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RESEARCH ARTICLE

Antioxidant Activity Profiling of Acetonic Extract of Jamun (*Syzygium cumini* L.) Seeds in Different *In-Vitro* Models

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Abstract:

Background:

Syzygium cumini L., commonly known as Jamun, black-plum, and Indian blackberry, is one of the most widely distributed trees in India with booming medical benefits and possesses antioxidant, anticancer and anti-diabetic properties. It belongs to the family *Myrtaceae*. Despite countless phytochemicals, seeds are not consumed and are the waste part of Jamun fruit.

Objective:

The objective of this study was to evaluate the antioxidant capacity of phenolics from Jamun seeds against a bundle of oxidant moieties.

Methods:

The 50% acetone extract of Jamun seeds was investigated for *in-vitro* antioxidant profiling. Assays include free radical scavenging activity, metal chelation activity, hydroxyl radical scavenging activity, hydrogen peroxide scavenging activity, total antioxidant activity, total reducing power, nitric oxide scavenging activity, and lipid peroxidation inhibition activity.

Results:

The extract depicted maximum DPPH radical scavenging activity followed by ABTS radical scavenging activity. Hefty metal chelation and nitric oxide scavenging activity were recorded while lipid peroxidation, H_2O_2, and OH^- scavenging activity was intermediate.

Conclusion:

Jamun seed showed ample antioxidant activity and certifies that it is the right candidate for exploitation as a source of natural antioxidants to counteract autoxidation-induced pathologies or diseases.

Keywords: Jamun, *Syzygium cumini*, Seeds, Antioxidant, Natural products, Phenolics.

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1. INTRODUCTION

The cellular level of Reactive Oxygen Species (ROS) is regulated by the interaction of complex antioxidant types of machinery in living systems. These natural antioxidants are well known to curtail the adverse effects of free radicals and withstand oxidative damage in biological entities. Antioxidants prevent the oxidation process that can damage cells in the body. They prevent the living system by scavenging the free radicals interactively and harmoniously and doing this get themselves oxidized instead of cells. When the ROS level rises, the damage becomes cumulative and results in incapacitating oxidative stress [1]. Dietary antioxidants can suppress the harmful effects of oxidative stress. Presently, most of the commonly used antioxidants like butylated hydroxyl toluene (BHT), Butylated Hydroxyl Anisole (BHA), and *tert*-butyl hydroquinone (TBHQ) are manufactured synthetically [2]. They are usually used to slow down the oxidative deterioration, but due to their probable toxic and carcinogenic effects, there has been increasing anguish over their use in fresh or processed foods [3]. Therefore, recent years have seen an increased surge of interest in finding naturally occurring antioxidants to substitute these synthetic antioxidants. As a result, the use of natural and safe antioxidants, especially of fruits and vegetable origin, has substantially increased among consumers and food scientists.

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Epidemiological studies have persistently shown that consistent consumption of fruits and vegetables is strongly correlated with a lowered risk of developing chronic diseases such as cancer and cardiovascular disease [4, 5]. Because of increased risk factors of various deadly diseases to humans, there has been a global trend towards the use of natural substances present in medicinal and dietary plants as the therapeutic antioxidants. It has been found in various studies that there is an inverse relationship between dietary intake of antioxidant-rich food/medicinal plants and the prevalence of human diseases. Wang *et al*. [6] affirmed that strawberry, orange, plum, red grape, kiwi fruit, and apple have healthy antioxidant activities. Studies conducted by Aqil *et al*. [7] have provided trustworthy evidence of Jamun's antioxidant potential and cancer-preventive properties. Worldwide, total production of Jamun is 13.5 million tonnes, out of which India contributes ~15.4% [8]. Jamun is an evergreen tropical tree and is native to Bangladesh, India, Nepal, Pakistan, Sri Lanka, Philippines, and Indonesia. It is an endemic and important commercial fruit of India. It has also been recognized as a nutraceutical fruit due to the presence of robust antioxidant compounds such as ascorbic acid, anthocyanins, and total phenols. Jamun fruit has been found rich in carbohydrates, minerals, and vitamins. Its seed weighs 1-3 g, and an average-sized fruit contains 68-86 mg of pulp [9]. Phytochemical analysis of Jamun seeds has revealed the presence of alkaloids, flavonoids, glycosides, phytosterols, saponins, tannins, and triterpenoids [10]. Its seeds contain albumen, fat, glycosides, jambosine, resin, ellagic acid, quercetin, gallic acid, as well as elements such as zinc, vanadium, sodium, and potassium [8]. Despite these phytochemicals, seeds are not consumed and are the waste part of Jamun fruit. In our laboratory, we are continuously exploring new sources of phenolics compounds [11, 12]. In continuation, we have explored Jamun seed as a good source of phenolic compounds and have devised an effective extraction protocol to extract them (unpublished data). The present work, herein, represents the antioxidant potential of phenolics rich Jamun extract.

