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PHYTOCHEMICAL COMPOSITION, ANTIOXIDANT, ANTIMICROBIAL AND ANTIGLYCATION EFFICACY OF *SYZYGIUM CUMINI*

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ABSTRACT

Syzygium cumini, a medicinal plant of Myrtaceae family, has worldwide presence. Previous literature has pointed out importance of this plant from medicinal point of view. This plant has shown its potential against bacteria, fungus, spleen enlargement, urinary infections, cancer and fever. The present work was designed to examine different therapeutic effects of its extracts. Aqueous macerated and decocted extracts of leaf, fresh fruit, dry fruit, fresh seed, dry seed, and bark were prepared for in-vitro analytical purposes. Phytochemical screening was done by qualitative and quantitative methods. Antiradical efficacy was assessed by DPPH (1, 1-diphenyl-2-picrylhydrazyl), ABTS (2, 2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) and metal chelation assays. Glycation inhibitory potential was examined by spectrophotometric and spectrofluorometric methods. Disc diffusion method was adopted to analyze antimicrobial activity. Phytochemical screening confirmed significant presence of tannins, saponins, terpenoids, cardiac glycosides, flavonoids, phenols and carbohydrates. Total phenolic contents (TPC) and total flavonoid contents (TFC) were shown as gallic acid and rutin equivalents respectively. Maximum presence of TPC was found in decocted dry seed extract (0.603±0.001mg/g) and maximum TFC were estimated in decocted leaf extract (0.09±0.012 mg/g). Decocted and macerated fresh fruit extracts showed best ability to reduce DPPH (93.1±0.6 % and 91.3±0.2 % respectively). Macerated fresh seed extract and decocted bark extract exhibited maximum antiradical potential (85.7±1.2 % and 84.3±0.8 % respectively) in ABTS assay. In metal chelation assay maximum radical Inhibition potential was observed for macerated bark extract (91.4±0.6 %) and decocted fresh seed extract (91.6±1.1 %). Against all studied microbial strains (*Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa*) highest inhibition zones were given by bark extract. Bark and dry seed extracts showed 86% and 88% glycation inhibition potential in spectrophotometric and spectrofluorometric analysis respectively. It is concluded that all parts of this plant possess antiradical, antimicrobial and glycation inhibition potential. So, this plant can be used to cure many diseases.

Key words: Metal Chelating Activity, Advanced Glycation End Products, Secondary Metabolites, Fluorescence, Polyphenols

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INTRODUCTION

Scientists are making head against diseases by considering medicinal plants instead of synthetic drugs because of their severe side effects. People are using medicinal plants to cure diseases and heal wounds because of their therapeutic effects against microbes (Russo *et al*., 2018). These plants have natural products which show greater efficacy against diseases and are part of traditional and complementary medicinal system of health care. According to World health organization only 250 out of 20,000 types of plants had been undergone scientific research to combat different diseases. Although 80% world population is dependent on plants for medicines yet only 25% of drugs have been prepared from plants (WHO., 2005). 600 medicinally important plants make flora of Pakistan distinctive and people use these plants for medication purposes against fever, cough, and cold and to heal wounds (Shinwari., 2010). Among medicinal plants *Syzygium cumini* is a traditional medicinal plant that is native to south Asian and east Asian countries. Its common names are black plum, java plum, jamun, Indian blackberry, purple plum and damson plum (Artanti *et al*., 2019). It belongs to Myrtaceae family which comprises of more than 100 genera and 3000 species. This plant has a lot of secondary metabolites such as anthocyanins, alkaloids, glucosides, flavonoids, ellagic acid, myrecetin, isoquercetin, terpenoids, polyphenols and kaempferol, which show antioxidant potential (Chhikara *et al*., 2018).

Traditionally it is used to treat numerous ailments such as stomach-ache, fever, diarrhea, dysentery, diabetes and asthma. Extracts of its seeds and pulp-fruit have shown antidiabetic and metabolic related activities (Soares *et al*., 2019). Stains left on skins after acne have been removed by using seed extracts (Gordon *et al*., 2011). Spleen enlargement and urinary infections are treated by its ripened fruit juice. Seeds and fruits extracts have also been utilized to cure diabetes and ring worm (Saravanan and Pari, 2008). Its leaf extracts have been found effective against dermopathy, constipation, fever and gastropathy (Ravi *et al*., 2005; Sagrawat, 2006). Its leaves are used to heal wounds, treat skin disorders, and cure diabetes. Its bark possesses diuretic effect (Agarwal *et al*., 2019). Stem bark have also been reported effective against burns and inflammation (Gordon *et al*., 2011). The mixture of its stem bark with yogurt can cure menorrhagia (Swami *et al*., 2012). Maximum efforts have been brought forth to examine effectiveness of *S. cumini* against diabetes but still extracts different parts of *S. cumini* have not been examined against this disease by using a number of solvents since phytochemicals behave differently to different solvents. Same is the case with investigations of effectiveness of extracts of different parts of S. cumini against free radicals generated in the body. It is also to our finest knowledge a little work has been reported about activity of *S. cumini* against protein glycation. Thus present work was designed to mark the significance of potential of *S. cumini* against free radicals and advanced glycation end-products by applying maceration and decoction methods of extract preparation to strengthen the pace of medicinal world against diseases.

MATERIALS AND METHODS

Plant Sample Collection: Leaf, Fresh fruit, fresh seed, dry fruit, dry seed and bark of *S. cumini* were collected from District Mirpur Azad Kashmir. These parts were washed with water to remove dust and mashed for further process.

