 hour. A lipidous material collects at the surface.Lipidous material will settle down indicatingOleic acidexistence.

2.3.2 Test for Proteins:(Biurette Test) 1% aqueous solution of Copper Sulphate:Add 1g of Copper Sulphate in 100ml of water to prepare 1% aq. sol. o Copper Sulphate. Take 3ml of sample solution in a test tube and add equal amount of NaOH solution to it. Mix well and add drop by drop 1% Copper Sulphate sol. (Mix b/w drops).Pink color indicates the presence of albumin. (27)

2.3.3 Evaluation of amino acids:(Ninhydrin Test) Ninhydrin Reagent: Add 2g of Ninhydrin in 100ml of ethanol to prepare 2% solution of Ninhydrin. Some few drops of the 2% ninhydrin reagent were added to 2 ml of the sample solution and heated in just a water bath for 5 minutes. Amino acids are found when blue or deep violet colour is formed. A deep purple colour is produced by ammonia, primary and secondary amines, and amino acids. A yellow colour is produced for proline and hydroxyproline. The sample doesn't really exhibit amino acids, amines, or ammonia if no colour change is seen.For asparagine, brown color is obtained. (28)

2.3.4 Test for Carbohydrates:(Fehling's Test) Fehling's Reagent: Fehling's Solution **A;** Dissolve 7g of $CuSO_4.7H_2O$ in 100ml H_2O. Fehling's Solution **B;** Dissolve 24g of KOH & 34.6g potassium sodium tartrate in 100ml H_2O. Add 2ml of Fehling's reagent A into 2ml of Fehling's reagent B and dissolve it completely. Take 4 ml of sample solution into a test tube and add the above reagent mixture into it. Heat the test tube in hot water for 1 to 2 minutes.Appearance of Reddish Brown Color showed existance of aldehyde or α-hydroxy ketone is present. (**Molisch's Test)** Molisch's Reagent**:**Prepare 5% alcoholic solution of α-naphthol. Take 2ml of sample solution in test tube. Add 2 to 3 drop Molisch's reagent in sample solution and mix well. Pour 2ml of conc. H_2SO_4 with sides of test tube.Appearance of pink / purple colored ring on junction of two liquids will indicate the Carbohydrates exisance.(29)(30)

Tests for Secondary Metabolites:

2.3.5 Test for Glycosides:(Erdmann's Test) Erdmann's Reagent: 10 drops of HNO_3 were added in 100ml of distilled water to prepare solution A reagent were made by combining 10 drops of solution A in 20ml conc. H_2SO_4. Take 2ml of alcoholic extract and add 2 to 3 drops Erdmann's reagent. A bright red color shows phenolic glycosides. (31)

2.3.6 Evaluation of Alkaloids:(Wagner's Test) To manufacture 100 ml of Wagner's Reagent, combine 2 g of iodine and 4 g of potassium iodide with enough distilled water. In a test tube, 1 ml of the extract was combined with 3 pre-made drops of the Wagner's reagent. Alkaloids were

present because brown precipitate developed. Hazarder's Test Hager's Reagent: One gramme of picric acid was dissolved in one hundred millilitres of distilled water to create a saturated solution. In a test tube, 1 ml of the extract was carefully combined with 3 drops of the just-made Hager's reagent. The appearance of yellow precipitates indicated a successful outcome and the extract's alkaloids content. (32)

2.3.7 Test for flavonoids: 2 ml dilute NaOH solution were added in 3 ml of extract. Yellow colour development is deemed to positive. (33)

2.3.8 Test for tannins:(Lead Acetate Test) Five millilitres of each kind of extract were combined with some drops of recently made, 1% lead acetate. Precipitation that is yellow indicates positive outcomes. (34)

2.3.9 Test for Phenols:(Lead acetate test) 3 ml of a 10% lead acetate solution was added after the extract (5 mg) had been dissolved in distilled water. The appearance of a large, white precipitate has allowed phenols to be identified.

