ORIGINAL ARTICLE

Antibacterial, antioxidant and antidiabetic activities of *Gmelina arborea* roxb fruit extracts

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Background: The plant *Gmelina arborea* has been traditionally used in India for several medicinal purposes like anthelmintic, diuretic, antibacterial, antioxidant and antidiabetic. **Aim:** The present study is an attempt to explore the antibacterial, antioxidant and antidiabetic activities of different extracts of fruits of plant *G. arborea* using ethanol, ethyl acetate, n-butanol and petroleum ether as solvents. **Materials and Methods:** A single dose (1000 µg/ml) of extract was evaluated for their antibacterial activities on human pathogens like *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *In-vitro* antioxidant activity of *G. arborea* fruits was determined by 1, 1-diphenyl-2-picrylhydrazil (DPPH) free radical scavenging and reducing power assay. Ascorbic acid was used as standard and positive control for both the analyses. The antidiabetic activity of above extracts was evaluated in alloxan induced diabetic model of Wistar rats. **Statistical Analysis:** All data are verified for statistically significant by using one way ANOVA at 1% level of significance ($P < 0.01$). **Results:** Only ethanol extract showed significant antibacterial activity against all pathogens and the activities were compared with the standard drug, streptomycin. The n-butanol extract did not show antibacterial activity against any pathogens, whereas ethyl acetate and petroleum ether extracts showed inhibitory action against *P. aeruginosa*. The extracts showed significant antioxidant activities in a dose dependent manner. The ethanol extract showed good antioxidant activity when compared to the other three extracts. All the extracts were able to reduce sugar level in blood. Ethanol extract was found to have good antidiabetic activity in comparison to other extracts. **Conclusion:** It can be concluded that the extracts of *G. arborea* possess antibacterial, antioxidant and antidiabetic activities.

Key words: Antibacterial, DPPH and alloxan, *gmelina arborea*, scavenging, streptomycin, *verbenaceae*

INTRODUCTION

Herbal medicines have received much attention as a source of new antibacterial drugs since they are considered as time-tested and comparatively safe both for human use and the environment.[1,2] Free radicals oxidation has been implicated in atherosclerosis, cancers, neuron degenerative diseases and inflammatory bowel diseases.[3,4] The two most commonly used synthetic antioxidants are butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) having side effect of toxicity and DNA damage induction. Therefore, natural antioxidants from plants and algal extracts have attracted increasing interest due to their non-toxicity and biosafety.[5]

Diabetes mellitus is one of the common metabolic disorders with micro-and macro vascular complications that results in significant morbidity and mortality. It is considered as one of the five leading causes of death in the world.[6,7] In modern medical fluid (Aroma), no satisfactory effective therapy is still available to cure diabetes mellitus.[8] There is increasing demand by patients to use natural products with antidiabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agents.[9-11] The plant, *G. arborea* was reported to several medicinal properties aphrodisiac, astringent, antidiabetic, diuretic, and tonic characteristics.

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Gmelina arborea roxb bark is light grey color in young stage. A blaze thick chlorophyll layer lies just under the outer bark whereas a pale yellow white layer is present inside the bark.[12,13] The fruit is oval in shape, 3/4 inches in length and is yellow in colour. The fruits are sweet in taste and sometimes astringent.[14,15]

MATERIALS AND METHODS

Drugs and chemicals

Streptomycin was procured as gift sample from Micro Lab. Ltd., Goa, India. The ethanol AR and ethyl acetate AR 60-80°C (Emsure® ACS) were procured from Merck Pvt. Ltd., Navi Mumbai, Maharashtra, India.

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n-butanol GR 80°C, petroleum ether AR 40-60°C, Alloxan (Hydrate-CAS: 2244-11-3) and DPPH were procured from Loba Chemie Pvt. Ltd., Mumbai, India. Agar, peptone and beef extract were procured from Himedia Laboratory Pvt. Ltd., Mumbai, India. All other chemicals and reagents used in present work were procured from authorized dealer.