2. MATERIALS AND METHODS

2.1. Fruits and Chemicals

Jamun fruits used in present investigations were procured from the local market of Hisar. All the chemicals used in this investigation were of high-quality analytical grade and were purchased from M/s Sigma Chemicals Co., USA, Sisco Research Laboratory, Hi-Media, and E. Merck, Mumbai.

2.2. Methods

Seeds were separated from the pulp, chopped, shade dried in the dark, and ground to powder. Total phenolic compounds were extracted from the powder under the pre-optimized conditions using 25 ml/g volume of solvent (50% acetone), 193 rpm agitation rate, and 150 min extraction time (unpublished data). The extract was evaluated for its *in-vitro* antioxidant potential using an array of oxidants.

2.2.1. DPPH Radical Scavenging Assay

The extract/TPC was assayed in terms of its radical-scavenging ability using the stable radical, DPPH (1, 1 diphenyl–2–picrylhydrazyl), following the method of Blois [13].

DPPH radical scavenging activity was calculated using the formula given below –

DPPH scavenging activity (%) = [Ac-As/Ac] x 100

Where Ac is the absorbance of control solution and As is the absorbance of the test solution. IC_{50} (concentration of sample required to scavenge 50% free radicals) was calculated from a regression analysis. BHA was used as the standard antioxidant.

2.2.2. Metal Chelation Activity

The chelating effect of extracts on ferrous ions was determined according to the method of Dinis *et al*. [14]. The results were expressed as IC_{50} calculated from a regression analysis. EDTA was used as a standard antioxidant.

2.2.3. ABTS Assay

The method used is based on the quenching of the most stable free cation (ABTS) decolorization assay [15]. The results were expressed as IC_{50} calculated from a regression analysis. BHA was used as a standard antioxidant.

2.2.4. Hydrogen Peroxide Scavenging Assay

The ability of sample extract to scavenge hydrogen peroxide was determined according to the modified method of Ruch *et al*. [16]. The results were expressed as IC_{50} calculated from a linear regression analysis. BHA was used as a standard antioxidant.

2.2.5. Hydroxyl Radical Scavenging Assay

The assay was performed following the method by Halliwell *et al*. [17] with slight modifications. Percent inhibition was calculated from the control without samples under similar conditions. IC_{50} was calculated from a regression analysis. BHA was used as a standard antioxidant.

2.2.6. Total Antioxidant Activity

The assay was performed as described by Prieto *et al.* [18]. The antioxidant capacity was expressed as $AO_{0.5AU}$ (amount of extract that produces 0.5 absorbance units at selected wavelength). Gallic acid was used as a standard antioxidant.

2.2.7. Total Reducing Power

The total reducing power of the extract was determined by the method of Oyaizu [19]. Increased absorbance of the reaction mixture indicates increased reducing power. The total reducing power was expressed as $RP_{0.5AU}$ (amount of extract that produces 0.5 absorbance units at selected wavelength). Gallic acid was used as a standard antioxidant.

2.2.8. Nitric Oxide Scavenging Activity

The procedure is based on the principle that sodium nitroprusside in aqueous solution at near-neutral pH spontaneously generates nitric oxide, which interacts with

oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to decreased production of nitrite ions [20]. The percent scavenging activity was calculated from the control without extract under similar conditions. IC_{50} was calculated from a regression analysis. Gallic acid was used as a standard antioxidant.

2.2.9. Lipid Peroxidation Inhibition Activity

A modified Thiobarbituric Acid Reactive Species (TBARS) assay [21] was used to measure the lipid peroxide formed using egg yolk homogenate as a lipid-rich medium. The absorbance of the upper organic layer was measured at 532 nm. Inhibition of lipid peroxidation (%) was calculated using control without samples under similar conditions. IC_{50} was calculated from a regression analysis. BHA was used as a standard antioxidant.

