Journal of Applied Pharmaceutical Science Vol. 2 (10), pp. 094-098, October, 2012
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2012.21019
ISSN 2231-3354

Evaluation of antidiabetic phytochemicals in *Syzygium cumini* (L.) Skeels (Family: Myrtaceae)

Md. Rashedul Alam[1], Akib Bin Rahman[2], Md. Moniruzzaman[1], Mohammad Fahim Kadir[3*], Md. Ahsanul Haque[3], Mohammad Razi-Ul-Hasan Alvi[4], Md. Ratan[4]

[1]Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.
[2]Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.
[3]Department of Pharmacy, University of Asia Pacific, Dhaka-1209, Bangladesh
[4]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.

ARTICLE INFO

Article history:
Received on: 15/09/2012
Revised on: 29/09/2012
Accepted on: 10/10/2012
Available online: 29/10/2012

Key words:
Syzygium cumini leaves, Phytochemical constituents, Plant extracts, NMR

ABSTRACT

Traditionally the leaves of Syzygium cumini (Myrtaceae) are widely used for treating diabetes. The present study was carried out to identify the putative antidiabetic constituents from the *S. cumini* leaves. From the NMR data four different compounds, Lupeol, 12-oleanen-3-ol-3ß-acetate, Stigmasterol, ß-sitosterol were identified from n-hexane fraction of plant extract. These compounds have potential antidiabetic activities which support the traditional use of the leaves as being remedy for treating diabetes.

INTRODUCTION

Syzygium cumini (L.) Skeels. a polyembryonic species (family-Myrtaceae), is a tropical fruit tree of great economic importance (Chase *et al*., 2009). It is a large evergreen tree up to 30 meters height and girth of 3.6 meters with a bole up to 15 meters. The fruit is commonly known as kalojum (Bangla), Jamun (Hindi), java plum, black plum and Indian blackberry. It is widely distributed forest tree of India, Bangladesh, Sri Lanka, Malaysia and Australia which is also cultivated for its edible fruits. The tree was introduced from India and tropical Asia to southern Africa for its edible and attractive fruits. *S. cumini* has been widely used for the treatment of various diseases in traditional and folk medicine. Unani system of medicine describes the use of the plant in liver tonic, enrich blood, strengthen teeth and gums and form good lotion for removing ringworm infection of the head (Ayyanar *et al*., 2012). Previous studies reported that, the bark of the plant has various properties like astringent, refrigerant, carminative, diuretic, digestive, antihelminthic, febrifuge, constipating, stomachic and antibacterial activity (Saravanan and Pari, 2008). The fruits and seeds are used to treat diabetes, pharyngitis, spleenopathy, urethrorrhea and ringworm infection (Saravanan and Pari, 2008). The leaves are antibacterial and used to strengthen the teeth and gums. The leaves have also been extensively used to treat diabetes, constipation, leucorrhoea, stomachalgia, fever, gastropathy, strangury, dermopathy and to inhibit blood discharge in the feces (Ravi *et al*., 2005; Sagrawat *et al*., 2006; Gowri and Vasantha, 2010). The barks, leaves and seeds extracts of *S. Cumini* have also been reported to possess anti-inflammatory antibacterial and antidiarraheal effects (Indira and Mohan, 1992; Chaudhuei *et al*., 1990; Bhuiyan *et al*., 1996). Powdered seeds are used as a remedy in diabetes and in menorrhagia (Sharma and Mehta, 1969). It has been also showed before that the leaf, bark, stem and pulp of *S. cumini* plants possess potent antidiabetic activity (Chaudhary *et al*., 2012; Kumar *et al*., 2008; Leelavinothan and Saravanan, 2006; Farswana *et al*., 2009; Bopp *et al*., 2009).

** Corresponding Author*
Mohammad Fahim Kadir, Assistant Professor, Department of Pharmacy, University of Asia Pacific, Dhaka-1209, Bangladesh.
Mobile: 008801816572691, Telephone: (880-2) 9664953
Fax: (880-2) 9664950

The major phytoconstituents are reported to contain vitamin C, gallic acid, tannins, anthocyanins, includes cyanidin, petunidin, malvidinglucoside and other components (Martinez and Del Valle, 1981; Wealth of India, 1976). Preliminary phytochemical analysis also showed the presence of Phenols, Terpenoids, Tannins, Saponins, Phytosterols, Carbohydrates, Flavonoids, Amino acids in stem bark of *S. cumini* (Kuncha *et al*., 2012). Previous investigations also revealed the bark of *S. cumini* contains butulinic acid, β-sitosterol, friedelin, epi-friedelanol (Wealth of India, 1982). It also contains new esters of epi-friedelanol (eugenin), D-glucoside, kaempterol-3-O-glucoside, quercetin, myricetin, astragalin and gallic acid (Sengupta and Das, 1965; Bhargava *et al*., 1974; Williamsen *et al*., 2002). The present study was designed to investigate the phytochemical bioactive compounds of the methanolic extract of *S. cumini* leaves to establish its antidiabetic activity.