2.3.10 Test for coumarins: One milliliter of 10% NaOH was added to one millilitre of the plant extract. Quinones were present as indicated by the development of a yellow tint. (33)

2.3.11 Test for Anthraquinones: To 0.5 ml of the extract, some drops of 2% HCL being added. Anthraquinones were present because of the precipitate's appearance in red.

2.3.12 Test for Phlobatannins: To 0.5 ml of the extract, some drops of a 10% ammonia solution was then added. Phlobatannins were present because of the pink colour of the precipitates.

2.3.13 Evaluationof Terpenes/Steroids :(Salkowaski's test) A similar volume of chloroform and a few drops of strong sulfuric acid are added to 1 ml of the test extract. When a brown ring appears, steroids are present, and when a bluish brown ring appears, phyto-steroids are present. (35)(36)

2.3.14 Test for Quinone: Each plant extract was incorporated with 1ml of conc. H_2SO_4.Quinones have been detected as revealed by the appearance of red color.

2.4Method of Anti-Oxidant Activity

2, 2-diphenyl 1-picryl hydrazyl inhibition test were conducted with some modifications. DPPH in the concentration of 100 µM was added in methanol. In a 96-wells plate, 100 µL were the entire test quantity containing 10 µL of the testing solution and 90 µL of DPPH solution. The contents were inserted as well as sprouted at 37°C for 30 min. To determine diminution in absorbance, Synergy HT BioTek® USA micro-plate reader were utilized at 517 nm. Ascorbic acid (0.1 mM) were standard antioxidant. For negative control 70% alcohol was used. Triplicate method was used for this experiment. The value of IC50 were evaluated by Ez-fit-5 Perrella Scientific Inc., Amherst, USA software. Reduction in absorbance indicated greater activity of radical scavenging the below formula were employed to calculate it

Percentage Inhibition of free radicals = 100 − [(Sample solution absorbance / control solution absorbance)] × 100

And,

Control solution absorbance = Total radical activity with solvent.

Samples Absorbance = Activity when the test substance is present. [24]

2.5 Alpha Glucosidase InhibitoryMethod

α-Glucosidase inhibition activity weredone with some modifications. In every well of the 96-well plate10 µL of sample, 70 µL of 0.1 M phosphate buffer, pH 6.8, and 10 µL of -glucosidase (0.5unit/µL) were added and heated for 15 min at 30°C. 10µL of p-Nitrophenyl-α-D-glucopyranoside substrate solution were inserted and heated to another 30 minutes. At 405 nm, the absorbance has been assessed using HT BioTek microplate reader.A positive control sample was acarbose, and that the wells without sample (only methanol) were employed as a negative sample. Every test has been carried out three times.

A sample's enzyme inhibitory rate has been estimated by using formula:

Percentage inhibition= 100- (Am.t / Ac) * 100

There,

Ac = Absorbance of negative control

Am.t = Absorbance of test sample [25]

2.6 Alpha amylase inhibitory Method

The starch-iodine assays were employed to evaluate the sample's inhibitory efficacy against alpha amylase in a 96-wells microtitre plate. Exactly 25 µl of assay buffer, 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mm sodium chloride), 20 µl of soluble starch (1%, w/v) and 20 µl of plant extracts/acarbose were heated for 5 minutes at 37 °C. So as positive control, Acarbose was used at the conc.0.5-10 mg/ml. Then 15 µl amylase solution (6 mg/ml) being inserted into every respond well and heated for 15 min at 37°C. After that 20 µl of 1M HCl was inserted preventing a enzymatic process, and 100 µl of iodine reagent (5 mm I2 and 5 mM KI) was inserted. So variations in color were observed and on microplate reader as absorbance were measured at 620 nm. The existence of starch was indicated by a dark blue colour(and a highly active inhibitor); the lack of starch was indicated by a yellow colour(and inhibitor) whereas a brownish coloration showed starch that had partially degraded (and active/ partially active inhibitor) in reaction mixture. %age inhibition of α-amylase were determined with

% inhibition= (absorbance of test/absorbance of control) 100 [26]

