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Research Article

Qualitative and Quantitative Screening of *Syzygium aromaticum* (Myrtaceae) and Evaluation of Anti-Hyperglycemic Effect

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ABSTRACT

The purpose of the present study was to trace out the presence of phytochemicals by in -vitro qualitative and quantitative screening methods and evaluate the anti-hyperglycemic effect of *Syzygium aromaticum* (Myrtaceae) on swiss albino mice. By performing qualitative screening of *Syzygium aromaticum* (SA_f) we confirmed the presence of phytochemicals. In quantitative screening, *in vitro* antioxidant potential, total antioxidant, total reducing power capacity and total phenolic content investigated and subsequent to evaluate the anti-hyperglycemic activity. The plant extract (SA_f) at the doses of 200 and 400 mg/kg body weight was orally administered for studying anti-hyperglycemic effect in alloxan-induced swiss albino mice. During 4-days of the study period in comparison with reference drug vildagliptin (50 mg/kg). In DPPH free radical scavenging assay found IC_{50} values of SA_f 13.204 µg/ml, total antioxidant of SA_f as 356.5 mg/g equivalent of ascorbic acid. %reducing power capacity (SA_f)of as 239.79±.075; and total phenolic content of (SA_f) as 407.69 mg/g equivalent of gallic acid, indicate potent phenolic content. Antioxidant study and quantification of phenolic compound of SA_f, the extracts revealed that they have high antioxidant capacity. Furthermore, by comparing glucose level with the standard drug with SA_f (8.35±2.07 and 8.77±1.71) mmol/L, the result showed that SA_f able to reduce blood glucose level with prolong dose administration. The present study suggests that methanolic extract of flower buds of *Syzygium aromaticum* (SA_f) could be used in managing oxidative stress and hyperglycemic condition.

Keywords: Antioxidant, Total Phenolic content, Reducing power capacity, *Syzygium aromaticum as* (SA_f), Anti-hyperglycemic test.

INTRODUCTION

In a normal cellular metabolism, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced in routine manner. They have power over to defense against pathogens to on cellular signal transduction within low to moderate concentration[1].Excessive production of ROS and RNS on one side during of oxidative stress and a deficiency of enzymatic and non-enzymatic antioxidant defense system on the other, resulting in degradation of, proteins, lipids, cellular components, DNA, and carbohydrates. Currently oxidative stress is suggested as mechanism underlying diabetes and diabetic complications[2]. A demoralizing illness with momentous morbidity and mortality, which has increased gradually worldwide[3]. For this instance, recent research searching superiors remedy as safe and natural antidiabetic and anti-oxidative plant products as extensive traditional medical treatment[4]. Even considering chemo preventive actions and costs effectiveness, herbs serve little or no toxicity during long-term oral administration and are relatively available at large scale, collectively encourage populations to use more herbs species traditionally[5]. The chemical and biological diversity of nature is vast enough to provide extra ordinary resources for discovery of disease remedy. When a plant is preferred as 'medicinal', it is implied that the assumed plant which, in one or more of its appendage, contains chemical substances that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs[6,7]. In Asian subcontinent, country like Bangladesh indigenous plant marked as medication of all diseases, locally named as "Kobiraji Ousodh". In this connection *Syzygium aromaticum* (SA_f) selected as common traditional recommendation and were subjected for studying their anti-hyperglycemic and oxidative stress inhibitory effect. *Syzygium aromaticum, loca*lly known as "lobongo" lavanga (Sanskrit) or laung, (family-Myrtaceae) is a bushy, evergreen tree with a medium-sized crown, growing 8-20 meters tall native to the Maluku Islands in Indonesia, and are commonly used as a spice, throughout the year it commercially harvested in Bangladesh, Indonesia, India, Madagascar, Zanzibar, Pakistan, Sri Lanka, and Tanzania .Its vast range of pharmacological activities has been well-researched and includes the treatment of analgesic, anesthetic effects[8,9]. Even the study found that eugenol component of Syzygium *aromaticum* (SA_f) possesses greater antipyretic properties

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than acetaminophen[10]. It is a remarkably versatile molecule incorporated as a functional ingredient that has shown bacterio-static and bactericidal/ germicidal effect on wide range of microorganism[11-14]. Antifungal properties of (SA_f) has also been reported[15-20]. In a research showed that, *Syzygium aromaticum* (SA_f) reduced levels of cytochrome P450 enzymes which specify the hepato-detoxification character of it[21.] It also referred as vaso-relaxant effects[22]. By considering all potent remedial properties, it also includes the anticancer properties[23]. At this juncture we studied the methanolic extract of *Syzygium aromaticum* (SA_f) for its qualitative and quantitative measurement of phytochemicals constituent and antidiabetic effects.