Collection of plant materials, identification and size reduction

The fruits of *G. arborea* were collected from local area of Koraput district (India) in the month of April and May 2008. The plant was identified and authenticated by the Biju Patnaik Medicinal Plants Garden and Research Centre, Dr. M. S. Swami Nathan Research Foundation, Jeypore, Koraput (District), Orissa (Letter No. MJ/DBT (08)/1067, dated 05.09.2008). The fruits were shade dried under normal environmental conditions. The dried fruits were pulverized to form coarse powder by using electrical grinder and stored in a closed air tight container for further use.

Preparation of solvent extracts

The dried fruits of *G. arborea* were made into coarse powder and extracted by Soxhlation method by Soxhlet apparatus, using ethanol, ethyl acetate, n-butanol and petroleum ether as solvents. A total amount of 800 g coarse powder was extracted with 1200 ml of each solvent. For each solvent, 10 cycles were run to obtain thick slurry. Each slurry was then concentrated under reduced pressure to obtain crude extract. All crude extracts were kept in closed air tight containers under cool and dark place for further study.

Phytochemical analysis

For the detection of the presence of carbohydrates and reducing sugars the standard tests, Molisch's test for carbohydrates and reduction of Fehling's solution for reducing sugars were done. In short, in Molisch's test, the gum was treated with α-naphthol and concentrated sulphuric acid, which gave violet ring at the junction of two layers. In case of the detection of reducing sugars to the *G. arborea* fruit mucilage, equal quantity of Fehling's solution was added. The presence of tannin was tested upon treating the gum with ferric chloride solution. There was no black precipitation for tannin with ferric chloride solution. The presence of mucilage was tested by treating the mucilage with ruthenium red solution and Benzidine solution, formation of pink colour with ruthenium red and blue colour with Benzidine solution indicated the presence of mucilage. The phytochemical properties such as the presence of proteins, flavonoids, sterols, alkaloids, saponins, glycosides, resins, phenols and terpenoids were also determined.[16-18]

Antibacterial Activity

Test organisms

Various gram positive and gram negative bacteria including both standard and clinical isolates were used as test organisms. The gram positive bacteria like *Staphylococcus aureus* (NCTC 3761) and *Bacillus subtilis* (NCTC 5677) were procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India. The gram negative bacteria like *Pseudomonas aeruginosa* (NCTC 8201) a clinical isolate was obtained from National Institute of Immunology (NII), New Delhi, India. The test organisms were maintained on soybean casein digest agar (SCDA) and transferred onto fresh slants on a regular basis.

Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of each extract was determined with the selected test organisms. The MIC of each extract was determined employing different concentrations of the extract by the paper disk diffusion technique. The reconstituted extract was diluted to give concentrations of 50, 100, 150, 200, 250, 300, 350 and 400 µg/ml. The lowest concentration of the extract that could inhibit the bacterial growth was considered as MIC.

Preparation of inoculums

The gram positive (*B. subtilis* and *S. aureus*) and gram negative bacteria (*P. aeruginosa*) were pre-cultured in nutrient broth incubated overnight in a rotary shaker at 37°C for 24 h and centrifuged at 10,000 rpm for 5 min. The resultant pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (At A610 nm).

Methodology

The antibacterial activity of the crude extracts was investigated against 3 bacterial strains by the paper disk diffusion technique.[19,20] Each extract was re-dissolved in ethanol to make a 1000 µg/ml solution and then filtered. From this solution, 80 µl aliquots were transferred onto blank paper disks (6 mm diameter) and dried. Dried disks were placed onto Mueller Hinton agar medium (Merck) previously inoculated with a bacterial suspension (*ca.* 108 CFU/ml) and incubated at 37 ± 1°C for 24 hrs. Plates were then examined for the presence of growth inhibition zones, and their diameters were measured. Streptomycin (125 µg/ml) was used as positive control. A disk loaded with 80 µl ethanol served as the negative control. The experiments were carried out three times and the results are presented as mean ± standard deviation.