3. RESULTS

The 50% acetone extract of Jamun seeds was investigated for the *in-vitro* antioxidant profile. Jamun seeds extract (JSE) was analyzed against a battery of oxidant moieties.

3.1. DPPH Radical Scavenging Assay

A dose-dependent increase in the quenching of free radicals was observed for JSE. In the present study, the IC_{50} value of JSE was calculated as 0.24 µg/ml (Table **1**), indicating it as a good scavenger of DPPH radical. The JSE showed 64.21% DPPH scavenging at 0.25 µg/ml while BHA exhibited only 14.69% activity at the same concentration, thus proving JSE a better scavenger of free radicals than BHA, a well-known synthetic antioxidant.

Table 1. DPPH radical scavenging activity of JSE.

JSE/TPC (µg/ml)	Percent inhibition	Linear regression equation (correlation coefficient)	IC_{50} (µg/ml)
0.082	16.5±0.7	-	-
0.164	32.5±0.9	-	-
0.246	64±1	y = 205.63x + 1.4189 (R^2 = 0.9645)	0.24
0.328	66±1	-	-
0.41	81.6±0.8	-	-

3.2. Metal Chelating Activity

A decrease in concentration-dependent color formation in the presence of the extract indicates iron-chelating potential. The metal chelating activity of JSE (IC_{50} value, 48.39 µg/ml) was calculated (Table **2**) and compared with standard anti-oxidant – EDTA (IC_{50} value, 37.99 µg/ml) which was used as a positive control.

3.3. ABTS Radical Scavenging Assay

Results show that JSE possesses remarkable ABTS scavenging activity, and it increases as the concentration rises. In the present study, the IC_{50} value of JSE was 0.31 µg/ml (Table **3**), indicating it a good scavenger of ABTS radicals.

Table 2. Metal chelating activity of JSE.

JSE/TPC (µg/ml)	Percent inhibition	Linear regression equation (correlation coefficient)	IC_{50} (µg/ml)
12.76	17 ± 1	-	-
25.53	26 ± 1	-	-
38.29	37 ± 1	y = 1.0085x + 1.2026 (R^2 = 0.992)	48.39
51.06	55 ± 1	-	-
63.82	65 ± 2	-	-

Table 3. ABTSradical scavenging activity of JSE.

JSE/TPC (µg/ml)	Percent inhibition	Linear regression equation (correlation coefficient)	IC_{50} (µg/ml)
0.1	22.1±0.8	-	-
0.19	32 ± 1	-	-
0.29	53 ± 1	y = 145.68x + 4.8059 (R^2 = 0.9686)	0.31
0.38	63 ± 1	-	-
0.48	68 ±1	-	-

3.4. Hydrogen Peroxide Scavenging Activity

When compared with BHA (IC_{50} 21.77 µg/ml) at equivalent concentrations, JSE exhibited better activity (IC_{50} 5.67 µg/ml), as depicted in Table **4**.

Table 4. Hydrogen peroxide scavenging activity of JSE.

JSE/TPC (µg/ml)	Percent inhibition	Linear regression equation (correlation coefficient)	IC_{50} (µg/ml)
1.91	26 ± 1	-	-
3.83	35.2 ± 0.9	-	-
5.74	49.1 ± 0.8	y = 8.012x + 4.588 R^2 = 0.984	5.67
7.66	66 ± 1	-	-
9.55	81 ± 1	-	-

3.5. Hydroxyl Radical Scavenging Activity

The IC_{50} value from Table **5** depicts that the plant extract (5.75 µg/ml) is a better hydroxyl radical scavenger than the standard BHA (18.01 µg/ml). In contrast, Pal *et al*. (2011) showed that gallic acid had better hydroxyl radical scavenging activity than ashwagandha root extracts.

3.6. Total Antioxidant Activity

The extract was found to possess substantial TAA with $AO_{0.5AU}$ value of 5.37 µg/ml (Table **6**) as compared with gallic acid ($AO_{0.5AU}$ 9.62 µg/ml).

3.7. Total Reducing Power

A rise in absorbance of the reaction mixture depicts an increase in reducing capacity due to the increased formation of the complex. The $RP_{0.5\ AU}$ was found to be 3.10 µg/ml (Table **7**), which was relatively more pronounced than that of gallic acid (5.47 µg/ml).