MATERIALS AND METHODS

Collection of the plant parts

The fresh dried flower buds of *Syzygium aromaticum* (SA_f) were collected from a local savory spice shop, Kawran Bazaar, Dhaka, Bangladesh in November 2012. The collected dried flower buds were primarily identified by assistant professor, Israt Jahan Bulbul, department of Pharmacy, Southeast University, Banani, and finally identified by the taxonomist of the Bangladesh national herbarium, Mirpur, Dhaka, Bangladesh and a voucher specimen was deposited in the herbarium unit.

Drying, Pulverization, Preservation of plant parts

The collected dried flower buds were washed with fresh water to avoid undesirable dirt and then cut into small pieces and dried for two weeks. The entire dried flower buds were ground into a coarse powder with the help of a suitable grinder machine and passing through sieve no 40, in pharmaceutical technology lab, Southeast University, Dhaka and finally stored in an airtight container, and kept in a cool, dark, and dry place until analysis commenced.

Chemicals and reagents

All chemicals and drugs were obtained commercially and were of analytical grade. Mercuric –Potassium Iodide TS, ferric chloride, lead acetate –Analytical Grade, DPPH (Sigma Chemical Co., USA.), Alloxan (Fluka, Germany), ammonium molybdate (Merck, Germany), sodium phosphate (BDH, England), potassium ferricyanide $K_3[Fe(CN)_6]$, Trichloroacetic acid (CCl_3 COOH), Folin–Ciocalteu reagent, GA [$C_6H_2(OH)_3$ COOH], ascorbic acid (AA) and Vildagliptin 50mg/tablet (Novartis) and glucose estimation kit (Human, Germany).

Instruments

HACH DR 4000U UV-visible spectrophotometer equipped with quartz cells of 1-cm light path used to determine the molecular absorption spectra and absorbance at specific wavelengths.

Experimental Animals

The swiss albino mice of either sex weighing (27-35g) were used to conduct the research and procured from the animal research branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDRB). They were kept in standard husbandry conditions (temperature 23±2 C relative humidity 55± 10 and 12 light and 12-hour dark cycle). The animals were fed with commercial diet pellets and water ad libitum. The animals were allowed to acclimatize to the atmosphere for 7 days prior to experimentation session. Animals were kept fasting overnight but allowed free access to water. Experiments on animals were performed in accordance with guidelines of the institutional animal ethics committee, Southeast University, Banani Dhaka, Bangladesh.

Ethical approval

The guidelines followed for animal experiment were accepted by ICDDRB which were approved by the institutional animal ethical committee[24].

Extraction of the Plant Material

By using soxhlet apparatus about 187 gm/each of dried plant material was refluxed with methanol, aqueous solvent and chloroform. The vapor flows through a coil where they condense back to liquid which is then collected in the receiving vessel. The whole mixture was successively filtered through a piece of clean, white cotton material and Whatman filter paper (Bibby RE200, Sterilin Ltd., UK).

Assay of Extractive values of plant sample

The extractive rate or the yield percentage of the plant sample is calculated before and after the extraction process using the formula

Extract yield % = (W1/W2) × 100

Where,

W1 = Net weight of powder in grams after extraction and,

W2= Total weight of powder in grams taken for extraction

Qualitative Screening

Test for Alkaloids

Mayer's reagent is an alkaloidal precipitating reagent used for the detection of alkaloids in natural products. Mayer's reagent (Mercuric –Potassium Iodide TS) is freshly prepared by dissolving a mixture of mercuric chloride (1.358 g) in 60mL of water and Dissolve 5g of potassium iodide in 10 ml of water and mix and dilute with water up to (100.0 ml). Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent (potassiomercuric iodide solution) to give a cream colored precipitate[25,26].

Test for Phenol

Ferric chloride Test: To 3 ml of extract, 3 ml of 5% w/v ferric chloride solution was added. The blue – black color indicates the presence of tannins and phenols. Lead acetate Test: To 3ml of extract, 3 ml of lead acetate solution was added. The occurrence of white precipitates indicates the presence of tannins and phenols.

Test for flavonoids

Sodium hydroxide test: About 5 mg of the compound is dissolved in water, warmed and filtered. 10% aqueous sodium hydroxide is added to 2 ml of this solution. This produces a yellow coloration. A change in color from yellow to colorless on addition of dilute hydrochloric acid is an indication for the presence of flavonoids[27].