3. Results

4. Phytochemical Analysis:

The ethanolic extract of Azadirachta indica leaves undergone phytochemical screening which revealed existence of lipids, carbohydrates, tannins, phenols, flavonoids, steroids/terpenes, alkaloids, glycosides, coumarins, and quinones. Phlobatannins, anthraquinones, amino acids, and proteins were absent in the Azadirachta indica leavesethanolic extract. Table 1

Table 1- Phytochemical Results

Sr. No	Phytochemicals	Results
	Primary metabolites	
1	Evaluation of Lipids	+
2	Test for Proteins	–
3	Evaluation of amino acids	–
4	Test for Carbohydrates	+
	Secondary metabolites	
5	Test for Glycosides	+
6	Evaluation of Alkaloids	+
7	Flavonoids	+
8	Tannins	+
9	Phenols	+
10	Coumarins	+
11	Anthraquinones	–
12	Phlobatannins	–
13	Terpenes/Steroids	+
14	Quinone	+

5. Antioxidant

The azadirachta indica leaf extract showed significant inhibitory activity (83.23 ± 0.1%) on conc. of 5mg/ml with an IC_{50} value of 1.44 mg/ml. Table 2

Table 2-Antioxidant Activity

Sample	Percentage inhibition	IC50
Azadirachta indica	83.23±0.1	0.488 mg/ml
Ascorbic acid	93.5±0.01	22.3 µg/ml

6. Alpha Glucosidase Inhibition

The azadirachta indica leaf extract showed significant inhibitory activityfor Alpha glucosidae enzymes that is 66.7±0.1% as compared to standard acarbose having 77±0.1% inhibition. Table 3

Table 3- Alpha Glucosidase Inhibitory Activity

Sample	Concentration (5mg/ml)	Percentage inhibition	IC_{50}
Azadirachta indica	5mg/ml	66.7±0.1	1.325 mg/ml
Acarbose (standard)	5mg/ml	77±0.01	2.59 µg/ml

7. Alpha AmylaseInhibition

The azadirachta indica leaf extract showed significant inhibitory activity for Alpha amylase that is 63.5± 0.01% as compared to standard acarbose having 73±0.1% inhibition. Table 4

Table 4- Alpha Amylase Inhibitory Activity

Sample	Concentration(5mg/ml)	Percentage inhibition	IC_{50}
Azadirachta indica	5mg/ml	63.5± 0.01	1.414 mg/ml
Acarbose (standard)	5mg/ml	73±0.01	2.59 µg/ml

8. Statistical analysis:

In order to analyze the data, one-way analysis of variances (ANOVA) and the Tukey test for multiple comparisons were used with IBM SPSS version 23.0 software, with the assumption that variances are equal. For each of the variables under consideration, the data were all represented as mean Standard error. It has been shown that P 0.05 qualifies as statistically significant.

9. Discussions

Azadirachta indica is a well-known wonderful herb having several biological effects that have reportedly been seen. Neem plant parts, including the leaf, bark, root, seed, and flowers, demonstrate a function in the control of disease through the modulation of diverse biological activities.Azardirachta indica the most effective percentage of DPPH radical inhibition is found in Neem oil, whose entire is the greaterphenol concentration. [37]In a study, the roots extract of the plant A. indica demonstrated 86.030.04% radical inhibitions at 100 g/ml, whereas the standards ascorbic acid and BHA shown 95.820.09 and 93.090.06% inhibitions, respectively, at the same dose. The ascorbic acid and BHA concentrations in the extract of A. indica were found to be 2.12 0.02 and 4.87 0.05 g/ml and 13.81 0.06 g/ml, respectively. The investigation revealed that the crude sample has high antioxidant qualities, which would support its long-standing use.