In Vitro Antioxidant Activity

Assay of reducing method

About 1 ml of ethanol plant extract solution (Final concentrations ranged from 100 to 500 mg/l) was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide [$K_3Fe(CN)_6$] (10 g/l). Then the mixture was incubated at 50°C for 20 min. About 2.5 ml

of trichloroacetic acid (100 g/l) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (1 mg/ml) and absorbance was measured at 700 nm in UV-Visible Spectrophotometer. Ascorbic acid was used as standard and phosphate buffer was used as blank solution. The same procedure was adopted for ethyl acetate, n-butanol and petroleum ether extracts. Increased absorbance of the reaction mixture indicates strong reducing power.[21-23]

DPPH radical scavenging activity

The DPPH radical scavenging activity was measured using the modified methods.[23-25] About 2.8 ml of test solution or standard ascorbic acid (in methanol), at different concentrations and 0.2 ml of DPPH (100 µM in methanol) were mixed and incubated at 37°C for 30 min. The absorbance of the resulting solution was measured at 517 nm using spectrophotometer. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with the control (not treated with extract) using the following formula.

$$\% \text{ DPPH radical-scavenging} = [(Ac - At)/Ac] \times 100 \quad (1)$$

Where, Ac and At are absorbances at 517 nm of the control and the test sample.

Data analysis

All the data are given as the mean ± SEM (Standard error of mean) of three individual measurements. 50% inhibitory concentration (IC_{50}) for DPPH radical scavenging activity was calculated by plotting the data in the graph as concentration versus percentage inhibition using Graph Pad Prism Software, version 4.03.

Acute Toxicity Studies

To study the toxic effect (if any) of *G. arborea* fruit extracts, Albino mice of either sex weighing 20-25 g were used. The animals were kept in the standard polypropylene cages at 25 ± 2°C/60% relative humidity in normal day and night photo cycle (12:12 h). The animals were acclimatized for a period of 14 days prior to performing the experiments. Prior to the study, the experimental protocols were approved by the Institutional Animal Ethics Committee of Gayatri College of Pharmacy, Gayatri Vihar, Jamadarpali, Sambalpur, Odisha (Ethical Committee No. 1339/ac/10/CPCSEA).

Acute oral toxicity study was performed as per OECD-423 guidelines.[26-28] The animals were kept fasting overnight but allowed free access to water *ad libitum*. The fasted mice were divided into different groups of six animals each. Each solvent extract solution was administered orally at a dose of 10 mg/Kg b.w., using normal saline water as vehicle and mortality in each group was observed for 14 days. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the same procedure was repeated in each group for each extract for further higher doses such are 100, 300, 600, 1000, 2000 and 3000 mg/Kg b.w. One tenth of this lethal dose was selected as the therapeutic dose for the evaluation of antidiabetic activities.

Antidiabetic Activity (Alloxan Induced Diabetic Model)

Animals

Healthy Wister rats of either sex were used in the present study. They were housed under standard conditions of temperature (25 ± 2°C) with 12 hours light per day cycle and relative humidity of 45-55 % in animal house of Gayatri College of Pharmacy, Odisha. They were kept in fasting condition for 16 h and prior to experiment they were fed with excess water *ad libitum*. Animals were caged and all operations on animals were done in aseptic condition.

Extracts and drugs

The extracts of *G. arborea* were tested in single doses in each group of experimental animals (300 mg/Kg b.w.). Glibenclamide was used as the standard drug in alloxan induced diabetic model at a dose of 5 mg/Kg of body weight of rat.

Experimental protocol

Animals were selected, weighed (150-180 g) and divided into seven groups ($n = 3$), namely normal control, diabetic control, standard drug and four groups belonging to four different extracts of *G. arborea*. Approval for the research work was obtained by the Institutional Animal Ethics Committee of Gayatri College of Pharmacy, Gayatri Vihar, Jamadarpali, Sambalpur, Odisha (Ethical Committee No. 1339/ac/10/CPCSEA).

Experimental Method

The alloxan induced diabetic model was used to evaluate the blood sugar level reducing capacity of various extracts. Here the blood sugar level of rats was raised by administration of alloxan.[29,30]

Wister rats were divided into seven groups of three animals in each group. The animals were fasted for 16 h with water *ad libitum*. The group - I was served as normal solvent control which received the normal saline water 2 ml/kg through oral route, the group - II was served as diabetic control which received alloxan (120 mg/Kg) with normal saline water subcutaneously, group-III was served as standard control which received alloxan 120 mg/Kg with glibenclamide at a dose of 5 mg/Kg orally, groups-IV to VII were served as

test groups which received alloxan (120 mg/kg) along with single dose (300 mg/Kg, b.w.) of ethanol, ethyl acetate, n-butanol and petroleum ether extracts respectively.

Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (120 mg/Kg).[31-33] Two days after of alloxan injection, rats with plasma glucose levels of more than 200 mg/dl were included in the study and at this stage the blood glucose level of each rat was consider as basal value in each group. Treatment with plant extracts and standard drug was started after 48 h of alloxan injection. Blood samples were drawn from tip of the tail on 1st, 3rd, 5th and 7th day respectively and estimated for fasting blood glucose level using electronic digital glucometer (Counter digital glucometer) which is previously validated for correctness.

Statistical Analysis

For determining the statistical significance, standard deviation, standard error mean and one way analysis of variance (ANOVA) at 1% level significance was employed followed by z-test. *P* values <0.01 were considered significant.[34,35]

RESULTS AND DISCUSSION

Phytochemical analysis

Table 1 shows the phytochemicals detected in *G. arborea* fruit extracts. The test for cardiac glycosides and steroids were positive for all the extracts. The tests for all phytochemicals were found to be positive for ethanol extract except proteins, amino acids triterpenoids and saponins. The tests for cardiac glycosides, proteins, amino acids, gums, mucilages, steroids and sterols were found to be positive for ethyl acetate extract. The tests for all phytochemicals were found to be positive for n-butanol extract except carbohydrate, proteins and amino acids. The tests for all phytochemicals were found to be positive for petroleum ether extract except gums, mucilages, tannins, phenolic compounds and flavonoids.

Antibacterial activity

The minimum inhibitory concentration for each extract was also determined using the disk diffusion technique and the results are presented in Table 2. The minimum inhibitory concentration (MIC) of ethanol extract was found to be 300 µg/ml against *S. aureus* and *P. aeruginosa*, while it was 200 µg/ml against *B. subtilis*. The ethyl acetate and petroleum ether extracts exhibited MIC of 400 µg/ml against *P. aeruginosa*. The inhibitory effects of ethanol, ethyl acetate, n-butanol and petroleum ether extracts against different test organisms are shown in Table 3. The ethanol extract exhibited significant antibacterial activity (Growth inhibition zone diameters ranging from 13 to 17.75 mm) against both Gram-positive and Gram-negative bacteria. The antibacterial activities shown by ethanol extract are somewhat comparable with the standard drug streptomycin. The n-butanol extract did not show any

Table 1: Phytochemical constituents of fruit extracts of *Gmelina arborea*

Phytochemicals	Ethanol extract	Ethyl acetate extract	n-butanol extract	Petroleum ether extract
Alkaloids	+	-	+	+
Carbohydrates	+	-	-	+
Cardiac glycosides	+	+	+	+
Anthraquinone glycosides	+	-	+	+
Gums and mucilages	+	+	+	-
Proteins and amino acids	-	+	-	+
Tannins	+	-	+	-
Phenolic compounds	+	-	+	-
Steroids and sterols	+	+	+	+
Triterpenoids	-	-	+	+
Saponins	-	-	+	+
Flavonoids	+	-	+	-

+: Present; –: Absent

Table 2: Minimum inhibitory concentration (µg/ml) of different extracts of *Gmelina arborea* fruits

Microorganism	Minimum inhibitory concentration (µg/ml)			
	EE	EAE	NBE	PEE
Gram-positive bacteria				
Staphylococcus aureus	300	–	–	–
Bacillus subtilis	200			
Gram-negative bacteria				
Pseudomonas aeruginosa	300	400	–	400

EE – Ethanol extract; NBE – n-butanol extract; EAE – Ethyl acetate extract and PEE – Petroleum ether extract

