Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2014-15; 7(1); 150-155

ISSN: 0975-4873

Research Article

In vitro Phytochemical, Antibacterial and Antioxidant Analyses in Different Plant Parts of *Syzium cumini*

Sanzida Mubassara[1*], Kalyan Kumar Biswas[1], Md. Muksedul Hasan[1], Md. Ibrahim Hossain[2], Sudip Paul[2]

[1]*Dept. of Botany, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh*
[2]*Dept. of Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh*

Available Online: 1st February, 2015

ABSTRACT

Syzium cumini has been traditionally used in folk medicine for various purposes. The present study was conducted in the leaves, barks, fruits and pulps of *S. cumini* plant to evaluate their phytochemical, antibacterial and antioxidant properties. Ethanolic extracts of all these plant materials were prepared using Soxhlet apparatus. Preliminary phytochemical studies revealed the presence of flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoids, cardiac glycosides and tannins as the chemical class present in the extracts. The results suggested the phytochemical properties of the plant for curing various ailments. The antimicrobial activity of the various extracts was tested against standard strains and clinical isolates of some bacteria using the disc diffusion method. The extracts showed inhibitory activity against Gram-positive bacteria *Bacillus subtilis, Bacillus cereus and Staphylococcus aureus.* Clinical isolates of the Gram-negative bacteria such as *Shigella flexneri, Pseudomonas aeruginosa* and *Vibrio cholera* also showed some vulnerability to the extracts. The leaf and bark extracts were found to be more potent antimicrobial than the pulp and seed extracts. Preliminary antioxidant activities of the extracts were also conducted and found that pulp extract has the highest antioxidant activity reflected by total polyphenol content, DPPH radical scavenging activity and total reducing power among the various parts of the plant followed by seed, bark and leaf extract. The findings from the study provide a support for the use of various parts of the plant in traditional medicine and for its further investigation.

Key words: *Syzium cumini*, traditional medicine, phytochemical, antibacterial, antioxidant

INTRODUCTION

Different parts of the plants contain various complex substances as secondary metabolites in different compositions which have been used in the treatment and prevention of various ailments since time immemorial[1]. Because of the side effects associated with synthetic drugs, health aware people are now moving towards different plant parts popularly known as traditional herbal medicines as remedies for different diseases. Modern scientific approaches would allow isolation, purification and characterization of bioactive compounds present in these plant parts and use these as safe drugs.

Syzygium cumini Linn (Family: Myrtaceae), commonly known as Jam is a popular seasonal fruit in Bangladesh, India and many other South East Asian countries. It is not only a delicious fruit but also an important traditional and modern medicine.

Different parts of the plant especially fruits, seeds and stem bark possess promising activity against diabetes mellitus and it has been confirmed by several experimental and clinical studies[2-5]. Despite tremendous advancements have been made in the field of diabetic treatments, several earlier investigations have been reported from the different parts of the plant with antioxidant[6,7], anti-inflammatory[8,9], hepatoprotective[10], antibacterial and antifungal[11], and many other therapeutic activities.

The plant possesses a large number of chemical compounds such as vitamin C, gallic acid, tannins, anthocyanins, acetyl oleanolic acid, triterpenoids, ellagic acid, isoquercitin, quercetin, kaempferol and myricetin in different concentrations[12-14]. Most of these compounds have been reported to possess nutritive and therapeutic potentials.

Although a large number of literature have been published regarding antioxidant and antimicrobial properties of *S. cumini*, there is scant information about comparative study including different parts of the plant. The aim of this study was to determine the organ-function relationship of the plant because various parts of this plant such as fruit pulp, seed, bark and leaves have already been reported to exhibit medicinal properties. Therefore, all parts of the plant should be analyzed to find out the relative bioactive properties of them which will ultimately establish its status as medicinal plant. This will ultimately help to find out potential sources in the plant which have anti-oxidative and anti-microbial activity so that it could be used as dietary supplements and drugs to improve physical health and prevent or treat different diseases.