Extract Preparation: Modified Goud *et al.,* (2020) was used for extracts preparation. Extracts were obtained by maceration and decoction processes. For maceration 10 gm of powder of each part was separately soaked in100 mL distilled water for one week and then filtered and evaporated at 80°C using rotary evaporator. Same process was used for decoction process except heating extract at 80 °C on hot plate before using rotary evaporator to obtain semisolid extract. Test solutions were prepared by dissolving 25mg of each extract in 15mL distilled water and kept stored for further analysis at the Department of Chemistry, Mirpur University of Science and Technology, Mirpur, Azad Kashmir.

Phytochemical screening of *S. cumini*: Standard protocols described by (Amir *et al*., 2018; Islam *et al*., 2016; Obouayeba *et al*., 2015) were followed for the screening of secondary metabolites.

Estimation of Total Phenolic Contents: Zhou (2006) method with slight modifications was adopted for the estimation of TPC. In a test tube, 100 µL of 10% Folin-ciocalteus reagent was mixed with 100 µL of plant extracts and incubated for a short duration at room temperature. After incubation, 1.5 mL of 20% Na_2CO_3 was added in the above mixture and incubated for further 30 minutes. After incubation period absorbance value was measured at 765 nm. TPC were determined as a gallic acid equivalent.

Estimation of Total Flavonoid Contents: TFC were estimated by following Zou method (2004). Reaction mixture containing 100µL of plant extracts, one mL distilled water and 0.5 mL 5% $NaNO_3$ was incubated for 2 minutes at room temperature. Then 0.5 ml of 10% $AlCl_3$ solution was added and again incubated for few minutes and after this period 1ml of 4% NaOH was added to it. TFC were determined as a Rutin equivalent.

Evaluation of Antioxidant Potential: Antioxidant potential of plant extracts was estimated by DPPH free radical scavenging assay, ABTS radical cation assay and metal chelating assay as described below.

DPPH Radical Scavenging Activity: DPPH free radical scavenging activity was assessed by Yu *et al* (2002) modified *Ghous et al* (2015) with further slight modifications. 1mL of 0.4 mM DPPH solution prepared in methanol was mixed with 25-125 µg/ml of plant extracts and vortexed vigorously followed by 30 minutes incubation period at room temperature. Absorbance was measured at 517nm in UV-visible spectrophotometer.
Following formula was used for the calculation of %age scavenging activity.
%age radical scavenging activity=$[(A_0-A_i)/A_0]*100$
where, A_0= Absorbance given by control
A_i= Absorbance given by extracts

ABTS Radical Cation Assay: Slightly modified Re *at al* (1999) by *Ghous et al* (2015) was adopted with more modifications to perform ABTS radical cation assay. According to this technique 3 mM $ABTS^{+-}$ solution prepared in 25 mL of distilled water was oxidized in 2.5 mM potassium persulphate solution (in 25 mL H_2O) for 1 day in darkness. Reaction mixture of 1ml $ABTS^+$ solution and 25-125 µg/ml of plant was incubated for 15 minutes at room temperature and marked volume up to 3 ml by the addition of water. Absorbance was recorded at 734 nm.

Following formula was used for the calculation of %age scavenging activity.
%age radical scavenging activity=$[(A_0-A_i)/A_0]*100$

Where, A_0= Absorbance given by control
A_i= Absorbance given by extracts

Metal Chelating Activity: Dinis *et al* (1994) modified by Ghous *et al* (2015) with further minor modifications was used to assess chelation activity of plant extracts. 25-125 µg/mL of plant extracts were mixed with 1 ml of 1.5 mM ferrous sulphate solution and 1 mL of 0.25 mM ferrozine solution. Mixture was shaken vigorously and incubated for at-least 10 minutes at room temperature. A stable magenta complex was formed by the reaction between ferrozine and divalent iron. Absorbance was recorded at 517 nm with the help of UV-visible spectrophotometer.
Following formula was used for the calculation of %age scavenging activity.
%age chelating activity= $[(A_0-A_i)/A_0]*100$
Where, A_0= Absorbance given by control
A_i= Absorbance given by extracts

Antimicrobial activity: Antimicrobial potential was examined gram negative and gram positive bacterial cultures as described in Table 1.

Table 1: Bacterial strains used for antimicrobial activity.

Serial No.	Bacterial strains	Type (gram positive/ negative)	Cell Shape
1	*Staphylococcus aureus*	Positive	Spherical
2	*Bacillus subtilis*	Positive	Rod-shaped
3	*Escherichia coli*	Negative	Rod-shaped
4	*Pseudomonas aeruginosa*	Negative	Rod-shaped

Disc Diffusion Method: The antibacterial activity was performed by disc diffusion method described by Bakht (2011) with minor modifications. Whatman filter paper NO.1 discs having diameter 6mm were formed and sterilized at 121 ^oC for 20 minutes by autoclave. The sterilized nutrient Agar medium was poured into the sterilized plates almost 15ml per plate and left it for 10-15 minutes for solidification. After solidification each bacterial culture provided by the department of Chemistry Mirpur University of Science and Technology was spread on separate plates. Sterilized discs were soaked into each extract and were placed in plates. For positive control solution of standard antibiotic Rifampicin was used. These plates were left in incubator for at least 24 hours at 37^oC, and after one day, zone of inhibition was measured with scale millimeter. All these steps were performed in laminar flow chamber.