[38]Azidirachta indica leaf extract in methanol was tested for its antioxidant potentialin different research, and it was discovered that the maximum inhibition attained was 71.23 percent when 500 g of the plant extract was taken. [39]A research was conducted for assessing the antioxidant potential of several extracts derived from different neem tree sections. Findings imply that leaf, flower& stem bark extracts has significant antioxidant activity. [40]In a different investigation, it was discovered that flowers and seed oil ethanolic extracts had superior free radical scavenging abilities. [41]Comparative research revealed that the bark had more complex phenolic contents and better antioxidant activity than the leaves. [42]It has also been determined how well the methanolic extract of the roots measures various flavonoids and their capacity to scavenge free radicals. [43] Furthermore, it was discovered in different research comparing the antioxidant activities of methanolic and chloroform extracts of neem leaves that methanolic extracts substantially outperformed chloroform extracts. [44]In a separate study, azadiradione and were tested for their ability to inhibit -amylase activity against the A.indica and had an IC50 value of 74.17 and 68.38 μM, respectively. [45]Raut, et al. 2015 reported that the Azadirachta indica bark aqueous extract exhibited 100 percent α-amylase inhibition. Azadirachta indica bark aqueous extract also has heat-stable amylase inhibitors. The extract was cooked at 100° C for 30 minutes with no change in their inhibitory action. [46]Alpha amylase and alpha glucosidase inhibitory activity of Ag-doped ZnO nanoparticles (NPs) was tested utilising an aqueous extract of Azadirachta indica (Neem) leaves as the reducing agent. At doses of 100 g/mL and 200 g/mL, respectively, Ag-ZnO NPs demonstrated 60% and 65% -amylase inhibition activity and 62% and 75% -glucosidase inhibition activity. [47]

A. indica-yogurt was produced and stored in the refrigerator for up to 28 days in another investigation. On day 7, the A. indica-yogurt water extract enhanced the inhibition to the highest levels of -amylase (47.4 5.8%) and -glucosidase (15.2 2.5%). For -glucosidase on day 14 of storage (15.9 10.1%) and for -amylase on day 21 of storage (54.8 3.2%), A. indica-yogurt water extract enhanced the inhibition to maximum levels. [48]The active ingredient of Azadirachta indica, Nimbandiolactone-23, demonstrated the strongest -glucosidase inhibitory action in the study, with an IC50 value of 38.7 μM. [49] Intestinal glucosidase was inhibited by A. indica aqueous and methanolic extracts by 62.44 and 41.07%, respectively. Murine liver glucosidase inhibition by A. indica was 69.29 percent. [50]The leaves of Azadirachta indica were used to isolate three limonoids: deoxyazadirachtolid, nimbandiol A, and azadirachtolid E. By using spectroscopic techniques, their structures were identified and compared to published works. With IC50 values of 38.7 mM, 85.76 mM, and 48.24 mM, respectively, three compounds (1-3) shown considerable a-glucosidase inhibitory effects against Saccharomyces cerevisiae a-glucosidase. [51]Biu, A., et al reported that in the extract, saponins scored well, while tannins and glycosides scored somewhat. Low scores were obtained for alkaloids, terpenes, flavonoids, reducing sugars, pentoses, and entire carbs. Ketones, monosaccharides, and anthraquinones were not found in the extract. [52]Galeane, et al reported that the presence of flavonoids and saponins is confirmed by thin layer chromatography and phytochemical screening. The hexane and ethyl acetate fractions that arise from the hydroethanol extract as well as the terpenes in the ethyl acetate extract all showed positive findings. [53]Susmitha, et al Reported that the ethanolic extract of Azadirachta indica revealed positive results for saponins, alkaloids, terpenes, flavonoids, tanins, and steroids according to the phytochemical study. [54]

10.Conclusions

It is suggested that Azadirachta indica have strongantioxidant activity which canplays pivotal role in the prevention and treatment ofoxidant induced pathologies. The findings of this study indicate that azadirachta indica leaves possesses goodalpha glucosidae and alpha amylaseinhibitorypotential showing that it can be utilized in the treatment of diabetesby preparing a powerful and inexpensive medicines. The precise mechanism of action needs to be clarified and confirmed, although, by additional study.The extract and its contents may make promising study subjects in the future because the outcomes are positive.

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