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EVALUATION OF *IN-VITRO* ANTI-DIABETIC AND ANTIOXIDANT ACTIVITES OF ETHANOLIC EXTRACT OF *SYZYGIUM CUMINI* SEEDS

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ABSRACT

Diabetes mellitus is one of the biggest public health problems in the world, and causes serious and increasingly prevalent complications such as heart disease, renal failure, nerve damage and vision and etc. Standard methods of treatment, including insulin and oral medications, are pricey and have negative effects. This has caused many interested in natural ways that can be used as a remedy for the disease. Jamun is also called Indian blackberry (Syzygium cumini), and ages have used its traditional medicine including antidiabetic activity. Despite its richness in nutrition, the fruit is sold to obtain its nutrients. The seeds are particularly sought after for their blood sugar balancing properties. Jamun seeds also contain numerous active compounds such as flavonoids, polyphenols, alkaloids and glycosides that help to improve insulin sensitivity, reduce oxidative stress and maintain a stable level of glucose in the blood. This is further solidified by science, which has found that extracts of Jamun seeds can inhibit the enzyme alpha-amylase and alpha-glucosidase to prevent the breakdown of

carbohydrates and have been reported to significantly reduce glucose spikes after eating. They also enhance insulin sensitivity and glucose utilization which offer benefits in treating both T1D and T2D. It has astringent, carminative, stomachic, diuretic, antidiabetic, anti-diarrheal, anti-inflammatory, radioprotective, gastro-protective, antioxidant, anti-allergic, anti-cancer, anti-bacterial, cardio-protective properties, etc.

KEYWORDS: Diabetes mellitus Jamun (Syzygium cumini) Antidiabetic properties Blood sugar regulation Natural treatment Jamun seed extract.

1. INTRODUCTION

Diabetes is now one of the fastest growing health problems in the world, affecting people of all ages and all regions. Its hallmark is persistently high levels of glucose in the blood, usually because the body either does not produce enough insulin or cannot use effectively the insulin it produces.[1-4] This metabolic disorder dramatically increases the risk of serious health complications, such as cardiovascular disease, kidney damage, nerve damage and others. It is also devastating at the individual and health system level.[5-6] Although drugs have made diabetes easier to manage, it is a chronic disease and there are still problems such as side effects and the rising costs of treatment. Therefore, there is a growing interest in exploring natural and holistic therapies which are safer, more affordable, and accessible.[7]

Reactive oxygen species (ROS) such as superoxide radicals (O−−2), hydroxyl radical (−OH) and peroxyl radicals (ROO−) have been implicated in carcinogenesis, coronary heart disease and many other health problems associated with advanced age.[8-11] There are in animals both endogenous and exogenous systems for cell protection. In many conditions, however, including with aging, the endogenous antioxidants are depleted and therefore an exogenous supply of antioxidants is essential. Antioxidants are important in biological systems[2] because they reduce hydroperoxides (ROO −), H 2 O 2 and scavenge free radicals, thus slowing down the formation of reactive oxygen species.[12-14]

Jamun (Syzygium cumini L.) is an important evergreen tropical and subtropical plant of the family Myrtaceous. These trees are also found growing in many parts of the world like Bangladesh, Pakistan, Philippines, West Indies, Eastern Africa, South America, etc. The fruit is native to India and is commonly known as Jambul, Black Plum, Java Plum, Indian Blackberry, Jamblang, etc. India is the second largest producer of this fruit in the world.[15] The main objectives of the present work were to study the effects of different parameters

(extraction temperature, extraction time and liquid to solid ratio), and to apply the RSM approach in order to optimize these conditions to obtain the aqueous extract of jamun seeds with maximum extraction yield, maximum purity and antioxidant activity, to identify and quantify the individual phenolic compounds in the optimally obtained jamun seed extract.[16,17]

1.1 Plant profile

Botanical Name: Syzygium cumini (L.) Skeels

Common Name: Jamun, Java plum, Black plum, Indian blackberry

Synonyms: Eugenia jambolana, Syzygium jambolanum

Family: Myrtaceae

Taxonomic hierarchy Kingdom: Plantae

Phylum: Angiosperms Order: Myrtales Family: Myrtaceae Genus: Syzygium

Species: Syzygium cumini (L.) Skeels

Other species of Syzygium

Syzygium aromaticum (Clove)