Antiglycation: This technique explained by Khan (2014) was adopted after minor modifications. Stock solution of plant extracts were made by mixing 1 mg each extract in 1 ml of Phosphate Buffer Solution (PBS-7.4 pH). With the help of micropipette 50-150 µg/ml of each extract was taken from stock solution and mixed with the solution containing 85 mg/ml of the Bovine Serum Albumin (BSA) and 1.5 M solution of glucose. After that reaction mixture was kept in water bath at 60^oC for ten days. BSA and glucose without plant extracts or inhibitor was used as control. The reaction mixture, after the incubation, was poured into separate eppendrof 1.5 mL tubes and then 10 µL of 100 % w/v tricholoroacetic acid (TCA) solution was added and this mixture was centrifuge at 14500 rpm for 5 minutes. Pellets formed after centrifugation were re-dissolved in alkaline PBS, centrifuged and supernatant was discorded. Aminoguanidine was used as standard reference. Analysis of this process was done by spectrophotometric and spectrofluorometric methods.

Spectrophotometric Method: Spectrophotometric method explained by Khan (2014) was used to assess antiglycation activity. Total 50-150 µg/mL of each sample was taken and 2 ml of distilled water was added. The absorbance was taken at 370 nm. UV-1900i Spectrophotometer was used to record absorbance values.

Spectrofluorometric Method: In spectrofluorometric method fluorescence was measured at emission of 440 nm and excitation at 370 nm of all the samples by using technique followed by Ramkissoon (2013). Schimadzu RF-600 Spectrofluorometer was used to record results.

Statistical Analysis: ALL results were analyzed by using MS-Excel 2016 and represented as Mean ± S.D (standard deviation).

RESULTS AND DISCUSSION

Phytochemical screening: Phytochemicals behave differently to different solvents (non polar to polar nature). In this study water was used as the primary solvent for maceration and decoction of extracts. All extracts of both the fractions showed the presence of flavonoids, carbohydrates, tannins, cumarins, anthocyanins, phenols, alkaloids, quinones, steroids, and glycosides and absence of anthraquinones, proteins and leucocyanins. Terpenoids were present in macerated extracts but completely absent in decocted extracts. Saponins were present only in fresh and dry seed extracts and bark extracts but absent in fresh and dry fruit extracts and leaves extracts (Table 2). Significant presence of

phytochemicals and important bioactive compounds have made medicinal plants a best source of life saving drugs. Presence of alkaloids in all fractions and saponins in seed fractions shows that these fractions can be used to treat hypertension as these phytochemicals have been reported to show antihypertension potential (Akinpelu and Onakoya., 2006). Polyphenols present in these plants have drawn much attention and regard because of their minimal adverse effects (Bahadoran *et al*., 2013). Important constituents of polyphenols are Phenolic and flavonoid contents (Mwamatope *et al*., 2020). They show various biological actions like antiglycation, antioxidant, anti-pathogenic, astringent, antiinflammation and anticancer activities (Yang *et al*., 2020). Defense system, against predators, parasites and microbes, is also strengthened by phenolic compounds (Reddy *et al*., 2021). Kumar (2020) has also attributed anti-viral, anti-bacterial and anti-tumor activities of plants to phenolic compounds. Antitumor activities of flavonoids and alkaloids have been reported (Zaman *et al*., 2020; Nantapap *et al*., 2017). Presence of flavonoids and tannins indicate that this plant can be used to treat diarrhea because these phytochemicals can inhibit development of diarrhea causing fluids (Krishnaiah., 2009). According to Khanam (2015) risk of cardiac diseases can be lowered by supplementing diet rich in phenolics, moreover, flavonoids can also show anti-allergic, antithrombotic, anti-pathogenic and hepato-protective properties because these compounds can outfit free radicals. Fractions of *S. cumini* can be used to handle cardiac diseases like cardiac arrhythmia and heart failure because of significant presence of cardiac glycosides. These phytochemicals inhibit Na^+/K^+ pump, increase Na^+ concentration in myocytes which in turn increases Ca ion concentration which in turn improves health of heart muscles (Krishnaiah., 2009). Reddy (2021) has reported antiseptic, insecticidal and anti-helminthic activities of terpenoids. Our research has confirmed huge presence of these phytochemicals in macerated and decocted extracts of *S. cumini*. Hence, various parts of this important plant possess therapeutic potential.

Table 2: Phytochemical screening of macerated and decocted extracts of *S. cumini*.

Phytochemicals	**Extracts**											
	Dedocted						Macerated					
	Leaves	Fresh fruit	Dry fruit	Fresh seed	Dry seed	Bark	Leaves	Fresh fruit	Dry fruit	Fresh seed	Dry seed	Bark
Phenol	+	+	+	+	+	+	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	-	-	-	+	+	+	-	-	-	+	+	+
Steroids	+	+	+	+	+	+	+	+	+	+	+	+
Quinones	+	+	+	+	+	+	+	+	+	+	+	+
Terpenoids	-	-	-	-	-	-	+	+	+	+	+	+
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-
Cumarins	+	+	+	+	+	+	+	+	+	+	+	+
Proteins	-	-	-	-	-	-	-	-	-	-	-	-
Anthocyanins	+	+	+	+	+	+	+	+	+	+	+	+
Leucocyanins	-	-	-	-	-	-	-	-	-	-	-	-