**Author for Correspondence*

Table 1: Phytochemical screening in ethanolic extract of different plant parts of *Syzium cumini*

Sl. No.	Name of the test	Result			
		Leaf Extract	Bark Extract	Seed Extract	Pulp Extract
01	Alkaloids	+++	++	+++	++
02	Tannins	+++	+++	+++	+++
03	Saponins	+++	+	+++	++
04	Flavonoids	+++	++	++	++
05	Anthraqinon glycosides	+	+	-	-
06	Cardiac glycosides	+	+	+	+
07	Phenols	++	++	++	++
08	Terpenoids	-	+	+	+
09	Phytosterols	-	+	+	+
10	Steroids	+++	++	+	++
11	Amino acids	+	+	+	+

"+++" = highly present, "++" = moderately present, "+" = slightly present and "-"= absent

Table 2: Antibacterial activity of ethanolic extract of different parts of *Syzygium cumini*

Microorganisms	Types	Zone of inhibition (mm)				
		Leaf Extract	Bark Extract	Seed Extract	Pulp Extract	Standard antibiotics
Escherichia coli	(-)	8	8	8	9	22.3
Shigella flexneri	(-)	10.3	10	9	10	15.7
Salmonella tyhpi	(-)	8	8	8	8	23.2
Pseudomonus aerus	(-)	12	12.5	12.5	11	18.3
Vibrio colerae	(-)	13.5	9.5	11.4	11.5	14
Bacillus subtilis	(+)	16.5	14.75	11.5	17	22.3
Bacillus cereus	(+)	21.25	20.5	18.5	18	25.3
Staphylococcus aureus	(+)	16.7	14.7	13.5	13	18.2

MATERIALS AND METHODS

Collection of plant materials

Fresh *S. cumini* plant materials were collected from Jahangirnagar University (JU) campus and were taxonomically identified by the specialist of JU herbarium.

Preparation of extracts

The plant materials were washed with tap water, prior to distilled water, shade dried and powdered. The powdered plant materials were subjected to extraction with 70% ethanol using soxhlet extractor. The extracts were dried in a vacuum pump (40^{o}C). The dried crude extracts were stored in a freezer (0^{o}C) for future use.

Phytochemical screening

The preliminary phytochemical screening tests were carried out to identify the useful constituents present in the plant materials by standard methods. In each test 10% (w/v) solution of the ethanol extract was used unless otherwise mentioned in individual test.

Test for alkaloids

A small amount of the extract was dissolved in water. If the sample did not dissolve in water, a drop of concentrated HCl or H_2SO_4 was added and gentle heat was applied. Different reagents were added in order to observe the precipitation and colour of the sample: If Hager's reagent showed yellow ppt., Mayer's reagent showed off-white ppt., and Wagner's reagent showed brown ppt., then this colour and precipitation indicated the presence of alkaloids.

Test for tannins

Few drops (2-3) of ferric chloride ($FeCl_3$) solution were added to a small amount of the extract. Formation of a black or blackish brown colour indicated the presence of tannins.

Test for saponins

A small amount of the extract was taken into a test tube and vigorously shaken with water. Appearance of foam persisting for 10 minutes indicated the presence of saponins.

Test for flavonoids

Few drops of concentrated HCl were added to a small amount of the extract. Immediate development of a red colour indicated the presence of flavonoids.

Test for anthraquinone glycosides

About 0.5 gm of powdered plant material was taken in a dry test tube. Five (5) ml of chloroform was added and the mixture was shaken at least for 5 minutes. The extract was filtered and the filter was shaken with an equal volume of 10% ammonia solution. Formation of a bright pink colour in the aqueous (upper) layer confirmed the presence anthraquinone glycosides.