Quantitative Analysis

Antioxidant activity measured by DPPH (1, 1-diphenyl-2-picrylhydrazyl) Free Radical Scavenging Assay

Antioxidants are suppressor of Free radicals or enable to donate hydrogen. In a chemical reaction with DPPH, Antioxidants stabilize the free radical and reduced to the

Table 1: Extractive Values in Different Solvent.

Type of extract	Yield (% w/w)
Methanol	27.89
Aqueous	17.04
Chloroform	12.83

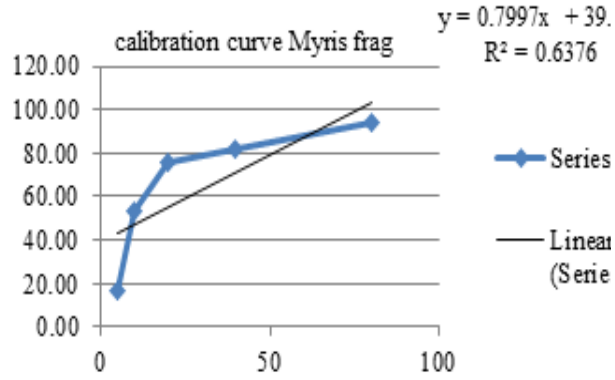

Figure 1: The calibration curve of Syzygium aromaticum for calculating IC_{50} Values.

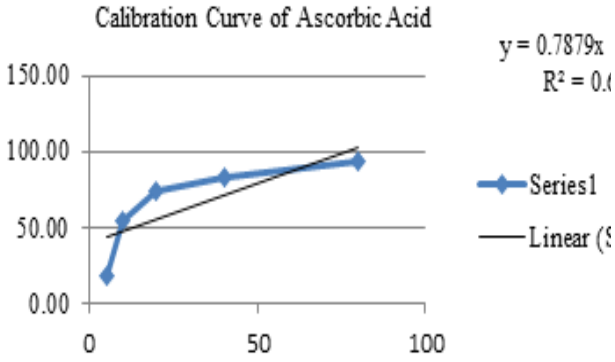

Figure 2**:** The calibration curve of Syzygium aromaticum for calculating IC_{50} values.

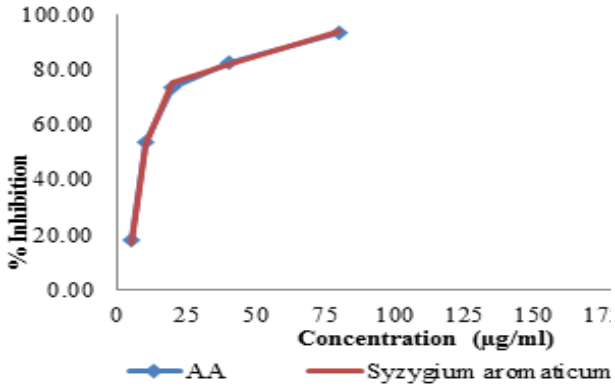

Figure 3: The antioxidant activity of methanolic extracts of Syzygium aromaticum (SA_f) and standard AA determined by using DPPH method.

DPPH-H. As a Consequence; the absorbance has decreased from the DPPH radical to the DPPH-H form. The degree of yellowing indicates the scavenging potential of the antioxidant (Hydrogen donating Capacity) compounds[28]. The scavenging reaction between (DPPH) and plant Extract [an antioxidant (H-A)] can be written as:

(DPPH) + (H-A) ----------DPPH-H + (A)
(Purple) (Extract) (Yellow)

The antioxidant effects of *Syzygium aromaticum* (SA_f) was performed according to the method of serial dilution method **(**Braca) by using DPPH radical scavenging method[29]. 1 ml of this solution (Freshly prepared solution of DPPH (0.004% w/v) in methanol) was added to 3 ml of extract's solution at diverse concentrations (5, 10, 25 and 50 µg/ml). After 20 min, absorbance was measured at 517 nm. Ascorbic Acid was used as a reference standard. The radical scavenging activity (RSA) or percentage inhibition was evaluated by comparing the values of absorbance for the investigational samples and control following the equation asindicated[30,31].

Percentage of inhibition = $[(A_0 – A_1) / A_0] \times 100$

Where, A_0 = Absorbance of the control; A_1 = Absorbance of the plant extract/ standard

IC_{50} value was calculated from the equation of line obtained by plotting a graph of concentration (µg/ml) versus % inhibition[32].

Total Antioxidant activity test (TAC)

The total antioxidant activity (TAC) or ORAC (oxygen radical absorbance capacity) is a quantitative process to investigate the reduction reaction rate between antioxidant, oxidant and molybdenum ligand. It involves in thermally generating auto-oxidation during delayed incubation period at higher temperature. The assay is based on the reduction of Mo (VI)–Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acidic pH exclusive of induction of free metal ions solution[33].