+ indicates presence; - indicates absence

Total Phenolic Contents (TPC) and Total Flavonoid Contents (TFC): Polyphenols can reduce generation of reactive oxygen species because polyphenols have ability to donate hydrogen atoms of phenolic hydroxyls (Waqas *et al*., 2021). Results in table 3 showed that 0.736 mg/g total phenolic contents (TPC) were present in macerated extract of fresh fruit as compared to 0.727 mg/g in its decocted extract. Macerated and decocted extracts of fresh seed contained 0.661 mg/g and 0.641 mg/g TPC respectively. 0.641 mg/g and 0.636 mg/g TPC were present in macerated and decocted leaves extracts respectively. Macerated extracts of dry fruit, dry seed and bark showed 0.670 mg/g, 0.631 mg/g, and 0.640 mg/g TPC presence respectively. TPC presence in macerated extracts was greater as compared to decocted extracts of these parts. All these results were measured as gallic acid equivalent. There are more than 8000 structures of polyphenols (Harbone., 1980) making them the most widely present phytochemicals of medicinal plants. Polyphenols serve as antioxidant agents. They have been

reported as best antiradical, antimutagenic, antimicrobial agents (Estruch., 2000). Minussi (2003) has reported effectiveness of polyphenols against cardiovascular diseases and cancer. Our study has unveiled significant presence of these important phytochemicals which means that this plant can be used to strengthen efforts of researchers agaisnt different diseases.

Table 3: Total phenolic and total flavonoid contents in different extracts of *S. cumini*

Plant parts	TPC mg/g		TFC mg/g	
	Extracts		Extracts	
	Macerated	Dedocted	Macerated	Dedocted
Leaves	0.641 ± 0.001	0.636 ± 0.006	0.130 ± 0.003	0.09 ± 0.012
Fresh fruit	0.736 ± 0.001	0.727 ± 0.007	0.396 ± 0.008	0.356 ± 0.009
Dry fruit	0.670 ± 0.001	0.660 ± 0.007	0.313 ± 0.009	0.308 ± 0.009
Fresh seed	0.661 ± 0.001	0.641 ± 0.006	0.296 ± 0.012	0.256 ± 0.009
Dry seed	0.631 ± 0.002	0.603 ± 0.009	0.291 ± 0.011	0.198 ± 0.019
Bark	0.640 ± 0.001	0.617 ± 0.008	0.273 ± 0.012	0.230 ± 0.013

Presence of TFC was estimated against rutin hydrate calibration curve. Fresh fruit extracts showed greater presence of TFC as compared to fresh seed extracts. TFC were 0.396 mg/g and 0.356 mg/g respectively in macerated and decocted extracts of fresh fruit as presented in the table 3. Macerated and decocted extracts of fresh seed respectively showed 0.296 mg/g and 0.256 mg/g presence of TFC. Dried fruit and dried seed extracts showed less presence of TFC as compared to fresh extracts. Minimum presence was estimated in both the macerated and decocted extracts of leaves.

DPPH scavenging assay: Reactive free radicals are generated in the body because of metabolic activities. These reactive radicals cause many dangerous diseases including aging, heart diseases, cancer, inflammation in organs and neurodegenerative diseases (Lian *et al*., 2019). Antiradical agents are necessary to outfit such reactive radicals. . Plants are plentiful source of natural antiradical agents. These antiradical agents, also named as antioxidants (Nimse and Pal, 2015).

Simplicity, swift estimation and direct quantification of scavenging potential are those characteristics because of which DPPH assay is frequently used (Kaewnarin *et al*., 2014). Those extracts which can furnish hydrogen atom to DPPH stable free radical are considered as best source of antioxidants.

Result of table 4 confirmed that all extracts of *S. cumini* have displayed significant scavenging potential in case of DPPH assay.

Table 4: Percent DPPH inhibition potency of extracts of *S. cumini*.

Extracts	**Macerated**					**Decocted**				
	Concentration µg/mL					Concentration µg/mL				
	25	50	75	100	125	25	50	75	100	125
Leaves	74.7±3.5	76±3.2	78.2±2.8	80.1±2.5	83.7±2.1	73±3.1	75.6±2.6	76.2±2.5	79.1±1.4	81.2±1.1
Fresh fruit	86.9±2.5	88.2±2.1	90.1±1.4	91.8±0.8	93.1±0.6	85.1±2.6	86.6±2.0	89.1±1.0	90.6±0.4	91.3±0.2
Dry fruit	80.6±2.7	81.5±2.5	83.1±2.2	84.5±2.1	87.5±1.8	78±3	79.2±2.6	81.5±1.9	83.1±1.6	85.5±1.2
Fresh seed	84.6±2.6	85.8±2.3	87.7±1.9	88.8±1.8	91.5±1.5	81.7±3.1	84.2±2.4	85.7±2.1	87.4±1.8	89.9±1.5
Dry seed	83.7±2.5	84.6±2.3	86.5±1.7	88.3±1.1	89.9±0.9	80.6±3.3	82.5±2.9	83.9±2.6	86.3±1.9	89.1±1.6
Bark	79.9±2.9	80.6±2.7	82.9±2.3	83.8±2.4	87.3±1.9	77.7±3	80.2±2.2	82.5±1.5	83.3±1.6	85.5±1.2

Initially scavenging potential, at 25 µg/mL concentration, was 86.9±2.5 % and 85.1±2.6 %for macerated and decocted extracts of fresh fruit respectively as compared to 80.6±2.7 % and 78±3.0 %for macerated and decocted extracts of dry fruit respectively. Scavenging efficacy of macerated fresh fruit extract increased to 88.2±2.1 %, 90.1±1.4 %, 91.8±0.8 %, 93.1±0.6 % and 86.6±2.0 %, 89.1±1.0 %, 90.6±0.4 %, and 91.3±0.2 for decocted fresh fruit extract at 50 µg/mL, 75 µg/mL, 100 µg/mL and 125 µg/mL respectively. This increased efficacy was according to the general observation of increased scavenging activity with increased concentration of extracts. Same trend was observed in case of extracts of other parts of *S. cumini*.