Table 3: Antibacterial activity of various fruit extracts of *Gmelina arborea*

Groups	Drugs	Dose (µg/ml)	Inhibition zone diameter (mm)		
			Bacillus subtilis (X±SEM)	*Staphylococcus aureus* (X±SEM)	*Pseudomonas aeruginosa* (X±SEM)
I	Normal Control (Ethanol)	80 µL	6.44±0.89	6.15±0.68	6.69±0.92
II	Streptomycin (Standard)	125	35.04±0.78	30.25±0.99	16.04±1.05
III	Ethanol extract	1000	16.0±0.85	17.75±1.08	13.0±1.11
IV	Ethyl acetate extract	1000	–	–	9.25±1.07
V	n-butanol extract	1000	–	–	–
VI	Petroleum ether extract	1000	–	–	14.5±0.93

Each data is expressed as zone of inhibition (mm) for 24 h of study. Each values is represented as mean±standard error of mean (*n*=3). Standard error of mean <0.641

activity against test organisms, where as ethyl acetate and petroleum ether extracts showed inhibitory action against *P. aeruginosa*. Among different organisms, *S. aureus* is found to be more sensitive to ethanolic extract while *P. aeruginosa* is sensitive to ethyl acetate and petroleum ether extracts.

Antioxidant activity

The reducing ability of a compound generally depends on the presence of reductant which has been exhibited antioxidant potential by breaking the free radical chain and donating a hydrogen ion. The presence of reductant (i.e., antioxidants) in *G. arborea* fruits extracts causes the reduction of the ferricyanide (Fe^{3+}) to the ferrous (Fe^{2+}) form. Therefore, the Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm by UV-Visible spectrophotometer. The ethanol, ethyl acetate and n-butanol fruit extracts exhibited significant reductive activities except petroleum ether extract as shown in Figure 1. The reducing capacity shown by the extracts is well comparable with standard antioxidant, ascorbic acid and it is in the order of ethanol> n-butanol> ethyl acetate> petroleum ether extracts.

The DPPH antioxidant assay is based on the ability of DPPH (A stable free radical), to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. The comparison of the antioxidant activities of the extracts and ascorbic acid is shown in Figure 2.

All other extracts showed significant DPPH scavenging activity except petroleum ether extract. The IC_{50} values for scavenging of free radicals were 41.5, 61.8, 82.6 and 60.1 µg/ml for ascorbic acid, ethanol extract, ethyl acetate extract and n-butanol extract respectively, which indicate the efficient DPPH scavenging activity. The order of DPPH scavenging activity was in the order of ethanol> n-butanol> ethyl acetate> petroleum ether extracts.

Acute toxicity study

Acute toxicity study revealed that no mortality was found in any solvent extract at any dose in Swiss albino mice, which confirmed that *G. arborea* fruits extract would be non-toxic in living body but whereas the LD_{50} of the extracts was found to be 1300 mg/kg body weight. One tenth of this lethal dose (i.e., 300 mg/kg b.w.) was selected as the therapeutic dose for the evaluation of antidiabetic activity.

Antidiabetic activity

The extracts produced a significant antidiabetic effect on first, third, fifth and seventh days at 300 mg/Kg body weight [Table 4]. These effects are comparable with the standard drug (Glibenclamide). It will be worth to mention that although different constituents were extracted in different solvents as per their polarities, ethanol extracts is more effective when compared to other extracts. The activity showed by this extract is of considerable importance and justified its use in the diabetic control in the folklore medicines. The antidiabetic activity of the extracts is in the order of ethanol> n-butanol> petroleum ether> ethyl acetate. By employing one-way ANOVA, all data were found to be statistically significant (F value <F crit) at 1% level of significant ($P < 0.01$ that is $P = 0.00417$) followed by z-test.

CONCLUSION

It can be concluded that the extracts of *G. arborea* fruits possess antibacterial, antioxidant and antidiabetic activities. The ethanol extract showed most potent antibacterial, antioxidant and antidiabetic activities. The plants may