Test for cardiac glycosides

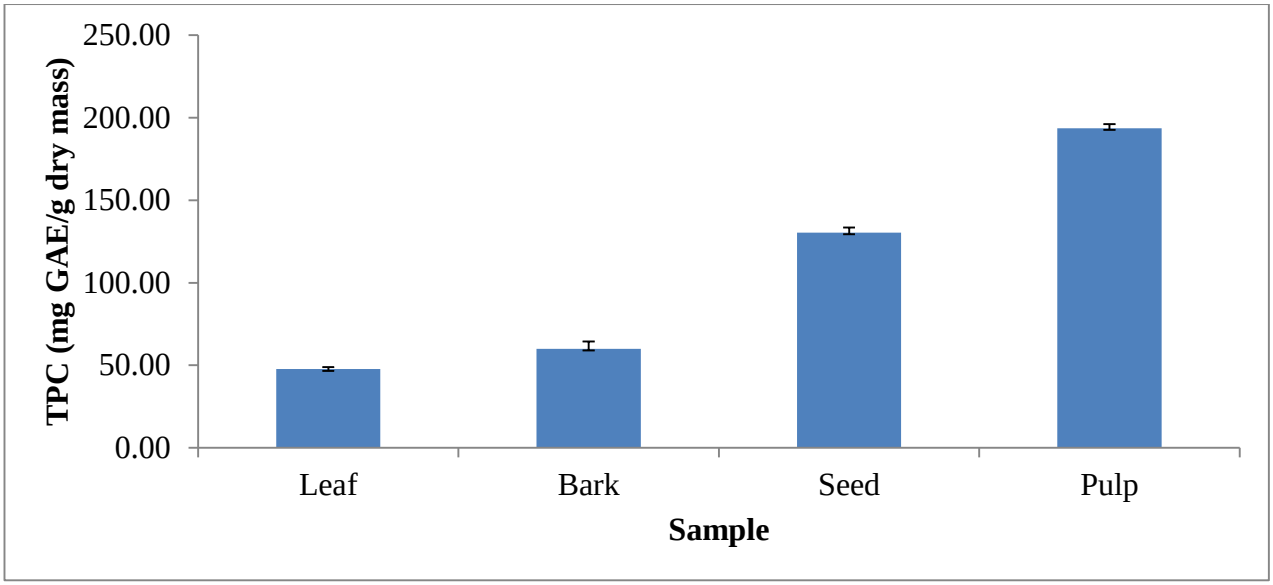

Fig 1: Total phenolic content of ethanolic extract of leaf, bark, seed and pulp of *Syzium cumini*. Data are presented as mean ± SE values of three replicates.