Syzygium malaccense (Malay rose apple) Syzygium jambos (Rose apple)[18,19]

- **Advantages of Jamun seeds:** Natural blood sugar regulation, high in antioxidants, supports digestive health, traditional medicinal use, minimal side effects.
- **Disadvantages of Jamun seeds:** May cause hypoglycaemia with anti-diabetic drugs, potential for allergic reactions, not recommended for pregnant or lactating women without medical supervision.
- **Chemical constituents of Jamun seeds:** Jambolana, Ellagic acid, Gallic acid, Corelating, Catechin, Flavonoids and Alkaloids with Antidiabetic and Antioxidant effect.[20,21]

1. MATERIALS AND METHOD

1.1. PLANT MATERIALS AND CHEMICALS

Dry jamun seed powder was purchased. Distilled water served as the solvent for the extraction process. The following chemicals were purchased from local stores 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), Folin-Ciocalteu phenol reagent, (+)-catechin hydrate (≥95%), (-)-epicatechin (≥90%), caffeic acid (≥98%), p-coumaric acid (≥98%), ellagic acid (≥95%), quercetin (≥95%), triethanolamine, and tannic acid. Additionally, bovine serum albumin (BSA), gallic acid, ascorbic acid, glacial acetic acid, sodium acetate

trihydrate, BHT, potassium acetate, methanol, ferrous sulfate, sodium hydroxide, sodium chloride, ferric chloride, sodium dodecyl sulfate (SDS), and aluminum chloride were used. All other chemicals were of analytical grade.

2.2 COLLECTION OF PLANT MATERIAL

Syzygium cumini seeds were obtained from ripe fruits that were readily available in the area. Jamun fruits were picked from trees within and nearby the area during the fruit production season ensuring their freshness and healthy appearance. Fruits were carefully picked to ensure that there was no disease, damage or contamination. The pulp from collected fruits was manually separated and the seeds were separated. The seeds were then washed well with clean water which is used to remove the pulp and impurities adhering to the seeds. Shade-drying process was done at room temperature, and clean seeds were kept dry for few days. Moisture related spoilage was prevented by proper drying. The dried seeds were kept in airtight containers for future use in making up powders and extracting.

2.3 PROCURMENT OF SEED POWDER

Jamun seeds were taken from the local areas and cleaned then shade dried, powdered in a grinder and stored in an airtight container for extraction.

2.4 EXRACTION

Extraction took place in a simple laboratory setup with a magnetic stirrer at 700 RPM.[22] The setup included a 1 L distillation flask and a condenser. Once the desired temperature was reached, the specified amount of dried jamun seed powder was added to the required volume of distilled water in the distillation flask. This marked the start of the extraction time. After extraction, the mixture was cooled to room temperature (25 ± 1°C) and then filtered through Whatman No. 1 filter paper. The extract was then used to measure extraction yield, extract purity, and antioxidant activities through various chemical tests.

2.5 PHYTOCHEMICAL ANALYSIS

Preliminary tests on the Jamun seed extract showed that it has several bioactive compounds, including flavonoids, phenolic compounds, tannins, alkaloids, saponins, and glycosides.[23] These phytochemicals are known for their antioxidant and antidiabetic effects. The detection of phenolic and flavonoid compounds suggests strong free radical scavenging activity. The results confirm that the extract contains important secondary metabolites with therapeutic value.

2.6. IN-VITRO EVALUATION

2.6.1. ANTI-DIABETIC ACTIVITY

1. Alpha- amylase inhibition assay

The α-amylase solution was prepared by dissolving 10 mg of α-amylase in phosphate buffer (pH 6.8) and making up the volume to 10 mL. The DNSA reagent was prepared by dissolving 1 g of 3,5-dinitrosalicylic acid (DNSA), 2 g of sodium hydroxide (NaOH), and 30 g of sodium potassium tartrate in distilled water, and the volume was adjusted to 100 mL. A 1% starch solution was freshly prepared using distilled water, and acarbose was used as the standard drug.[24] For the assay, 0.5 mL of the ethanolic extract was mixed with 0.5 mL of α-amylase solution and incubated at 37°C for 10 minutes. Then, 0.5 mL of starch solution was added and incubated again at 37°C for 10 minutes. The reaction was terminated by adding 1 mL of DNSA reagent, followed by heating the mixture in a boiling water bath for 5 minutes. After cooling, the reaction mixture was diluted with 10 mL of distilled water, and the absorbance was measured at 540 nm using a UV–Visible spectrophotometer to determine the α-amylase inhibitory activity.[25]