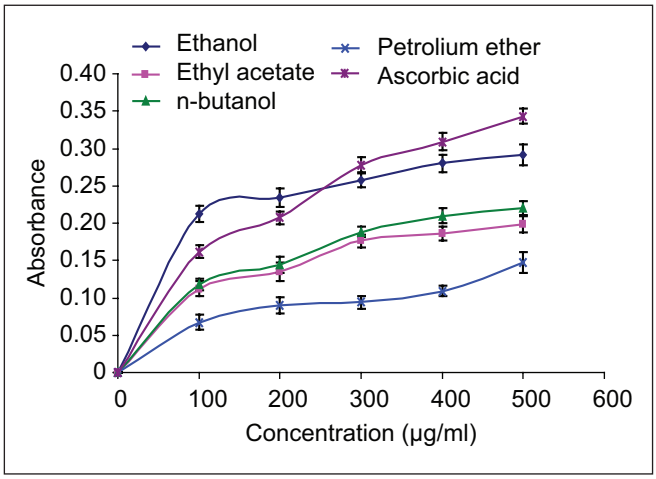

Figure 1: Reducing power of different extracts of *G. arborea* (Fruits) as compared to ascorbic acid. (Each point represents as mean ± standard error of mean [*n*=3])

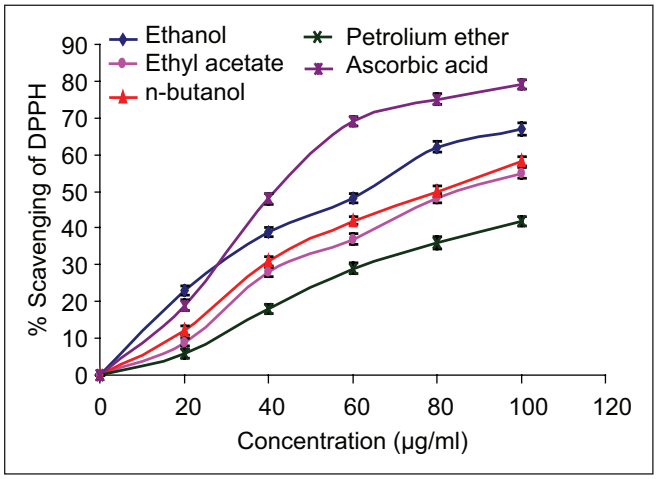

Figure 2: 1,1-diphenyl-2-picryl hydrazil scavenging activities of different extracts of *G. arborea* compared to ascorbic acid. (The data represent the percentage of DPPH inhibition. Each point represents as mean ± standard error mean [n=3])

Table 4: Antidiabetic activities of fruits extracts of *G. arborea* by alloxan induced diabetic model

Groups	Blood glucose level (mg/dL) (X±S.D.)				
	Basal value	1st day	3rd day	5th day	7th day
I	77±0.34	82±0.41	80±0.52	83±0.22	81±0.19
II	337±0.58	337±0.75	334±0.81	301±0.49	300±0.88
III	283±0.62	251±0.92	155±0.85	131±0.95	119±1.06*
IV	389±0.97	252±1.11	166±1.08	114±1.17	92±1.21***
V	335±1.15	208±1.13	177±0.96	146±0.90	124±1.10*
VI	371±1.18	258±1.06	180±1.01	160±1.14	97±0.92**
VII	308±0.99	238±1.06	152±1.13	136±0.89	118±1.19*

ANOVA

Source of variation	*SS*	*df*	*MS*	*F*	*P*-value	*F* crit
Between groups	127568.07	4	31892.0196	1.7863	0.0041	2.689
Within groups	199894.69	30	6663.15635			
Total	327462.76	34				

Each values is represented as mean±standard deviation (n=3). Standard error of mean <0.6986. *P<0.05, **P<0.01 and ***P<0.001 (test of significance between two proportions by z-test) in comparison to control in seven days study. Data are found to be significant (F value <F crit) by testing through one way ANOVA at 1% level of significance (P<0.01 that is P=0.00417). Group I-Control (Normal saline water), group II-Diabetic control (Alloxan-120 mg/kg), group III-Standard control (Glibenclamide 5 mg/kg), groups IV to VII-Alloxan (120 mg/kg) with ethanol, ethyl acetate, n-butanol and petroleum ether extracts respectively (300 mg/kg of b.w.)

be considered as a source of natural antioxidants for medicinal use. However, the components responsible for the antibacterial, antioxidant and antidiabetic activities are currently unclear. Therefore, further investigation is needed to isolate and identify the constituents present in the fruits extracts. Furthermore, the *in vivo* antioxidant activity of this extract needs to be assessed prior to clinical use.

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