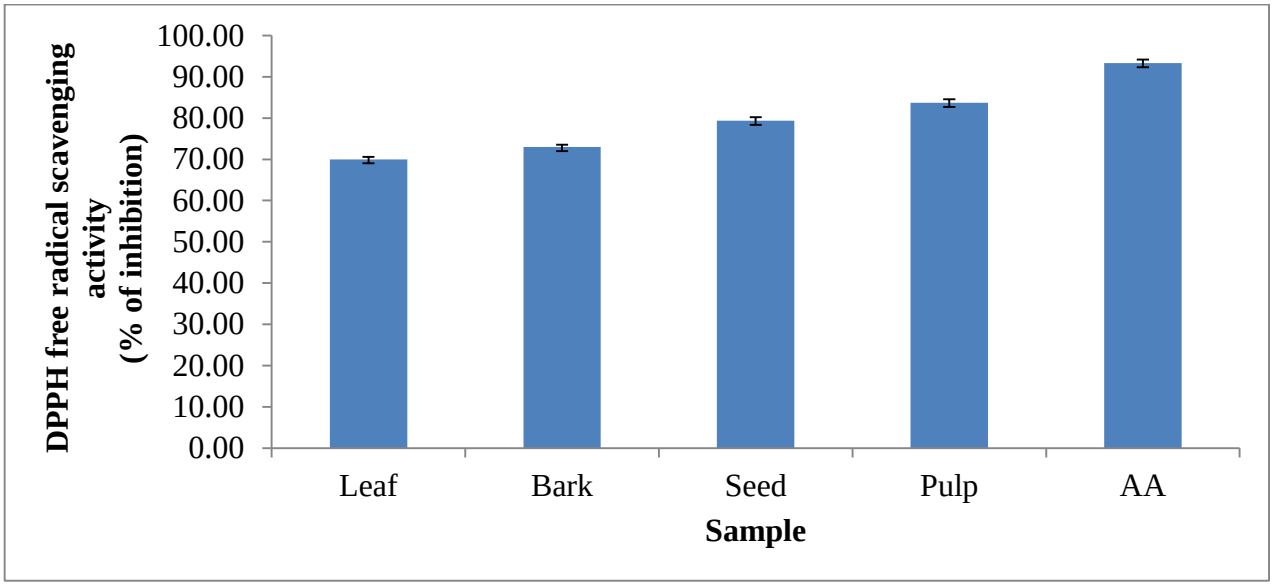

Fig 2: DPPH free radical scavenging activity of ethanolic extract of leaf, bark, seed and pulp of *Syzium cumini*. Data are presented as mean ± SE values of three replicates. AA, Ascorbic acid.

About 1 gm of the powdered plant material was boiled with 70% alcohol for about 3 minutes and then filtered. To the filtrate, 5 ml of water and 0.5 ml of a strong solution of lead acetate were added. The mixture was shaken well and filtered. The filtrate was extracted with an equal volume of chloroform and was evaporated to dryness. The residue was dissolved in 3 ml of glacial acetic acid and 2 drops of ferric chloride solution were added to it. The mixture was then poured slowly into a test tube containing 2 ml of concentrated H_2SO_4 to form an upper layer. A reddish brown colour was found to be formed at the junction of the low layers and the upper layer turned bluish green on standing. This was an evidence for the presence of cardiac glycoside in the plant material.

Test for phenols

About 1 ml of the extract was taken into a test tube; and 2 ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution was added. Formation of blue or green color indicated the presence of phenols.

Test for terpenoids

About 2 ml of chloroform and 1 ml of conc. H_2SO_4 were added to 1 ml of extract and observed for reddish brown color that indicated the presence of terpenoids.

Test for phytosterols

The extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted and extracted with ether. The ether layer was evaporated and the residue was tested for the presence of phytosterol. The residue was dissolved in few drops of diluted acetic acid; 3 ml of acetic anhydride