The following equation: used to determine the total antioxidant activity is expressed as the number of equivalents of AA

A = (C x V)/m

A = Total content of antioxidant compounds, mg/g plant extract, in Ascorbic acid

C = the concentration of ascorbic acid established from the calibration curve, mg/ml

V = the volume of extract, ml; m = the weight of pure plant methanolic extract, g.

Reducing power assay

For the measurement of the reductive ability, transformation of fe^{3+} to fe^{2+} was investigated in the presence of extract like the antioxidant activity, the reducing power of *Syzygium aromaticum* (SA_f) increased with increasing concentration of the sample. Potassium Ferri-cyanide + Ferric chloride -----→Potassium Ferro cyanide + ferrous chloride. The reducing power of *Syzygium aromaticum* was determined according to the method previously described by Oyaizu[34]. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as the standard. Phosphate buffer (pH 6.6) was used as blank solution. The absorbance of the final reaction mixture of two parallel experiments was taken and is expressed as mean ± standard deviation.

Total Phenolic content test

The content of total phenolic compounds in plant methanolic extracts was determined by folin-ciocalteu

Table 2: List of phytochemicals tests performed

Test for	Performed Test	Methanol	Aqueous	Chloroform
Alkaloid	Mayer Regent Test	+++	++	+-
Phenol	Ferric Chloride Test	+++	++	++
	Lead acetate Test			
Flavonoids	Sodium hydroxide test	+++	++	++

Table 3: The % of inhibition of methanolic extracts of *Syzygium aromaticum (SA$_f$).*

Conc.	Abs 1	Abs 2	Abs 3	% of Inhibition			Avg %	SEM	IC_{50}
0	1.750	1.757	1.749	0.000	0.000	0.000	0.00	0.00	
5	1.437	1.454	1.490	17.886	17.245	14.808	16.65	0.94	
10	0.821	0.826	0.829	53.086	52.988	52.601	52.89	0.15	13.20400501
20	0.427	0.429	0.433	75.600	75.583	75.243	75.48	0.12	
40	0.311	0.317	0.309	82.229	81.958	82.333	82.17	0.11	
80	0.104	0.101	0.107	94.057	94.252	93.882	94.06	0.11	

Table 4: The % of inhibition of methanolic extracts of AA.

Ascorbic acid (AA)									
Conc.	Abs 1	Abs 2	Abs 3	% of inhibition			AVG%	SEM	IC_{50}
0	1.785	1.810	1.779	0.000	0.000	0.000	0.00	0.00	
5	1.481	1.479	1.450	17.031	18.287	18.494	17.94	0.46	
10	0.829	0.824	0.827	53.557	54.475	53.513	53.85	0.31	12.78
20	0.469	0.471	0.472	73.725	73.978	73.468	73.72	0.15	
40	0.319	0.301	0.318	82.129	83.370	82.125	82.54	0.41	
80	0.109	0.111	0.113	93.894	93.867	93.648	93.80	0.08	

Table 5: Absorbance of ascorbic acid at different concentration**.**

Total Antioxidant capacity assay				
Standard: Ascorbic acid				
Conc.	Abs	Abs	Abs	AVG
200	0.941	0.949	0.952	0.947
100	0.421	0.429	0.427	0.426
50	0.187	0.191	0.192	0.190
5	0.029	0.031	0.033	0.031
0	0.000	0.000	0.000	0.000

reagent (FCR). The FCR actually measures a samples reducing capacity. The exact chemical nature of FCR is not known. Nevertheless, it is believed to contain hetero-polyphosphotunstates-molybdates. Sequences of reversible one or two electron reduction reactions lead to blue species possibly $(PMoW_{11}\ O_{40})^4$. The total phenolic content of methanolic extract and several organic fraction were determined using Folin-ciocalteu reagent[35] and aluminum chloride method[36] based on the method of Woisky and Salatino (1998)[37] respectively. The content of phenolic in the extract of Syzygium aromaticum was calculated from regression equation of the calibration curve and is expressed as Gallic acid standard /equivalent (GAE)[38].

In vivo Test

Acute toxicity test

Acute toxicity performed to categorize the LD_{50} median lethal dose (LD_{50}) value - an estimate of the dose of a test substance that kills 50% of the test animals. The classification of acute toxicity is primarily based on a dose or concentration which is considered to causes mortality and this is called the acute toxicity estimate (ATE).