This change displayed incredible increase in results. Less scavenging activity was given by macerated and decocted extracts of leaves. Similarly bark extracts also showed lower level of scavenging potential as compared to fresh fruit and seed extracts and dry fruit and seed extracts. Antiradical potency of plant extracts are thought to be because of phenolic and different biologically active natural compounds (Ivanovic *et al*., 2021). Different parts of medicinal plants have presence of different but important bioactive compounds, so, these can be used for health benefits because of their antiradical effects. These compounds start their antiradical action by preventing or inhibiting oxidation of molecules (oxidizable) by quenching free radicals, setting antioxidant enzymes active, inducing apoptosis and chelating metal catalysts to halt oxidative stress. This stress damages human tissues triggering degenerative disorders such as cancer in the body (Fang *et al*., 2010; Mwamatope *et al*., 2020). Main sources for free radical stabilizing agents in plants are phenolic and flavonoid compounds (Park *et al*., 2020).

ABTS Assay: Since ABTS is sensitive to only acidic pH, so, considering its applications at number of pH values, it can be adopted to outfit free radicals (Waqas *et al*., 2021). Antiradical efficacy of *S. cumini* extracts was also examined by this assay as detailed in Table 5.

At all concentrations maximum antiradical efficacy was given by decocted and macerated bark extracts as compared to rest of extracts except at 125 µg/mL concentration at which macerated extract of fresh seed showed maximum potential (85.7±1.2 %) as an antiradical agent. At all concentrations macerated extract of dry fruit gave minimum antiradical activity but this trend observed an interesting irregular shift in case of decocted extracts of all fractions.

At 25 µg/mL, for decocted extracts, minimum radical inhibition potential was observed for leaf extract (71.1±4.1 %) and dry seed extract (71±4.6 %) and in case of macerated extracts at the same concentration, minimum potential was given by dry fruit extract (71.9±3.9 **%).** Decocted leaf extract and dry seed extract also exhibited minimum potential at 50 µg/mL which was 73±3.5 % for leaf extract and 73.1±4.2 % for dry seed extract. At 75 µg/mL minimum inhibition potential was given by decocted dry seed extract (74.6±3.9 %) and macerated dry fruit extract (76.8±2.6 %).

Table 5: Percent Inhibition of free radicals by extracts of *S. cumini* by ABTS assay.

Extracts	Macerated					Decocted				
	Concentration µg/mL					Concentration µg/mL				
	25	50	75	100	125	25	50	75	100	125
Leaves	72.2±4.1	75.4±3.2	78.1±2.6	79.7±2.4	83.2±1.9	71.1 ± 4.1	73 ± 3.5	76 ± 2.6	78.7 ± 1.7	81.2 ± 1.1
Fresh fruit	74.2±3.7	77±3.0	79±2.5	81±2.2	84.1±1.8	73.1 ± 3.0	74.9 ± 2.5	76.7 ± 1.9	79 ± 1.1	80.6 ± 0.7
Dry fruit	71.9±3.9	74.6±3.2	76.8±2.6	79.4±1.9	82.1±1.3	71.4 ± 3.7	74 ± 3.1	75.5 ± 2.7	78.3 ± 1.9	81 ± 1.1
Fresh seed	76.4±3.7	78.3±3.1	81.1±2.3	83.6±1.5	85.7±1.2	73.5 ± 4.1	75.8 ± 3.4	78.7 ± 2.6	80.8 ± 2.2	84 ± 1.8
Dry seed	75.6±3.5	76.2±3.4	77.9±3.0	80.8±2.3	84.1±1.8	71 ± 4.6	73.1 ± 4.2	74.6 ± 3.9	79.5 ± 1.9	82.3 ± 1.3
Bark	77.7±3.0	78.8±2.7	81.2±1.8	84±.0.5	84.7±0.3	74.8 ± 4.1	77.1 ± 3.9	76.1 ± 4.2	82.1 ± 1.5	84.3 ± 0.8

Similarly, at 100 µg/mL concentration minimum antiradical efficacy was observed for macerated dry fruit extract (79.4±1.9 %) and decocted dry fruit extract (78.3±1.9%). At 125 µg/mL concentration minimum potential was recorded for macerated dry fruit extract (82.1±1.3 %) and decocted fresh fruit extract (80.6±0.7 %).

Metal chelating activity: Another antiradical activity is the evaluation of metal chelating ability because it may catalyze fenton type reactions and hydroperoxide decomposition (Zengin *et al*., 2016). This method is based on measurement of ferrous ion-ferrozine complex absorbance after treating test material with Fe^{2+} solution. Fe^{2+} ion can move single electron allowing the creation and circulation of radical reactions. Complex formation takes place between Ferrozine and free Fe^{2+} however, other chelating agents disrupt complex formation thus a decreased complex formation after treatment specifies the existence of antioxidant chelators (Mohamed *et al*., 2021). Chelating agents had been reported as effective secondary antioxidants since they reduce the redox potential and stabilize the metal ion oxidized form. Fe^{2+} ions are regarded as the most competent pro-oxidants in pharmacology. The presence of ferrous chelating compounds breaks down the formation of ferrozine-Fe^{2+} complex resulting in red colour appearance (Turan *et al*., 2016). The results of our work show that fruits and seeds of *S. cumini* possess greater metal chelating ability.

Table 6: Percent metal chelating efficacy of extracts of *S. cumini*.