Table 5. Hydroxyl radical scavenging activity of JSE.

JSE/TPC (µg/ml)	Percent inhibition	Linear regression equation (correlation coefficient)	IC_{50} (µg/ml)
1.64	25 ± 2	-	-
3.28	33.2 ± 0.9	-	-
4.92	43 ± 1	y = 7.5982x + 6.293 (R^2 = 0.9676)	5.75
6.56	57 ± 2	-	-
8.21	66 ± 2	-	-

Table 6. Total antioxidant activity of JSE.

JSE/TPC (µg/ml)	Absorbance	Linear regression equation (correlation coefficient)	$AO_{0.5AU}$
1.74	0.124 ± 0.005	-	-
3.48	0.319 ± 0.008	-	-
5.22	0.46 ± 0.01	y = 0.0978x - 0.0253 (R^2 = 0.9957)	5.37
6.96	0.66 ± 0.03	-	-
8.7	0.85 ± 0.02	-	-

3.8. Nitric Oxide Radical Scavenging Assay

The nitric oxide radical scavenging activity of JSE (IC_{50} value, 37.01 µg/ml), as shown in Table **8**, was compared with standard antioxidant – gallic acid (IC_{50} value, 33.2 µg/ml).

3.9. Lipid Peroxidation Inhibitory Activity

The decrease in MDA level by JSE illustrates its role as an antioxidant. The present study shows that JSE (IC_{50}, 5.38 µg/ml) (Table **9**) has more influential lipid peroxidation inhibitory activity as compared to BHA (IC_{50}, 18.97 µg/ml).

4. DISCUSSION

4.1. DPPH Radical Scavenging Assay

The DPPH scavenging activity of the Jamun Seed Extract (JSE) was taken as a parameter to estimate its antioxidant potential. DPPH is a relatively stable radical, and bleaching of its absorption (515 nm) by a test compound is representative of its capacity to scavenge free radicals by donating a hydrogen atom to become a stable diamagnetic molecule. DPPH radical has the undeniable advantage of being unconcerned by the side reactions, *i.e*., enzyme inhibition and metal chelation [22]. Our results indicate distinct scavenging activity of the extract towards DPPH radicals in comparison with BHA. A high DPPH scavenging activity has also been reported in the acetonic extract of *S. cumini* leaves [23].

Table 7. Total reducing power of JSE.

JSE/TPC (µg/ml)	Absorbance	Linear regression equation (correlation coefficient)	$RP_{0.5AU}$
0.76	0.126 ± 0.005	-	-
1.53	0.257 ± 0.009	-	-
2.3	0.38 ± 0.01	y = 0.1594x + 0.007 (R^2 = 0.9988)	3.10
3.06	0.50 ± 0.03	-	-
3.83	0.61 ± 0.03	-	-

Table 8. Nitric oxide radical scavenging activity of JSE.

JSE/TPC (µg/ml)	Percent inhibition	Linear regression equation (correlation coefficient)	IC_{50} (µg/ml)
11.49	25.5 ± 0.2	-	-
22.98	337.4 ± 0.9	-	-
34.46	51 ± 1	y = 1.1513x + 7.3879 (R^2 = 0.9611)	37.01
45.95	59 ± 1	-	-
57.44	70 ± 2	-	-

Table 9. Lipid peroxidation inhibitory activity of JSE

JSE/TPC (µg/ml)	Percent inhibition	Linear regression equation (correlation coefficient)	IC_{50} (µg/ml)
1.42	20 ± 1	-	-
2.83	28 ± 1	-	-
4.25	40 ± 1	y = 8.6211x + 3.5496 (R^2 = 0.9883)	5.38
5.66	53 ± 1	-	-
7.09	63 ± 2	-	-

4.2. Metal Chelating Activity

Iron an acutely reactive metal and causes oxidative damage to lipids, proteins, and other cellular components. Metal chelating capacity is desirable to decrease the concentration of transition metals, causing lipid peroxidation. In this assay, EDTA had slightly higher chelating activity as compared to JSE, and similar results were found by Bholah *et al.* [24] in *Moringa oleifera* Lam. extracts and Pal *et al*. [12] in red rose petal extracts.