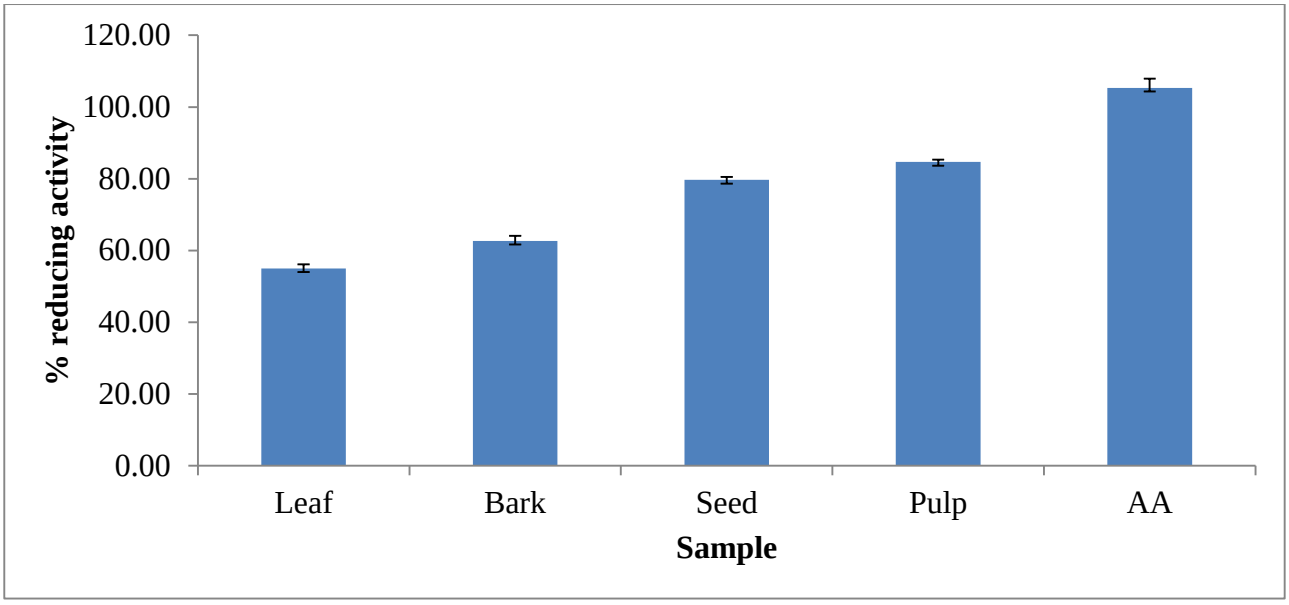

Fig 3: Total reducing power of ethanolic extract (100 µg/ml) of leaf, bark, seed and pulp of *Syzium cumini* compared with 100 µg/ml ascorbic acid (AA). Data are presented mean ± SE values of three replicates.

was added followed by few drops of Conc. H_2SO_4. Appearance of bluish green color indicated the presence of phytosterol.

Test for steroids

One ml of the extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turned into red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Test for amino acids

One ml of the extract was treated with few drops of Ninhydrin reagent. Appearance of purple colour indicated the presence of amino acids.

Screening of antibacterial activity

The agar disc diffusion method[15] was used to evaluate the antibacterial activity of the plant extracts by measuring the zone of inhibition against the test organisms. The microorganisms were inoculated into nutrient agar medium. Sterile filter disc (8mm in diameter) were impregnated with 40 µl of sample extract (250 mg/ml) and placed on the inoculation plates. The plates were incubated at 37ºC for 24 hours. Microbial growth inhibition was determined as the diameter of the inhibition zones around the discs.

Analysis of antioxidative activity

The antioxidative activity of the plant extracts was evaluated by means of determining total phenolic content (TPC), DPPH radical scavenging activity and total reducing power.

Determination of TPC

The TPC of the ethanolic extract of different parts of *S. cumini* plant was determined by Folin-Ciocalteu method[16] with gallic acid (GA) as the standard and expressed in terms of gallic acid equivalent (mg/g of dry mass).

Determination of DPPH radical scavenging activity

The antioxidant activity of the each sample extract was assessed by the ability of the extract to scavenge 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radicals. DPPH free radical scavenging activity was monitored by measurement of decline in absorbance at 517 nm. Vitamin C was used as the standard compound[17].

Determination of total reducing power

The reducing power of the extracts was estimated following Oyaizu[18] using vitamin C as standard.

Statistical analyses

Statistical analyses were performed by Statistical Package for Social Sciences (SPSS) version 13.0 where needed. Microsoft Office Excel 2007 was used to generate the bar charts.

RESULTS

Phytochemical screening of the samples

Phytochemical screening was carried out on different parts of the *S. cumini* plant using standard procedure to identify the phytoconstituents and results are presented in Table 1. The extracts revealed the presence of alkaloids, tannins, saponins, flavonoids, cardiac glycosides, phenols, steroids and amino acids in different concentrations. Terpenoids and phytostrols were present in bark, seed and pulp extract but absent in leaf extract. Anthraquinon glycosides were present in leaf and bark extracts but absent in seed and pulp extracts.