Extracts	Macerated					Decocted				
	Concentration µg/mL					Concentration µg/mL				
	25	50	75	100	125	25	50	75	100	125
Leaves	78.1±3.1	80.4±2.4	82.8±1.6	84.1±1.4	86.1±0.8	77.4±3.1	79.7±2.4	82±1.7	83.2±1.6	85.5±1.2
Fresh fruit	80.9±3.0	83.1±2.5	84.4±2.2	86.4±1.6	88.8±0.7	80.4±2.9	82.4±2.5	83.5±2.4	85.2±2.1	88.2±1.6
Dry fruit	79.9±3.1	82.2±2.5	84±2.1	85.6±1.7	88.1±0.8	79.1±2.9	81.5±2.3	83.2±2.0	84.1±1.9	87.1±1.3
Fresh seed	83.6±2.9	85±2.5	87±2.1	88.5±1.8	88±1.1	81.9±3.3	84.4±2.6	86.3±2.1	87.8±1.9	91.6±1.1
Dry seed	82.7±3.0	84.5±2.5	86.4±2.1	87.8±1.9	90±0.7	81.5±3.2	83.3±2.7	85.5±2.0	87.5±1.4	91±0.8
Bark	83.7±2.9	85.3±2.5	87.1±2.1	88.8±1.8	91.4±0.6	83.3±2.8	84.2±2.6	86.4±1.9	88.2±1.5	90.4±0.7

Meta chelating activity is another important assay to assess the antiradical potency of plants. At 25 µg/mL the maximum activity was given by extracts of bark (83.7±2.9 % for its macerated extract and 83.3±2.8 % for it decocted extract) and minimum activity was exhibited by extracts of leaves (78.1±3.1 % for its macerated extract and 77.4±3.1 % for its decocted extract). Macerated extract of bark gave 85.3±2.5 %, 87.1±2.1 %, 88.8±1.8 % and 91.4±0.6 % chelating potential as compared to 84.2±2.6 %, 86.4±1.9 %, 88.2±1.5 % and 90.4±0.7 % chelating potential of decocted extract at 50 µg/mL, 75 µg/mL, 100 µg/mL, and 125 µg/mL respectively. Similarly macerated extract of leaves gave 78.1±3.1 %, 80.4±2.4 %, 82.8±1.6 %, 84.1±1.4 % and 86.1±0.8 % chelating potential as compared to 77.4±3.1 %, 79.7±2.4 %, 82±1.7 %, 83.2±1.6 % and 85.5±1.2 % chelating potential of decocted extract at 25 µg/mL, 50 µg/mL, 75 µg/mL, 100 µg/mL, and 125 µg/mL respectively. Macerated extracts (ME) and decocted extracts (DE) of fresh and dried fruits showed interesting results. At 25 µg/mL the chelating potential was 80.9±3 % (ME) and 80.4±2.9 % (DE) of fresh fruit and 79.9±3.1 % (ME) and 79.1±2.9 % (DE) of dried fruit, at 50 µg/mL it was 83.1±2.5 % (ME) and 82.4±2.5 % (DE) of fresh fruit and 82.2±2.5 % (ME) and 81.5±2.3 % (DE) of dried fruit, at 75 µg/mL it was 84.4±2.2 % (ME) and 83.5±2.4 % (DE) of fresh fruit and 84±2.1 % (ME) and 83.2±2.0 % (DE) of dried fruit, at 100 µg/mL it was 86.4±1.6 % (ME) and 85.2±2.1 % (DE) of fresh fruit and 85.6±1.7 % (ME) and 84.1±1.9 % (DE) of dried fruit and at 125 µg/mL it was 88.8±0.7 % (ME) and 88.2±1.6 % (DE) of fresh fruit and 88.1±0.8 % (ME) and 87.1±1.3 % (DE) of dried fruit (Table 6).

Antimicrobial activity: Inappropriate, multiple and self-prescribed usage of synthetic drugs have triggered resistance of microbes against various antibiotics. This alarming situation forced scientist to look for herbal medicines to combat the growing resistance of pathogens (Waqas *et al*., 2021). Our study has revealed presence of important bioactive agents, in *S. cumini*, which can counter pathogens successfully.

Table 7: Antimicrobial activity of extracts of *S. cumini* extracts.

Pathogens	**Zone of Inhibition (mm)**						
	Control (Rifampicin)	Leaves	Fresh fruit	Dry fruit	Fresh seed	Dry seed	Bark
Escherichia coli	25	8	11	9	11	10	16
Staphylococcus aureus	20	14	13	12	15	12	13
Bacillus subtilis	18	12	14	9	10	10	16
Pseudomonas aeruginosa	15	10	11	9	11	11	12

Against *E. coli* all extracts except bark showed moderate combat ability. Bark extract showed higher sensitivity with 16 mm zone of inhibition (ZI). Against *S. aureus* moderate level inhibition potential was given by dry fruit and dry seed extracts and higher sensitivity was given by all other extracts. Against *B. subtilis* fresh fruit and bark extracts showed significant resistance with 14 mm and 16 mm ZI respectively but other extracts exhibited moderate level of sensitivity. Against *P. aeruginosa* none of the extracts gave higher resistance. All extracts exhibited moderate level of inhibition potential. A lot of novel antimicrobial agents have been discovered because of phytochemical constituents of plant extracts. Potential of plant extracts against microbes is attributed to presence of TFC (Adamczak *et al*., 2020; Yang *et al*., 2020). Ozcelik (2011) and Liu (2011) have also reported inhibitory potential of flavonoids against *E. coli* and *S. aureus*. As none of the extracts gave low level

of sensitivity against any of these pathogens, so, it can easily be inferred from it that *S. cumini* is a good natural source of bioactive compounds which can change trend of growing resistance of pathogens (Table 7).