MATERIALS AND METHODS

Collection and Identification of plant materials

Fresh plant leaves of *Syzygium cumini* was collected from Savar, Dhaka, Bangladesh in November, 2011. This plant was identified by Bangladesh National Herbarium (DACB). The collection number for the plant was HM09 and accession number was 423498.

Preparation of plant extract

The collected leaves of *Syzygium cumini* were washed generally and then kept for drying in the sun for seven days. The plant materials were then oven dried for 24 hours at low temperature. Powdered material of *S. cumini* leaves was macerated with methanol in round bottom flask. The containers were sealed with cotton plug and aluminum foil at room temperature for 15 days with occasional shaking. The mixture was filtered through cotton and then evaporated to dryness (45^0C) under reduced pressure by rotary evaporator. The percentage yield of the extract was calculated by using the formula below:

% yield= (weight of extract/weight of plant material) ×100%

Mother solution was prepared by mixing 15 gm of methanolic extract by triturating with 270 ml of methanol containing 30 ml distilled water. This solution was partitioned successfully by four solvents of different polarity. The mother solution was taken in a separating funnel. 100 ml of n-hexane was added here and the funnel was shaken and kept undisturbed. Then the organic portion was collected and repeated thrice. Carbon tetrachloride (CCl_4) and dichloromethane (CH_2Cl_2) extract was collected with the help of aqueous mother fraction adding 38 ml and 48 ml distilled water respectively keeping the other procedure unchanged. Finally *n*-hexane, CCl_4, CH_2Cl_2 and aqueous extract were obtained.

Detection of chemical compounds by NMR spectroscopy

In order to isolate different types of compounds *n*-hexane soluble fraction was subjected to TLC screening. This revealed a considerable number of compounds and required further fractionation. Sephadex was soaked in a mixed solvent of *n*-hexane: Dichloromethane: Methanol at the ration of 2:5:1 for swelling. Then slurry found from the extraction of the plant was added into glass column. The previous solvent mixture was used as initial mobile phase. The column was eluted with the same solvent mixture and finally washed with dichloromethane and methanol mixture with increasing polarity. Different fractions were collected in 28 test tubes and these were rendered for evaporation to dryness. 1 to 14, 15 to 22, 23 to 26, 27 to 28 test tubes had solvent systems n-hexane: Dichloromethane: Methanol (20:50:10), Dichloromethane: Methanol (90:10), Dichloromethane: Methanol (50:50) and methanol (100%) respectively. These column fractions were screened by TLC under UV light after spraying with vanillin-sulfuric acid reagent. Fractions having significant result were selected for further investigations with small column and suitable solvent systems. Crystal found in the end was analyzed by NMR for detection of isolated compound. ^{1}H NMR spectra were recorded using a Bruker AMX-400 (400 MHz) instrument and the spectra were referenced to the residual non-deuterated solvent ($CDCl_3$) signals. Column chromatography (CC) was conducted over (Merck, Germany) sephadex (LH-20). Spot on TLC plates were visualized under UV light (254 and 366 nm) after spraying with vanillin-sulfuric acid, followed by heating at 110^{o}C for 5-10 minutes.

RESULTS

Plant extraction

The yield of the methanolic extract of leaves of *S. cumuni* was 9.87 % (w/w) dry matter.