2. ALPHA-GLUCOSIDASE INHIBITION ASSAY

The 100 mM potassium phosphate buffer (pH 6.8) was prepared and used to prepare the α-glucosidase enzyme solution.[26] A 5 mM p-nitrophenyl-α-D-glucopyranoside (pNPG) solution and 0.2 M sodium carbonate (Na_2CO_3) solution were also prepared, while acarbose was used as the standard drug. For the assay, 50 µL of the ethanolic extract was mixed with 100 µL of α-glucosidase solution and incubated at 37°C for 10 minutes. Then, 50 µL of 5 mM pNPG solution was added, and the mixture was further incubated at 37°C for 20 minutes. The reaction was terminated by adding 2 mL of 0.2 M sodium carbonate, and the absorbance was measured at 405 nm using a UV–Visible spectrophotometer to evaluate the α-glucosidase inhibitory activity.[27,28]

2.6.2. IN-VITRO ANTI-OXIDANT ACTIVITY

1. TOTAL PHENOLIC CONTENT

The 10% Folin–Ciocalteu reagent was prepared by diluting 10 mL of Folin–Ciocalteu reagent to 100 mL with distilled water, and 7.5% sodium carbonate (Na_2CO_3) solution was prepared by dissolving 7.5 g of sodium carbonate in distilled water and making up the volume to 100 mL.

Ascorbic acid was used as the standard, and serial dilutions were prepared to obtain different standard concentrations.[29,30,31] For the assay, 0.5 mL (or 1 mL) of the ethanolic extract was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent, allowed to stand for 5 minutes, followed by the addition of 2.0 mL of 7.5% sodium carbonate solution. The mixture was incubated in the dark at room temperature for 30 minutes, and the absorbance was measured at 765 nm using a UV–Visible spectrophotometer to determine the total phenolic content of the extract.[32]

2. HYDROGEN PEROXIDE SCAVENGING ASSAY

The 50 mM phosphate buffer (pH 7.4) was prepared by dissolving 6.8 g of disodium hydrogen phosphate (Na_2HPO_4) and 1.0 g of sodium dihydrogen phosphate (NaH_2PO_4) in 1 L of distilled water.[33,34] A 40 mM hydrogen peroxide (H_2O_2) solution was prepared by diluting 4.1 mL of 30% H_2O_2 and making up the volume to 1 L with distilled water. The ethanolic extract was dissolved in the phosphate buffer to obtain the required concentrations. For the assay, 1.0 mL of the sample extract was mixed with 0.6 mL of 40 mM H_2O_2 and 1.4 mL of phosphate buffer (pH 7.4). The mixture was allowed to stand for 10 minutes at room temperature, and the absorbance was measured at 230 nm using a UV–Visible spectrophotometer to evaluate the hydrogen peroxide scavenging activity.[35]

3. RESULTS AND DISCUSSION

3.1. DETERMINATION OF PERCENTAGE YEILD

Percentage Yield (%) = (Weight of dried extract / Weight of powdered drug) × 100 Weight of powder = 50 g

Weight of china dish = 67.66 g

Weight of china dish + dried extract = 68.66 g Weight of dried extract = 68.66 − 67.66 = = 1.00 g **Calculation**