Antimicrobial activity of the samples

Ethanolic extracts of the leaves, barks, seeds and pulps of the plant were tested for their antibacterial activity by using disc diffusion method at 250 mg/ml concentration against 8 different bacterial strains collected from a reference laboratory (Table 2). All of the extracts displayed the highest level of activity against *Bacillus cereus*. Significant antimicrobial activity was also exerted by the plant extarcts against *Bacillus subtilis, Staphylococcus aureus* and *Vibrio cholerae.* Lesser amount of antibacterial activity was shown against Gram negative bacteria *Pseudomonas* and *Shigella flexneri,*

whereas *Escherichia coli* and *Salmonella typhi* showed resistance against the plant extracts.

Antioxidative activity of the samples

Total phenolic content, DPPH scavenging activity and total reducing power of the extracts of leaves, barks, seeds and pulps of *S. cumini* were determined and presented in Figure 1-3. Pulp extract had the highest total polyphenol content, DPPH scavenging activity and total reducing power, followed by pulp, bark and leaf extracts.

DISCUSSION

Most of the people in Asia and Africa rely on the use of different traditional herbal formulation for therapeutic purposes [19]. Among these, *S. cumini* in one of the most important ones. A number of studies have been conducted to elucidate the therapeutic and nutritional activity of its different plant parts. Antidiabetic activity of the plant is well established but there are also published data on potent activity against other diseases. The seeds of this pant are pharmacologically the best studied but others also possess pharmacological potential. But comprehensive studies regarding all plant parts of *S. cumini* are scare. Therefore, in the present study *in vitro* phytochemical, antibacterial and antioxidant potentials of the leaves, barks, seeds and pulps of the plant were analyzed.

In the present study, most of the biologically active phytochemicals such as flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoids, cardiac glycosides and tannins were found to be present in the ethanolic extracts of different parts of the *S. cumini* plant. The medicinal properties of *S. cumini* plant extracts may be due to the presence of above mentioned phytochemicals.

Studies on the efficiency of medicinal plants with respect to the control of infectious diseases are more essential to know their therapeutic value and hence in pharmaceutical arenas. Plants having antimicrobial compounds have enormous therapeutic potential as they can act without any side effect as often found with synthetic antimicrobial products. In the present study, all of the extracts showed antibiotic activity against the bacterial strains except *E. coli* and *S. typhi*. The antimicrobial activity of the different extracts of *S.cumini* may be due to the presence of various poplyphenols and other phytoconstituents[20]. The results obtained from the study suggest a potential application of *S. cumini* plant for treatment of infectious diseases.

Plant materials rich in antioxidants play an essential role in the prevention of various diseases. Anti-oxidative compounds are thought to be beneficial to suppress the reactive oxygen species which may cause aging or carcinogenesis. The ethanolic extract of different parts of *S. cumini* plant showed considerable antioxidant potential as reflected by TPC, Total polyphenol, DPPH radical scavenging activity and total reducing power. Among the extracts, the pulp one exhibited the highest antioxidant potential. The presence of chemical compounds such as vitamins, phenolics, tannins and anthocyanins in the plant materials would be responsible for the antioxidant property showed by the extracts. The pulp of the plant was already reported to contain vitamin C, gallic acid, tannins, anthocyanins cyanidin glucoside, petunidin, malvidin and other components[21].

CONCLUSIONS

The present findings support the applicability of different parts of *S. cumini* plant in traditional system as this plant appears to be a rich source of different phytoconstituents, antibacterial and antioxidant compounds. These suggest that the plant could be used as curative for different ailments. Further *in vivo* study will allow better confirmation of these findings.

ACKNOWLEDGEMENTS

The financial support provided by University Grants Commission of Bangladesh to conduct the research work has been greatly acknowledged.

CONFLICT OF INTERESTS

There is no conflict of interests.