Table 6: Absorbance of methanolic extract of Syzygium aromaticum (SA_f)

Conc.	Abs	Abs	Abs	AVG	ST. DE
200	0.927	0.924	0.929	0.927	0.001

According to the acute toxic classic method by Lorke[39], the animals were divided into 4 groups containing 4 animals each. The Syzygium aromaticum (SA_f) suspension was administrated orally in increasing dose up to 200 mg/kg to 1500 mg/kg. These animals were observed for mortality and toxicity for 14 days.

Study on Anti-hyperglycemic activity on Alloxan-induced diabetic mice

To perform antidiabetic activity, randomly separated group of mice were injected intra-peritoneally (I.P.) at a dose of 120 mg/kg b.w. alloxan monohydrate (fluka, Germany) freshly prepared in normal saline solution. After an hour of alloxan administration, animals were given feed ad libitum and 1ml of (100 mg/ml) glucose i.p. to combat ensuring severe hypoglycemia after 72 hr of Alloxan injection; the animals were tested for evidence of diabetes by estimating their blood glucose level using gluco-meter. Mice shown FBG > 150 mg/dl considered diabetic and selected for studies.

Animal grouping and experimental design

Animals selected were fasted overnight and then divided into four groups (n=4) as follows:

Group-I**:** Normal control mice (non-alloxanized) that administered distilled water only; Group-II: Diabetic control mice (Untreated, alloxanized); Group-II:Diabetic mice administered with Syzygium aromaticum (SA_f) (200 mg/kg/day) respectively: Group-IV: Diabetic mice administered with Syzygium aromaticum (400 mg/kg/day)

Table 7: % reducing power capacity of Syzygium aromaticum (SA_f) in different conc.

Conc.	Abs 1	Abs 2	Abs 3	% Reducing Power			Avg	SEM
0	0.249	0.248	0.247	0.00	0.00	0.00	0.00	0.00
5	0.260	0.267	0.264	4.42	7.66	6.88	6.32	0.98
25	0.489	0.481	0.497	96.39	93.95	101.21	97.18	2.13
50	0.584	0.579	0.589	134.54	133.47	138.46	135.49	1.52
100	0.791	0.790	0.795	217.67	218.55	221.86	219.36	1.28
200	0.843	0.846	0.839	238.55	241.13	239.68	239.79	0.75

Table 8: % reducing power capacity of AA in different conc.

Conc.	Abs 1	Abs 2	abs 3	% Reducing Power			Avg	SEM
0	0.251	0.255	0.253	0	0	0	0	0
5	0.285	0.287	0.285	13.55	12.55	12.65	12.91	0.317
25	0.521	0.525	0.522	107.57	105.88	106.32	106.59	0.505
50	0.639	0.647	0.651	154.58	153.73	157.31	155.21	1.082
100	0.804	0.807	0.805	220.32	216.47	218.18	218.32	1.113
200	0.911	0.917	0.915	262.95	259.61	261.66	261.41	0.973

Table 9: Absorbance of Gallic acid at different concentration.

Conc.	Abs	Abs	Abs	Avg
200	2.890	2.870	2.930	2.897
150	2.110	2.190	2.170	2.157
100	1.390	1.420	1.450	1.420
50	1.021	1.040	0.990	1.017
0	0.000	0.000	0.000	0.000

Table 10: Absorbance of methanolic extract of Syzygium aromaticum (SA_f).

Total Phenolic content					
Syzygium aromaticum (SA_f)					
Conc.	Abs	Abs	Abs	AVG	ST. DE
0.0002	1.190	1.152	1.171	1.171	0.019

respectively: Group-V: mice administered once with Vildagliptin (50 mg /kg) as reference standard drug.

RESULTS

Extractive values

The extractive values of, methanol, chloroform, and aqueous extracts are given in Table 1. The highest extractive yield was found in the methanolic dried flower buds extract. Consecutive extractive values discovered the solubility and polarity specifics of the metabolites in the plant. Methanolic extract showed high extractive yield 27.89% w/w when compared to Aqueous extracts.

Qualitative Screening

By performing qualitative screening through different analytical test, founded following indication of presence (Table 2).

DPPH free radical scavenging activity

Like a potent inhibitor of oxidation, methanolic extract of *Syzygium aromaticum* (SA_f) demonstrated H-donor activity and showed potent scavenging rate of DPPH. The percentage (%) of scavenging was found to be concentration dependant, i.e., scavenging capacity increases with the increase of concentration of both the extracts. By contrasting % of inhibition for SA_f and AA for the concentration of 80 µg/ml, found 94.06±011% and 93.80±0.08% (Table 3 and Table 4) respectively, which indicate potent antioxidant capacity of sample (SA_f). Furthermore, the IC_{50} values of SA_f and AA is 13.204 µg/ml. and 12.78 µg/ml calculated by using regression equation curve Fig.1 and Fig.2 respectively.