Chemical Compounds

Test tube 6, 7, 8 and 9 were selected for further investigation because of their significant result . The column elution was separated in 22 beakers with the help of a small column and mobile phase consisting of ethyl acetate and hexane in 14:86 ratios. These were kept at room temperature covered with aluminum foil to dryness. White crystals were observed in different beakers after four days,. These crystals were then analyzed by NMR. Different fractions contained different compounds which are presented in Table 1. The structure of the compounds found after analyzing in NMR is presented in Figure 1. ^{1}H NMR spectrum of compound 1 showed a double doublet (J = 11.5, 5.03 Hz) of one proton intensity at δ 3.21 ppm, typical of an oxymethine proton at C-3 of a triterpene. The splitting pattern of this proton confirmed the ß orientation of the C-3 oxygenated substituent. The spectrum also displayed two singlets at δ 4.68 and 4.56 ppm (^{1}H each) assignable to the vinylic protons at C-29. The ^{1}H NMR spectrum showed seven singlets at δ 0.95, 0.79, 0.83, 1.02, 0.93, 0.799 and 1.68 ppm (3H each) assignable to methyl group protons at C-4 (H_3-23, H_3-24), C-10 (H_3-25), C-8 (H_3-26), C-14 (H_3-27), C-17 (H_3-28) and C-20 (H_3-30), respectively. By comparing the ^{1}H NMR data with previously published data, compound 1 was identified as lupeol (Aratanechemuge *et al*.,

2004). The identity of 1 was further substantiated by co-TLC with an authentic sample of lupeol. The ^{1}H NMR spectra of compounds 2 and 3 readily demonstrated the steroidal nature of these compounds. The spectral data of compounds 2 and 3 were super imposable to the ^{1}H NMR spectral data published for ß-sitosterol and stigmasterol (Morales *et al*., 2003; Kolak *et al*., 2005). Additionally, thin layer chromatographic analysis of 2 and 3 with authentic samples of ß-sitosterol and stigmasterol, respectively, also confirmed their identity. ^{1}H NMR spectrum of compound 4 displayed a proton broad singlet at δ 5.18 which indicates the presence of olefinic proton. Eight singlets each of three proton intensity at 1.12, 1.06, 1.02, 1.00, 0.97, 0.93, 0.87, 0.84 ppm (3H each) assignable to methyl group protons at C-4 (H_3-23, H_3-24), C-10 (H_3-25), C-8 (H_3-26), C-14 (H_3-27), C-17 (H_3-28) and C-20 (H_3-29, H_3-30) respectively. By comparing the ^{1}H NMR data with previously published data and compound 4 was identified as 12-oleanen-3-ol-3ß-acetate (Krishnaswamy *et al*., 1975). Spectral analysis is shown in Table 2.