REFERENCES

1. Tanaka H, Sato M, Fujoiwara S. Antibacterial activity of isoflavanoids isolated from Erythrina variegate against methicillin resistant Staphylococcus aureus. Leet. *Appl. Microbiol.* 2002; 35: 494-8.
2. Bopp A, De Bona KS, Bellé LP, Moresco RN, Moretto, MB. Syzygium cumini inhibits adenosine deaminase activity and reduces glucose levels in hyperglycemic patients. *Fundam Clin Pharmacol* 2009; 23:501–7.
3. Bhuyan ZA, Rokeya B, Masum N, Hossain S, Mahmud I. Antidiabetic effect of Syzygium cumini (L) seed on type II diabetic rats. *DUJBS* 2010; 19:157–64.
4. Pandey M, Khan A. Hypoglycaemic effect of defatted seeds and water soluble fibre from the seeds of Syzygium cumini (Linn.) Skeels in alloxan diabetic rats. *Indian J Exp Biol* 2002; 40:1178–82.
5. Mandal S, Barik B, Mallick C, De D, Ghosh D. Therapeutic effect of ferulic acid, an ethereal fraction of ethanolic extract of seed of Syzygium cumini against streptozotocin-induced diabetes in male rat. *Methods Find Exp Clin Pharmacol* 2008; 30:121–8.
6. Banerjee A, Dasgupta N, De B. *In vitro* study of antioxidant activity of *Syzygium cumini* fruit. *Food Chem* 2005; 90:727–33.
7. Sultana B, Anwar F, Przybylski R. Antioxidant activity of phenolic components present in barks of*Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica* and *Eugenia jambolana* Lam. trees. *Food Chem* 2007; 104:1106–14.
8. Chaudhuri AKN, Pal S, Gomes A, Bhattacharya S. Antiinflammatory and related actions of *Syzigium cumini* seed extract. *Phytother Res.* 1990; 4:5–10.
9. Muruganandan S, Srinivasan K, Chandra S, Tandan SK, Lal J, Raviprakash V. Anti-inflammatory activity of *Syzygium cumini* bark. *Fitoterapia* 2001; 72:369–75.
10. Hossain S, Chowdhury IM, Basunia MA, Nahar T, Rahaman A, Choudhury BK, Choudhuri SK, Mahmud I, Uddin B. Syzygium cumini seed extract protects the liver against lipid peroxidation with concurrent

amelioration of hepatic enzymes and lipid profile of alcoholic rats. *J Compl Integr Med* 2011; 8:1–17.
11. Chandrasekaran M, Venkatesalu V. Antibacterial and antifungal activity of *Syzygium jambolanum* seeds. *J Ethnopharmacol* 2004; 91:105–8.
12. Gupta GS, Sharma DP. Triterpenoid and other constituents of *Eugenia jambolana* leaves. *Phytochemistry* 1974; 13:2013–4.
13. Bhatia IS., Bajaj KL. Chemical constituents of the seeds and bark of *Syzygium cumini*. *Plant Med.* 1975; 28:347–52.
14. Noomrio MH, Dahot MU. Nutritive value of *Eugenia jambosa* fruit. *J Islam Acad Sci.* 1996; 9(1):9-12.
15. Bauer AW, Kirby WM, Sherres JC, Turk M. Antibiotic susceptibility testing by standardized single disc method. *Am. J. Clin. Pathol.* 1966; 44:493-6.
16. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin–ciocalteu reagent. *Methods in Enzymology* 1999; 299:152-78.
17. Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I. Antioxidant principles from Bauhinia terapotensis. *Journal of Natural Products* 2001; 64:892–5.
18. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. *Japanese Journal of Nutrition* 1986; 44:307-15.
19. World Health Organization. Traditional Medicine. WHO Fact Sheet No. 134. Geneva. Available at: http://tinyurl.com/5mrd5 (accessed 11 December 2008).
20. Timbola AK, Szpoganicz B, Branco A, Monache FD, Pizzolatti MG. A new flavonoid from leaves of*Eugenia jambolana*. *Fitoterapia* 2002; 73:174–6.
21. Wealth of India. Raw materials, New Delhi: CSIR, 1976; 10: 100-104.