50 g → 100%

1 g → X

X = 100 × 1 ÷ 50 = 2%

Percentage Yield = 2%

3.2. PHYTOCHEMICAL SCREENING

TEST	OBSERVATION	RESULT
ALKALOIDS **Mayer's Test:** Took 2 mL of the ethanolic extract in a clean test tube and added 2–3 drops of Mayer's reagent. Mixed gently and observed for precipitate formation.	Pale yellow precipitate was formed.	**Positive (+)**
Drage Dorff's Test: Took 2 mL of the ethanolic extract in a clean test tube and added 2–3 drops of Drage Dorff's reagent. Mixed gently and observed the reaction.	Reddish-brown precipitate was formed.	**Positive (+)**
FLAVONOIDS **Alkaline Reagent Test:** Took 2 mL of the ethanolic extract in a clean test tube and added 2–3 drops of sodium hydroxide solution followed by 2–3 drops of dilute hydrochloric acid.	Intense yellow colour appeared and disappeared after the addition of dilute acid.	**Positive (+)**
PHENOLICS **Lead Acetate Test:** Took 2 mL of the ethanolic extract in a clean test tube and added 2–3 drops of 10% lead acetate solution. Mixed gently and observed the reaction.	Bulky white precipitate was formed.	**Positive (+)**
Ferric Chloride Test: Took 2 mL of the ethanolic extract in a clean test tube and added 2–3 drops of 5% ferric chloride solution. Mixed gently and observed the colour change	Dark brown colour developed.	**Positive (+)**
GLYCOSIDES Keller–Killiani Test: Took 2 mL of the ethanolic extract in a test tube and added 2 mL of glacial acetic acid containing one drop of ferric chloride solution. Carefully added 1 mL of concentrated sulphuric acid along the side of the test tube.	Brown ring appeared at the interface.	**Positive (+)**
PROTEINS **Biuret Test:** Took 2 mL of the ethanolic extract and added sodium hydroxide solution followed by 2–3 drops of copper sulphate solution	No violet or purple colour developed.	**Negative (−)**
TANNINS **Ferric Chloride Test:** Took 2 mL of the ethanolic extract and added 2–3 drops of 5% ferric chloride solution.	Blue-black colour developed.	**Positive (+)**
TERPENOIDS **Salkowski Test:** Took 2 mL of the ethanolic extract, added 2 mL of chloroform followed by concentrated sulphuric acid carefully along the side of	Reddish-brown ring appeared at the interface.	**Positive (+)**

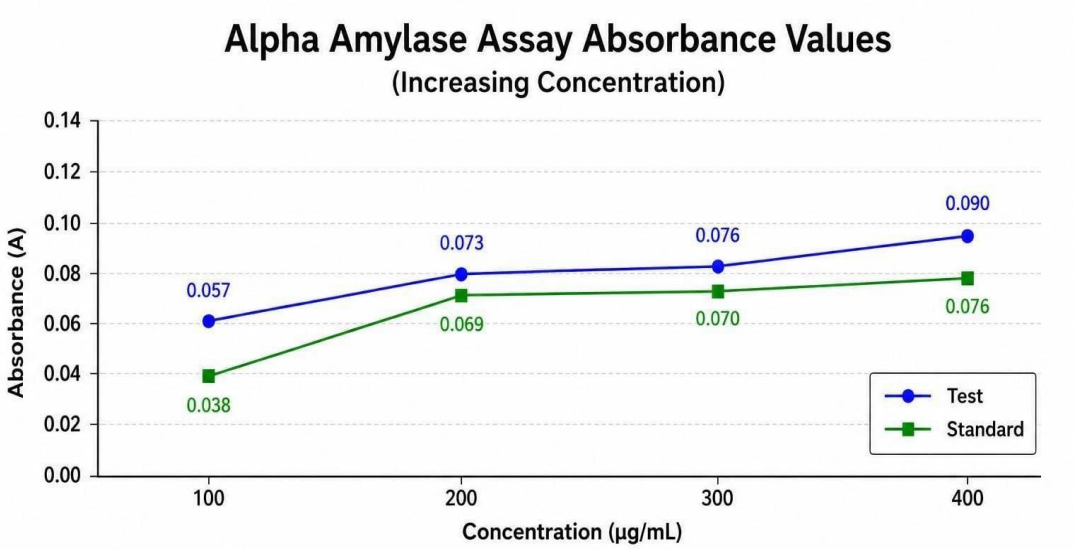
the test tube.		
SAPONINS **Foam Test:** Took 2 mL of the ethanolic extract and added 5 mL of distilled water. Shook vigorously for 30 seconds and allowed it to stand.	Stable persistent froth was observed.	**Positive (+)**

3.3. IN-VITRO ANTI-DIABETIC ACTIVITY

1. Alpha amylase inhibition asssay

Table no. 1: Alpha amylase inhibition assay.