Total antioxidant activity

Total antioxidant capacity of methanolic extract of *Syzygium aromaticum* (SA_f) evaluated by Prieto P procedure and expressed as the number of equivalent of ascorbic acid (AA). The Total antioxidant activity was calculated from regression equation curve ($y=0.004x-0.024$, $R^2=0.994$) and expressed as ascorbic acid equivalent (AA) and found *Syzygium aromaticum* (SA_f) as 356.5 mg/g equivalent of AA (Table 5, Table 6 and fig 4).

Calculation

$y = 0.004x - 0.024$ $y=0.927$; $m=0.004$; $c=0.024$

$X=237.67$ microgm/ml

$C= 0.2377$ microgm/ml; $V=0.3$ ml; $m=.0002$

$A = (C \times V)/m$

$=356.5$

Where,

$A = (C \times V)/m$

A = Total content of Antioxidant compounds, mg/g plant extract, in Ascorbic acid

C= The concentration of ascorbic acid established from the calibration curve, mg/ml

V = The volume of extract, ml;

m = The weight of pure plant methanolic extract, g

Reducing power Assay

The reducing power of methanolic extract of *Syzygium aromaticum* (SA_f) was measured using FRAP assay. It measures the antioxidant effect of any substance in the reaction medium in term of its reducing ability and it reflects total antioxidant power involving the single electron transfer reaction. Table 7, Table 8& Fig.5 shows the reductive capabilities of SA_f compared to AA at different concentration. The reducing power of *Syzygium aromaticum* (SA_f) rise as the concentration gradually increased and founded remarkable % reducing power capacity as 239.79±.075 compared to ascorbic acid 261.41±.0973 respectively.

Determination of Total Phenolic content

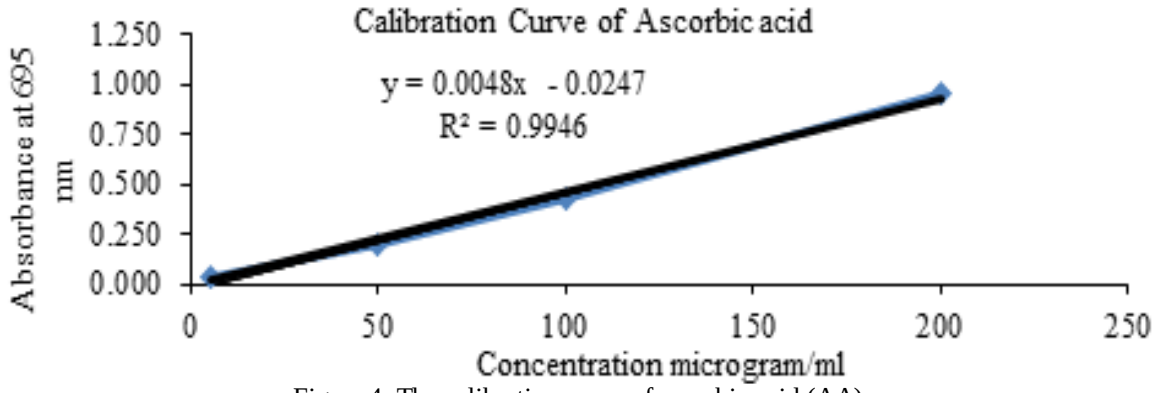

Figure 4: The calibration curve of ascorbic acid (AA)

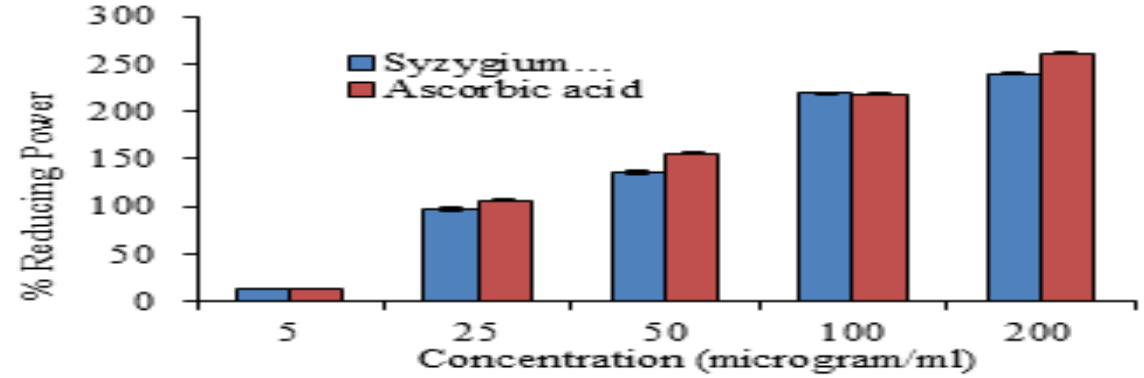

Figure 5: The comparative reducing power capacity activity of Syzygium aromaticum (SA_f) and standard AA.