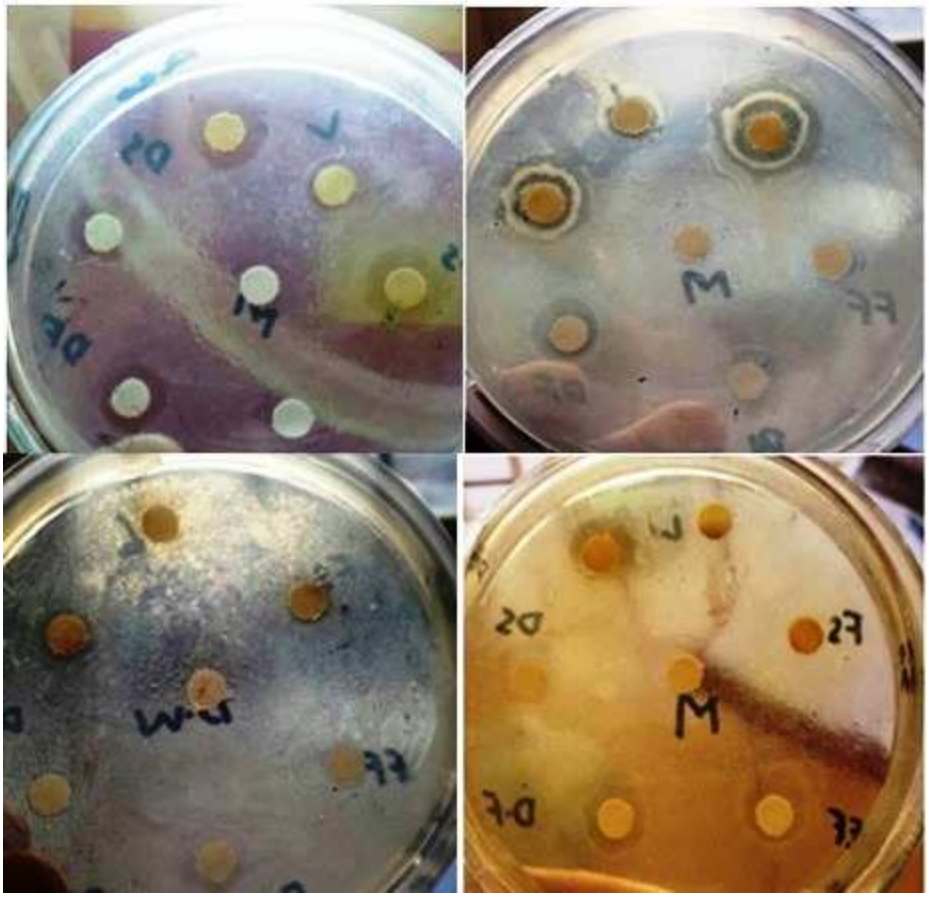

Figure 1: Antimicrobial activity of extracts of *S. cumini*

Antiglycation activity: Non-enzymatic reaction of circulating proteins with reducing sugars results in the formation of AGEs. These products result in cause of diabetic complications, impaired enzymatic action, odd molecular capacity, decreased degradation capacity and interloping in receptor recognition (Vasarri *et al*., 2020). Membrane basal proteins and human serum are subjected to in vivo non-enzymatic glycation. This susceptibility of human serum is because of its higher concentration and 20 days half-life. AGEs being fundamental markers of diabetes mellitus are core cause of all diabetic complications (Chaudhuri *et al*., 2018). Thus inhibitors of AGEs are necessary to deal with all the complications. A lot of plants have been scrutinized for their glycation inhibitory and radical inhibitory potential. Medicinal plants exhibiting glycation inhibitory and radical inhibitory efficacy possess higher remedial and defensive potential against different complications (Win *et al*., 2019). In the present study potential of *S. cumini* extracts to serve as natural inhibitors of AGEs was assessed by spectrophotometric and spectrofluorometric methods. Glycation process is affected by phenolic and polyphenolic compounds because of -OH group present in them. The glycation inhibitory effect of *S. cumini* thus can be clearly associated with abundance of polyphenols found in its extracts. In addition to antiradical and glycation inhibitory potential, this medicinal plant has also shown its potential against microbes which are main cause of fatal diseases in human beings. Thus *S. cumini* can be used to treat a lot of health complications to improve health and care facilities for human beings.

Aminoguanidine was used as a standard inhibitor in both spectrophotometric and spectrofluorometric methods. At 50 leaf extract exhibited minimum glycation inhibitory potential (30%). Fresh fruit extract gave 43%, 59%, and 76% inhibition at 50 µg/mL, 100 µg/mL and 150 µg/mL concentrations respectively in spectrophotometric analysis and 48% at 50 µg/mL, 74% at 100 µg/mL and 87% at 150 µg/mL in spectrofluorometric analysis. Similarly fresh seed extract exhibited 39%, 60% and 81% at 50 µg/mL, 100 µg/mL and 150 µg/mL concentrations respectively in

spectrophotometric analysis and 47%, 63%, 81% at the same concentrations in spectrofluorometric analysis. Maximum inhibitory potential was exhibited by bark extracts at all concentrations when examined by spectrophotometric method but in case of spectrofluorometric analysis bark and dry seed extracts showed 49% inhibition potential collectively at 50 µg/mL while at 100 µg/mL 74% inhibition potential was given by fresh fruit extract and at 125 µg/mL 88% inhibition potential was observed for dry seed extract.