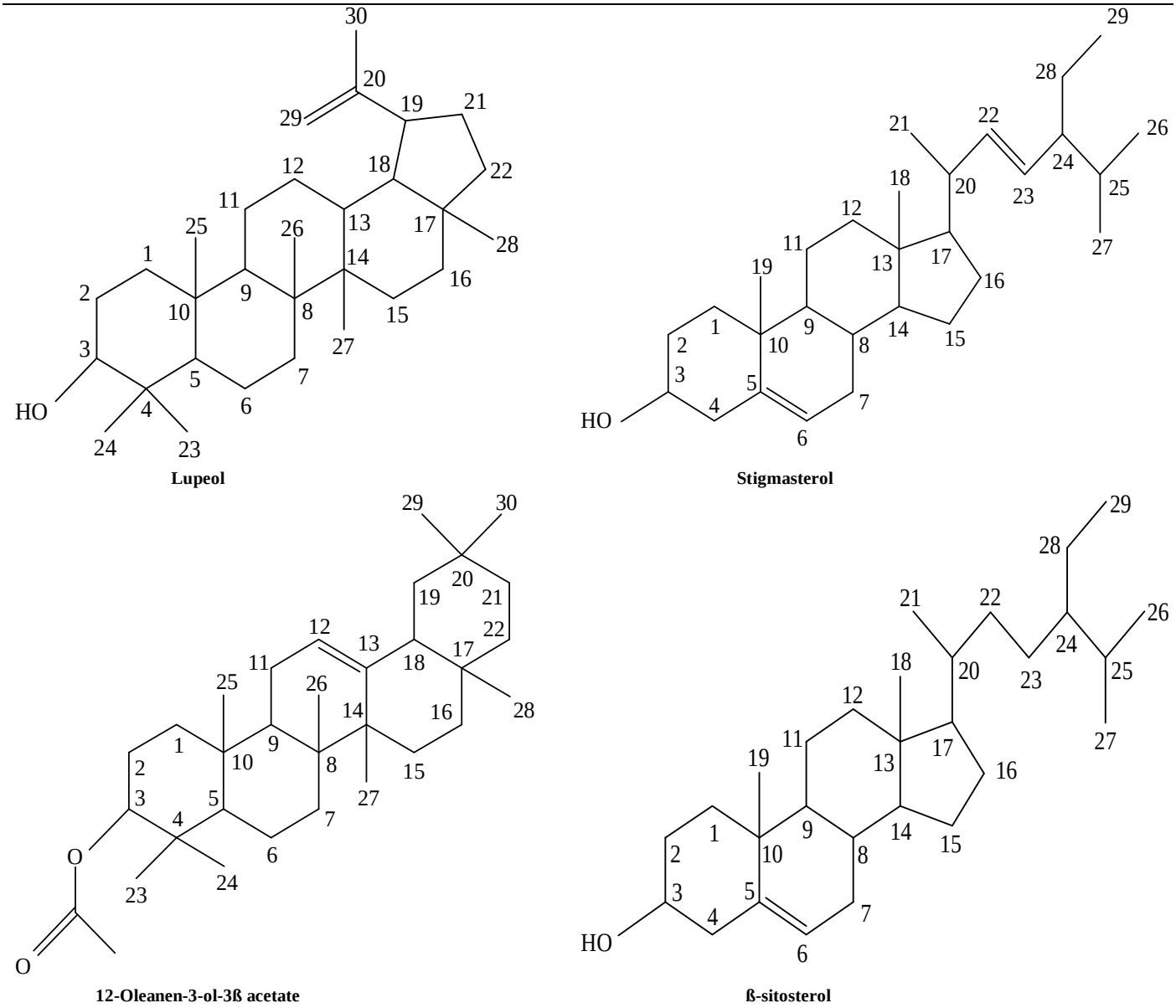

Fig. 1: The structures of the Compounds found in *S. cumuni.*

Table. 1: Constituents in different Solvent Systems

Fraction no.	Developing solvent system	Name of compounds
11-12	Ethyl acetate: Hexane = 14:86	12-oleanen-3-ol-3ß-acetate
16-17	Ethyl acetate: Hexane = 14:86	12-oleanen-3-ol-3ß-acetate
18-19	Ethyl acetate: Hexane = 14:86	Stigmasterol
20	Ethyl acetate: Hexane = 14:86	Stigmasterol
21-22	Ethyl acetate: Hexane = 14:86	Stigmasterol, ß-sitosterol
Rest of 16	Ethyl acetate: Hexane = 14:86	Lupeol

Table. 2: Physicochemical and ^{1}H NMR spectral data of lupeol (1), ß-sitosterol (2), stigmasterol (3) and 12-oleanen-3-ol-3 ß-acetate (4) in $CDCl_3$

	1 (lupeol)	2 (ß-sitosterol)	3(Stigmasterol)	4(12-oleanen-3ol-3 ß-acetate)
Physical appearance	**Colorless crystalline mass**	**Amorphous powder**	**Colorless niddles**	
Proton position		^{1}H NMR mult δ (ppm), J (Hz)		
2				
3	3.21, dd (11.5, 5.03)	3.51, m	3.51, m	4.46, m
6		5.35, m (7.0)	5.35, m	
18		0.67, s	0.67, s	
19	2.38, m	1.01, s	1.01, s	
21		0.91, d (6.4)	0.92, d (6.0)	
22			5.14, dd (15.0, 6.5)	
23	0.95, s		5.35, dd (15.0, 9.0)	1.12, s
24	0.79, s			1.06, s
25	0.83, s			1.02, s
26	1.02, s	0.83, d (6.0)	0.84, d (6.0)	1.00, s
27	0.93, s	0.80, d (6.0)	0.82, d (6.0)	0.97, s
28	0.799, s			0.93, s
29	4.68, br. s 4.56, br. s	0.85, d (6.0)	0.82, t (6.5)	0.87, s
30	1.68, s			0.84, s