4.3. ABTS Radical Scavenging Assay

The ABTS scavenging activity is taken as a parameter for desolvation of chain-breaking antioxidants in case of lipid peroxidation and antioxidant activity of hydrogen donating antioxidants [25]. The ABTS method is established as a rapid method for the determination of antioxidant activity and could be a convenient tool to screen samples and cultivars to obtain great content of natural antioxidants in foods. A high ABTS scavenging activity has also been found in Jamun fruit peel

extract than standard compounds such as gallic acid, caffeic acid, sinapic acid, and quercetin [26].

4.4. Hydrogen Peroxide Scavenging Activity

The generation of even low levels of H_2O_2 in biological systems may be pernicious since naturally occurring iron complexes react with H_2O_2 to produce highly reactive hydroxyl radicals in a superoxide-driven 'Fenton' reaction [27]. Pal *et al*. [11] showed that ascorbic acid had higher hydrogen peroxide scavenging activity than ashwagandha root extracts. Tchimene *et al*. [28] reported that leaf extract of *Crateva adansonii* had better hydrogen peroxide scavenging activity than ascorbic acid.

4.5. Hydroxyl Radical Scavenging Activity

This radical can cause strand damages in DNA, causing carcinogenesis, mutagenesis, and cytotoxicity. Moreover, hydroxyl radicals are capable of quick inception of the lipid peroxidation process by abstracting hydrogen atoms from unsaturated fatty acids [29, 30]. The hydroxyl radicals were produced in the present study by incubating ferric-EDTA with ascorbic acid and H_2O_2 at pH 7.4 and allowed to react with 2-deoxy-2-ribose to produce malondialdehyde (MDA)-like product. This compound then formed a pink chromogen upon heating with TBA at low pH.

4.6. Total Antioxidant Activity (TAA)

Since the assay is simple and unaffected by other antioxidant measurements commonly employed, its application has been expanded to plant extracts [18]. The assay is successfully used in the quantification of vitamin E in seeds. Pal *et al*. [11, 12], differently, stated that gallic acid had higher TAA than ashwagandha root and red rose petal extracts.

4.7. Total Reducing Power (TRP)

The reducing capacity of a compound can be estimated by the direct reduction of Fe^{3+} to Fe^{2+}. The addition of free Fe^{3+} to the reduced product leads to the formation of intense Perl's Prussian blue complex, which possesses strong absorbance at 700 nm. This method is often used as an indicator of electron-donating activity, which is an important mechanism for testing the antioxidant activity of plant extracts [31]. Many reports have affirmed that there is a direct correlation between antioxidant activities and reducing the power of certain plant extracts [31, 32]. Our present results imply that JSE has the potential to donate electrons to reactive free radicals, hence, converting them to more stable non-reactive species and terminating the free radical chain reaction.

4.8. Nitric Oxide Radical Scavenging Assay

Nitric oxide is a dynamic pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation, and regulation of cell-mediated toxicity [33]. The excessive production of NO is associated with various diseases like adjuvant arthritis, cancer, *etc*. Our results are following those reported by Pal *et al.* [11], where ascorbic acid showed higher nitric oxide radical scavenging activity than different extracts of ashwagandha root. The mechanism involved in the scavenging activity of the extract may be associated with its phenolic compounds, as indicated in earlier studies [34].

4.9. Lipid Peroxidation Inhibitory Activity (LPIA)

Lipid peroxidation is a crucial biological consequence of oxidative cellular damage and aging. The polyunsaturated fatty acyl side chains, due to their susceptibility to oxidative damage in membrane phospholipids, pose a consistent threat to cellular integrity and functions [35]. In the process, cyclic peroxides, lipid peroxides, and cyclic end peroxides are produced, which ultimately get fragmented into aldehydes like malondialdehyde (MDA). MDA forms a pink chromogen with TBA that has a strong absorbance at 532 nm. JSE inhibited the amount of MDA generated in the egg homogenate model. Anjum *et al.* [36] also showed that walnut extracts could prevent oxidative deterioration of linoleic acid. It may be due to their polyphenolic compounds which protect the fatty acids from getting rancid.

CONCLUSION

In-vitro antioxidant studies depict that the radical scavenging capacity of JSE for DPPH radical is almost similar to ABTS radical and much higher than that of metal chelation activity and nitric oxide scavenging activity. Moderate scavenging activity was recorded against lipid peroxidation, H_2O_2, and OH^- radicals. Overall, it is essential to evaluate more than one antioxidant assay when natural plant extracts are being evaluated for their antioxidant potential.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial, or otherwise.

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