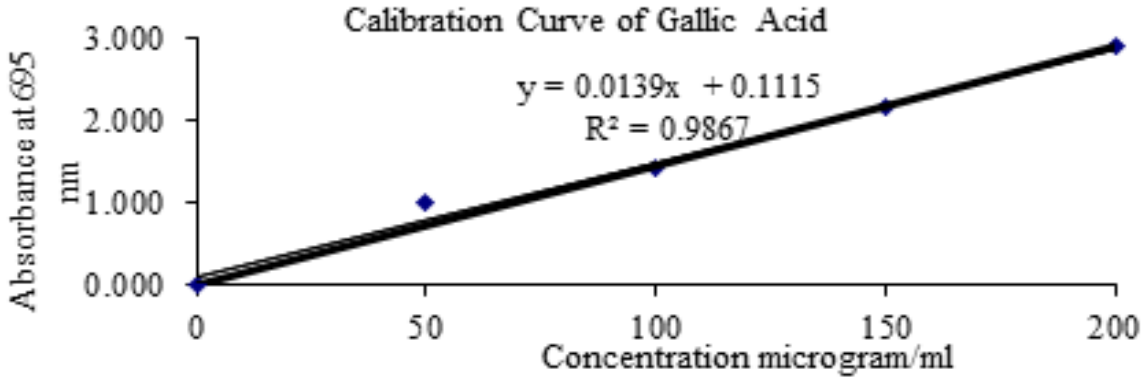

Figure 6: The Calibration Curve of Gallic Acid (GA)

The total phenolic content in the extracts of *Syzygium aromaticum* (SA_f) was determined according to the colorimetric Folin-Ciocalteu assay with Gallic acid as a standard compound. The total phenolic content was calculated from regression equation curve ($y=0.013x+0.111$, $R^2=0.986$) and expressed as Gallic acid equivalent (GAE) (Table 9 and fig 6). A potent range of total phenolic content was found in the plant materials to be 407.69 mg/g plant extract (in GAE) for *Syzygium aromaticum* (SA_f) (Table 10).

Calculation

Regression equation y = 0.013x + 0.111; y=1.171; m=0.013; c=0.111

x= 81.538 micro gm/ml; C= 0.0815 mg/ml;

V=1 ml; m=.0002

A = (C x V)/m

= 407.69 mg/g Gallic Acid

The amount of total phenol content of Syzygium aromaticum is 407.69 mg/g Gallic Acid

Where, A = Total phenol content, mg/g plant extract, in Gallic acid

C= the concentration of Gallic acid established from the calibration curve, mg/ml

V = the volume of extract, ml; m = the weight of pure plant methanolic extract, g

Acute toxicity

During the acute toxicity estimate (ATE) study of the extracts, either mortality or any considerable symptoms of toxicity was not found after the oral administration of *Syzygium aromaticum* (SA_f) up to a dose of 1.5 g/kg body weight in mice. Even no significant changes in general behavior was observed in mice up to 14-days study.

Table 11: Blood glucose level of alloxan induced diabetic mice after treatment with the methanolic extracts of SA_f 200mg/kg and 400mg/kg -body weight for consecutive 4 days.

Blood glucose level mmol/L								
Group	Mice no	Day 1	Day 2	Day 3	Day 4	Avg	SD	SEM
	M1	5.3	5.5	5.2	5.1	5.27	0.1707	0.085
Normal control	M2	5.1	5.3	5.2	5.4	5.25	0.129	0.065
	M3	5.6	5.7	5.4	5.2	5.47	0.221	0.110
	M4	5.7	5.3	5.6	5.7	5.57	0.189	0.094
Alloxanized	M1	12.3	13.5	15.9	17.7	14.82	2.43	1.21
Control mice	M2	12.7	14.6	15.6	16.7	14.9	1.69	0.85
Untreated	M3	12.5	15.4	17.1	18.4	15.85	2.54	1.27
	M4	12.8	15.6	16.5	17.9	15.7	2.15	1.07
Syzygium	M1	12.4	10.6	8.5	7.7	9.8	2.12	1.06
aromaticum	M2	13.1	11.4	8.9	7.3	10.17	2.578	1.28
200mg/kg	M3	13.5	11.1	8.7	7.3	10.15	2.72	1.36
	M4	12.8	11.4	9.1	8.2	10.37	2.10	1.05
Syzygium	M1	13.1	9.8	6.9	5.3	8.77	3.43	1.71
aromaticum	M2	12.9	9.6	6.4	5.1	8.55	3.42	1.71
400mg/kg	M3	13.5	9.2	6.4	5.3	8.6	3.65	1.82
	M4	12.7	9,7	7.1	5.2	8.7	3.22	1.61
Vildagliptin	M1	12.9	7.9	6.3	4.8	7.97	3.51	1.75
50mg/kg/day	M2	13.1	8.1	6.7	4.4	8.07	3.68	1.84
	M3	14.1	8.5	6.1	4.7	8.35	4.14	2.07
	M4	13.6	8.2	6.3	4.3	8.2	3.90	1.95