DISCUSSION

NMR data indicated the presence of Lupeol, 12-oleanen-3-ol-3ß-acetate, Stigmasterol, ß-sitosterol in n-hexane portion. According to Panda *et al*. (2009) stigmasterol has significant effect on lowering serum glucose concentration with a concomitant increase in insulin level indicating it's hypoglycemic and insulin stimulatory activity. Further, Batta *et al*. (2006) found that this plant sterol has been found to compete with cholesterol for intestinal absorption and thus results in lowering the plasma concentration of cholesterol level. It was reported that stigmasterol supress cholesterol biosynthesis via inhibition of sterol Δ24-reductase in human Caco-2 and HL-60 cell lines thus reducing hepatic cholesterol. Again, study of Li *et al*. (2004) and Gupta *et al*. (2011) reported that beta-sitosterol has antidiabetic activity though there was no evidence about the exact mechanism. However, Jamaluddin *et al*. (1994) showed that the structure elucidation of the hypoglycaemic fractions proved the presence of stigmasterol along with beta-sitosterol. When these constituents were tested individually they showed no activity which concluded that synergism between these two is necessary to produce the antidiabetic effect. Lupeol, a phytoconstituent is known to suppress the progression of diabetes. Serum insulin level is elevated with lupeol treatment. Concomitantly it causes reduction of glycated haemoglobin, serum glucose and nitric oxide. Thus lupeol works as a potential antidiabetic constituent (Gupta *et al*., 2012).

CONCLUSION

From the above information it is clear that S. cumini possesses potential antidiabetic compounds which are of utmost importance and is therefore imperative to further investigation. Out of four valuable constituents found, stigmasterol is one of the major one and has been isolated from many plants till date and evaluated for antidiabetic activities. Further studies should be carried out in order to explore the exact mechanism of these compounds to use *Syzygium cumini* (L.) Skeels. as a potential antidiabetic medicinal plant.

STATEMENT OF CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

REFERENCE

Aratanechemuge Y., Hibasami H., Sanpin K., Katsuzaki H., Imail K., Komiya T. Induction of apoptosis by lupeol isolated from mokumen (Gossampinus malabarica L. Merr) in human promyelotic leukemia HL-60 cells. J. Oncol, Rep. 2004; 11: 289-292.

Ayyanar M., Subash-Babu P. Syzygium cumini (L.) Skeels: A review of its phytochemical constituents and traditional uses. Asian Pacific Journal of Tropical Biomedicine. 2012;(2) : 240-246.

Batta AK., Xuab G., Honda A., Miyazaki T., Salen G. Stigmasterol reduces plasma cholesterol levels and inhibits hepatic synthesis and intestinal absorption in the rat. Journal of Pharmaceutical Sciences. 2006; 55(3): 292-299.

Bhargava KK., Dayar R. Seshadri TR. Chemical components of *Eugenia jambolona* stem bark. Current Science. 1974; 43(20): 645-646.

Bhuyan MA., Mia MY. Rashid MA. Antibacterial principles of the seed of Eugenia jambolana. Bangladesh J. Botany. 1996; 25: 239–241.

Bopp A., Bona KS., Bellé LP., Moresco RN., Moretto MB. *Syzygium cumini* inhibits adenosine deaminase activity and reduces glucose levels in hyperglycemic patients. Fundamental & Clinical Pharmacology. 2009; 23: 501–507.

Chase MW., Reveal JL. A phytogenetic classification of land plants to accompany APG III. Botanical Journal of Linnean Society. 2009; 161: 122-127.

Chaudhary B., Mukhopadhyay K. Syzygium cumini (L.) skeels: a potential source of nutraceuticals. Intrnational J. of Pharm. and Bio. Sci. 2012; 2(1): 46-53

Chaudhuei AKN., Pal S., Gomes A., Bhattacharya S. Antiinflammatory and related actions of *Syzygium cumini* seed extract. Phytotherphy Research. 1990; 4: 5–10.

Chiu HL., Wu JH., Tung YT., Lee TH., Chien SC., Kuo YH. Triterpenoids and Aromatics from Derris laxiflora. J. Nat. Prod. 2008; 71 (11): 1829-1832.

Farswana M., Mazumder P., Parcha V. Modulatory effect of an isolated compound from *Syzygium cumini* seeds on biochemical parameters of diabetes in rats. International Journal of Green Pharmacy. 2009; 3: 128-133.

Gowri SS. Vasantha K. Phytochemical Screening and Antibacterial Activity of *Syzygium cumini* (L.) (Myrtaceae) Leaves Extracts. International Journal of PharmTech Research. 2010; 2:1569-1573.

Gupta R., Sharma AK., Dobhal MP., Sharma MC., Gupta RS. Antidiabetic and antioxidant potential of β-sitosterol in streptozotocin-

induced experimental hyperglycemia. Journal of Diabetes. 2011; 3(1):29-37.