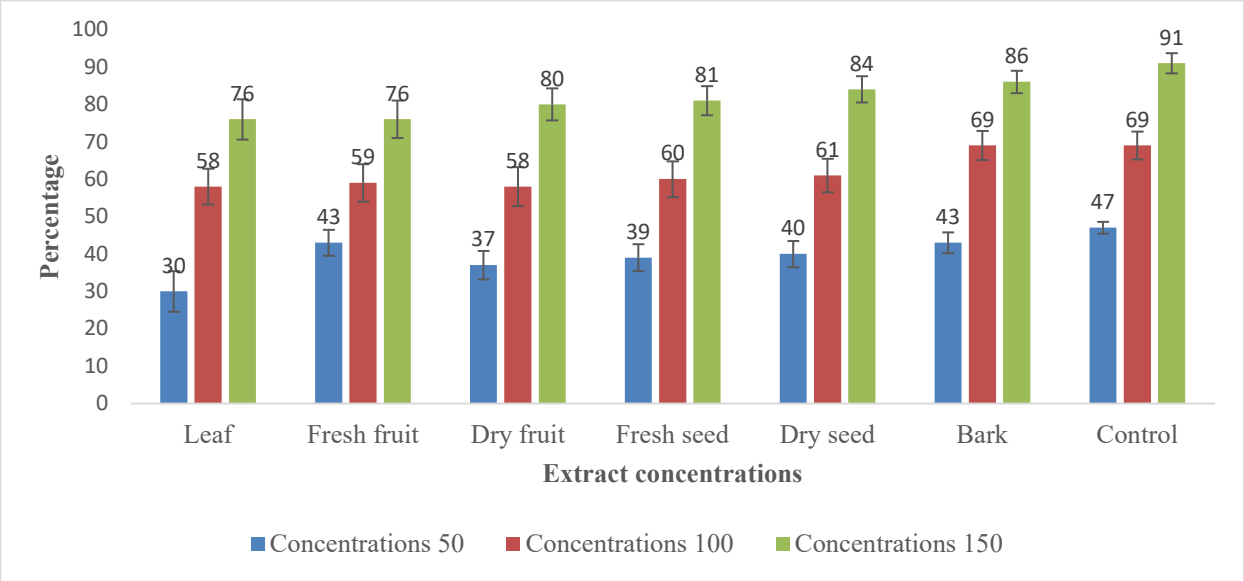

Figure 2: Spectrophotometric analysis of glycation inhibitory potential of *S. cumini* extracts

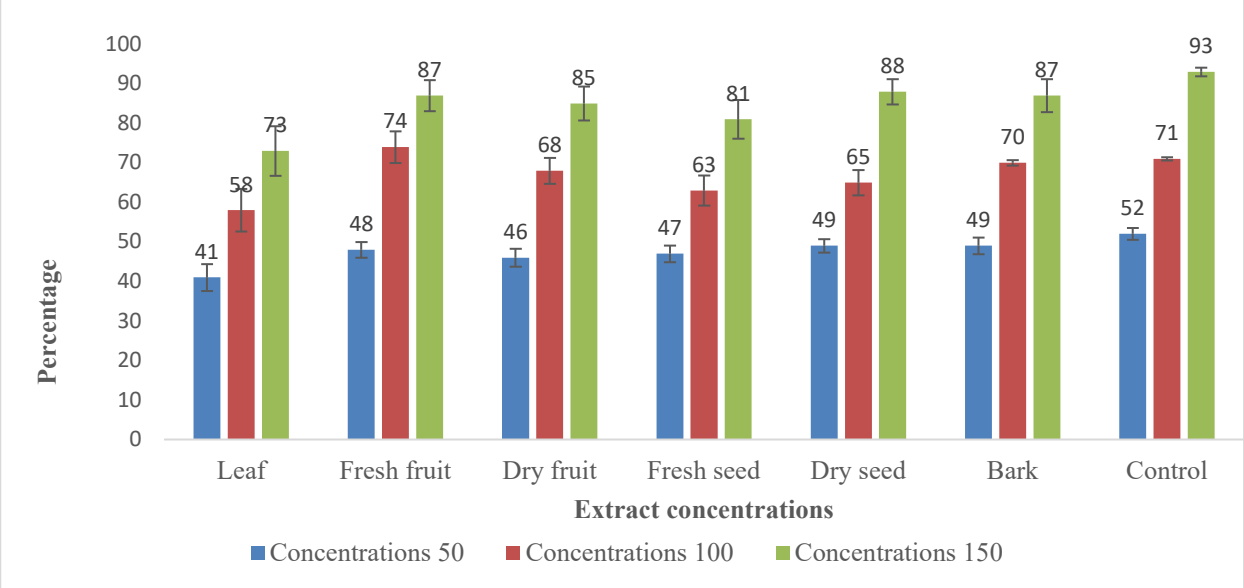

Figure 3: Spectrofluorometric analysis of glycation inhibitory potential of *S. cumini* extracts

Conclusions: These results show that extracts of *S. cumini* can outfit reactive radical species which means that important bioactive compounds are present in this plant. These bioactive compounds, according to this study, also served as glycation inhibitory agents strengthening medicinal importance of this plant especially its fruit and seed. Additional studies must be carried out isolate those bioactive compounds which can bring revolutionary changes in medicinal research works ultimately making life more protective against many dangerous diseases.

Authors Contributions: Muhammad Waqas and Beenish Akram designed and performed these activities and wrote the paper under supervision of Tahseen Ghous.

Beenish Akram and Tahseen Ghous analyzed the data. Mukhtar Ahmed wrote and reviewed the manuscript.

Conflict of Interests: The authors declare no conflict of interest.

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