Study on Antidiabetic activity on Alloxan-induced diabetic mice

The consequences after chronic administration of SA_f summarized in (Table 11), showed significant anti-hyperglycemic action between experimental and diabetic control mice. At a dose of 200 mg/kg body weight, SA_f significantly lowered blood glucose level and showed reduction of 41.11 % and at 400 mg/kg SA_f body weight dose, produced maximum reduction of 59.96 % of blood glucose level, respectively, inhibition of blood glucose level 66.11 % was found for vildagliptin (50 mg /kg) on day 4 as a peak.

DISCUSSION

In vitro antioxidant activity

In vitro findings indicated that *Syzygium aromaticum* (SA_f) attributed their anti-oxidative abilities in terms of total scavenging activity, Reducing power Capacity, total antioxidant capacity and DPPH radical Total Phenolic Content.

DPPH radical scavenging activity

Potential DPPH radical scavenging activity revealed by SA_f might confirm its hydrogen donating capacity. Furthermore, the IC_{50} values of *Syzygium aromaticum* (SA_f) contrast with AA is 13.204 µg/ml. and 12.78 µg/ml proposed ability to protect the consumers' health from various free radical-related diseases.

Total antioxidant activity

The experiential total antioxidant activity of SA_f as 356.5 mg/g equivalent of AA might be contribute for investigating phenolic compounds in (SA_f) extracts. Due to potent antioxidant activity SA_f extracts may be accountable for its anti-inflammatory and chemo protective mechanism. As well as justifying the basis of using this plant's extract as folkloric remedies.

Reducing power assay

Dose-dependent high yield of reducing ability of the extracts exerts antioxidant action by breaking the free radical chain by donating hydrogen atom[40]. From our investigation we founded remarkable % reducing power capacity as 239.79±.075 compared to ascorbic acid 261.41±.0973 respectively, so this reducing capacity of SA_f is an important approach for the management of oxidative stress ailment.

Total phenol content

Plants are the major source of phenolic compound; have several biological activities including antioxidant properties[41].Many studies focused on the correlation of antioxidant activity to phenolic compounds content. The results of Kumar *et al.*[42] Yao *et al.*[43] and Hinnenburg *et al.*[44] stated a strong correlation between phenol and antioxidant activity. Through our evaluation we found the amount of total phenol content of Syzygium aromaticum (SA_f) is407.69 mg/g Gallic Acid which indicate potent phenolic content and help to initiate anti-hyperglycemic test.

Acute toxicity

In respect to peak nonfatal doses studied exposed the nontoxic nature of SA_f. Until the end of the study period there was no lethality or any noxious reactions originate at any of these doses selected. According to toxicity classification[45] the SA_f extract is nontoxic.

In vivo Anti-diabetic effect

Type -1 (Insulin-dependent) diabetes study model has been developed here to investigate the anti-hyperglycemic activity of (SA_f). By using Alloxan (a beta-cytotoxin) insulin-secreting pancreatic β-cells destructed - (a reactive

oxygen species-dependent oxidative damage[46]) and resulting diminished level of serum insulin[47]. Vildagliptin, 50 mg/kg/day were treated on experimental groups as standard and test samples SA_f to evaluate and compare anti-hyperglycemic activity. Chronic administration of SA_f in diabetic mice resulted into efficient lowering of blood glucose level suggesting that the extracts might possess insulin-like effect on peripheral tissues either by promoting glucose uptake and metabolism or inhibiting hepatic gluconeogenesis since alloxan treatment causes permanent destruction of β-cells.

CONCLUSION

The consequences of entire experimentation indicated that *Syzygium aromaticum* (SA_f) have both hypoglycemic and antioxidant potential. The present study suggests that by lowering serum blood glucose level in diabetic mice, SA_f ensure higher antioxidant or reducing capacity with huge amount of phenolic content.

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COMPETING INTERESTS

The authors have declared that no competing interests exist.

CONSENT

Not applicable**.**

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