Gupta R., Sharma AK., Sharma MC., Dobhal MP., Gupta RS. Evaluation of antidiabetic and antioxidant potential of lupeol in experimental hyperglycaemia. Nat Prod Res. 2012; 26(12):1125-1129.

Indira G., Mohan RM. National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India. 1992; 34-37.

Jamaluddin F., Mohamed S., Lajis MN. Hypoglycaemic effect of *Parkia speciosa* seeds due to the synergistic action of β-sitosterol and stigmasterol. Food Chemistry 1994; 49(4): 339-345.

Kolak U., Topcu G., Birteksoz S., Otuk G., Ulubelen A. Terpenoids and steroids from the roots of Salvia blepharochlaena, Turk. J. Chem. 2005; 29: 177-186.

Krishnaswamy NR., Prasanna S., Seshandri TR., Vedantham TNC. α- and β-Amyrin esters and sitosterol glucoside from Spilanthes acmella. Phytochemistry. 1975; 14: 1666–1667.

Kumar A., Ilavarasan R., Jayachandran T., Deecaraman M., Aravindhan P., Padmanabhan N., Krishan MRV. Anti-diabetic activity of *Syzygium cumini* and its isolated compound against streptozotocin-induced diabetic rats. Journal of Medicinal Plants Research. 2008; 2: 246-249.

Kuncha J., Ayyachamy M., Mariappan M. In-vitro evaluation of nitric oxide scavenging activity of methanolic and aqueous extract of syzygium cumini Linn. Bark (Myrtaceae). Int. J Pharm Sci Res. 2012; 3(2): 615-619.

Leelavinothan P., Saravanan G. Effects of *Syzygium Cumini* bark on blood glucose, plasma insulin and C-peptide in streptozotocin-induced diabetic rats. International Journal of Endocrinology and Metabolism 2006; 4: 96-105.

Li WL., Zheng HC., Bukuru J., De Kimpe N., Natural medicines used in the traditional Chines medical system for therapy of diabetes mellitus. J Ethnopharmacol. 2004; 92: 1-21.

Martinez SB. Del Valle MJ. Storage stability and sensory quality of duhat *Syzygium cumini* Linn. anthocyanins as food colorant. UP Home Economic Journal. 1981; 9(1).

Morales G., Sierra P., Mancilla A., Paredes A, Loyola LA., Gallardo O., Borquez J. Secondary metabolites from four medicinal plants from northern Chile: antimicrobial activity and biotoxicity against Artemia salina, J. Chile Chem. Soc. 2003; 48 : 13-18.

Panda S., Jafri M., Kara A., Meheta BK. Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from Butea monosperma. Fitoterapia. 2009; 80 (2): 123-126.

Ravi K., Rajasekaran S., Subramanian S. Antihyperlipidemic effect of *Eugenia jambolana* seed kernel on streptozotocin-induced diabetes in rats. Food Chem. Toxicol. 2005; 43: 1433-1439.

Sagrawat H, Mann AS, Kharya MD. Pharmacological potential of *Eugenia jambolana*: A review. Pharmaco. Mag. 2006; 2: 96-105.

Saravanan G., Pari L. Hypoglycaemic and Antihyperglycaemic Effect of *Syzygium cumini* Bark in Streptozotocin-Induced Diabetic Rats. Journal of Pharmacology and Toxicology. 2008; 3: 1-10.

Sengupta P. Das PB. Terpenoids and related compounds IV, Triterpenoids in the stem bark of *Syzygium cumini* bark. Indin journal of Chemical Science. 1965; 42(4): 255-258.

Sharma P., Mehta PM. In Dravyaguna vignyan. (The Chowkhamba Vidyabhawan), Part II & III, Varansi. 1969; 586.

Wealth of India. Ed. 10, CSIR, New Delhi. 1982; 100-104.

Wealth of India. Raw materials, New Delhi: CSIR. 1976; 10: 100–104.

Williamsen EM. Major Herbs in Ayurveda. Churchill Livingstone. 2002; 279-282.

How to cite this article:

Md. Rashedul Alam, Akib Bin Rahman, Md. Moniruzzaman, Mohammad Fahim Kadir, Md. Ahsanul Haque, Mohammad Razi-Ul-Hasan Alvi, Md. Ratan. Evaluation of antidiabetic phytochemicals in *Syzygium cumini* (L.) Skeels (Family: Myrtaceae). J App Pharm Sci. 2012; 2 (10): 094-098