Concentration (µg/mL)	Test (Absorbance)	Standard (Absorbance)
0.1	0.057	0.038
0.2	0.073	0.069
0.3	0.076	0.070
0.4	0.090	0.076

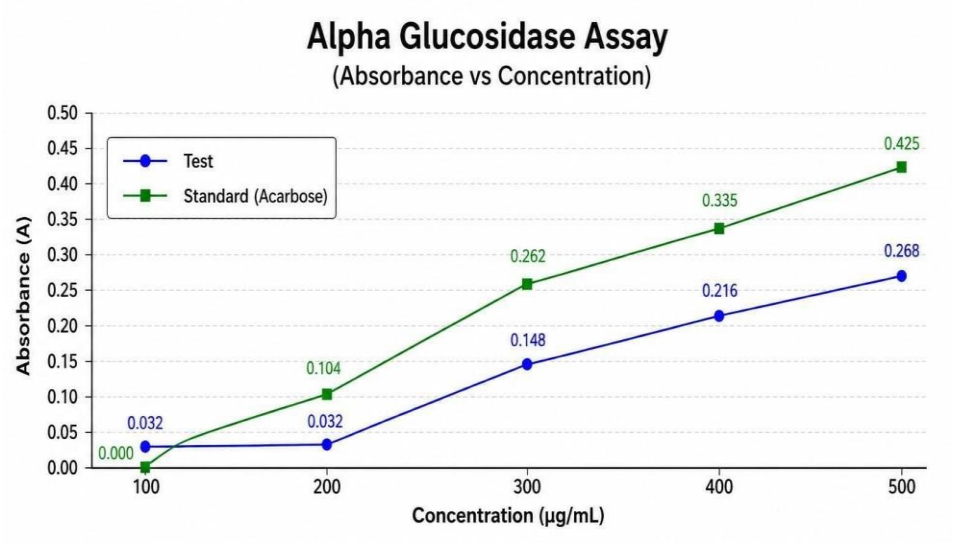


Graph 1: Graph of Alpha amylase inhibition assay.

2. ALPHA-GLUCOSIDASE INHIBITION ASSAY

Table no. 2: Alpha Glucosidase inhibition assay.

Concentration (µg/mL)	Test (Absorbance)	Standard (Acarbose) (Absorbance)
0.10	0.000	0.000
0.20	0.032	0.104
0.30	0.148	0.262
0.40	0.216	0.335
0.50	0.268	0.425



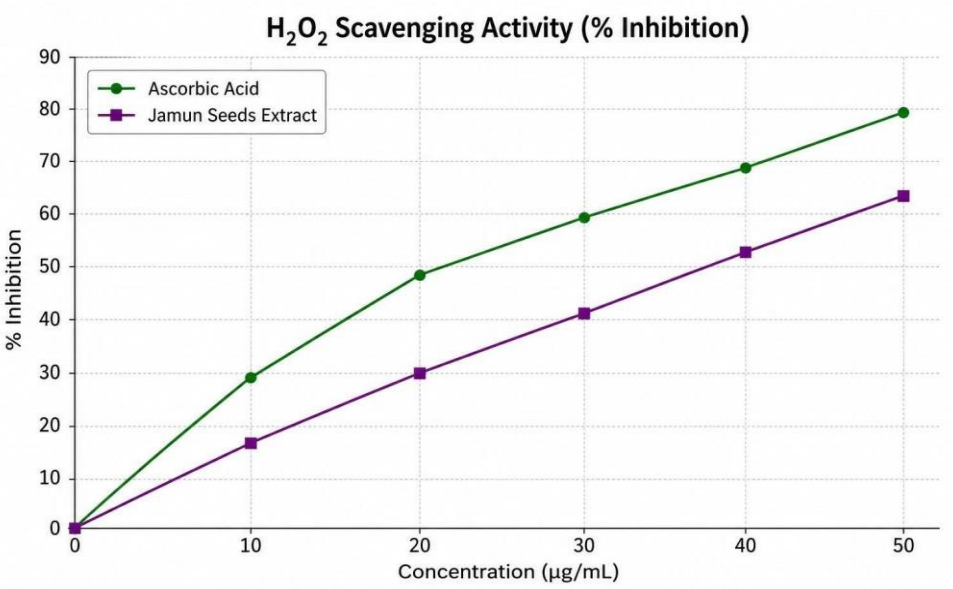
Graph 2: Graph of Alpha glucosidase inhibition assay.

3.4. IN-VITRO ANTI-OXIDANT ACTIVITY

1. Hydrogen peroxide scavenging assay

Table no. 3: Scavenging Activity: Ascorbic acid vs Jamun SeedsExtract.

Concentration (µg/ml)	Absorbance (Ascorbic acid)	%Inhibition (Ascorbic acid)	Absorbance (Jamun seed Extract)	%Inhibition (Jamun seed Extract)
0 (Blank)	0.800	0.0%	0.800	0.0%
10	0.565	29.2%	0.665	16.9%
20	0.410	48.8%	0.560	30.0%
30	0.325	59.4%	0.470	41.3%
40	0.250	68.8%	0.380	52.5%
50	0.165	79.4%	0.300	62.5%

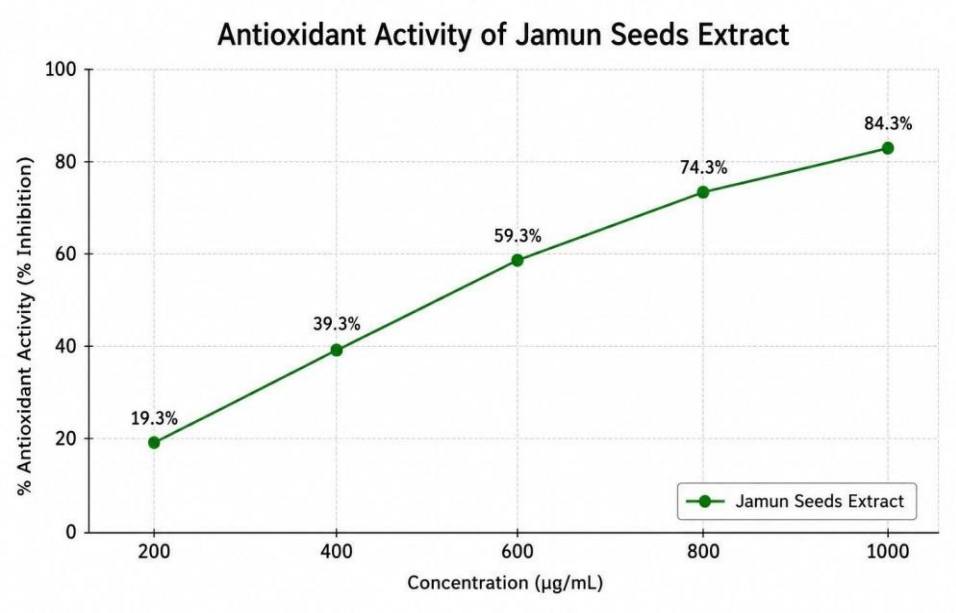


Graph 3: Graph of Hydrogen Peroxide Scavenging Activity.

2. Total phenolic content

Table no. 4: Total Phenolic Content.

Concentration (µg/mL)	Absorbance (control)	Absorbance (Jamunseed Extract)	% Antioxidant (%Inhibition)
200	0.700	0.565	19.3%
400	0.700	0.425	39.3%
600	0.700	0.285	59.3%
800	0.700	0.180	74.3%
1000	0.700	0.110	84.3%



Graph 4: Graph of Total phenolic content.

4. CONCLUSION

The present study aimed to explore the in-vitro anti-diabetic and antioxidant potential of Syzygium cumini seed ethanolic extract through the standard laboratory methods. The results demonstrated that the extract possesses noteworthy inhibitory effects against α-amylase and α-glucosidase, indicating its potential to help regulate carbohydrate digestion and glucose release. Additionally, the extract showed strong antioxidant properties due to its phenolics content and hydrogen peroxide scavenging properties, indicating its potential in mitigating oxidative stress. The results of this study suggest that the extract of S. cumini seeds is a good natural source of bioactive molecules that have anti-diabetic and antioxidant properties. The study validates the traditional use of seeds of Syzygium cumini in the management of diabetes.

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