ANTIDIABETIC, ANALGESIC, ANTIOXIDANT AND ANTIMICROBIAL POTENTIALS OF METHANOLIC EXTRACTS OF FRUITS AND SHOOTS OF *Brassica oleracea L. var. italic.*

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Authors' contributions

We all are the research group members from different university. All authors have equal contribution on this research work. SMMH contributed in the conception and design of study; works in drafting and revising the manuscript. MMI, JI, MSH, FA, AKA & RTT was involved to carry put the experimental research work. TSN, RAS helped during the drafting the manuscript.

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ABSTRACT

Background: Brassica oleracea L. var. italic conjointly known as “broccoli” is a crucial ayurvedic medication in traditional medicine mostly cultivated in Italy, France, England and USA. The aim of the present research work was to determine the antidiabetic, analgesic, antioxidant and antimicrobial potentials of fruits and shoots of broccoli.

Methods: In this study antidiabetic activity was investigated using alloxan induced diabetic model. Analgesic activity was manifested using acetic acid-induced writhing reflex. Antioxidant activity was evaluated by DPPH scavenging method while antimicrobial activity screening was carried out by disc diffusion method.

Results: Qualitative phyto analysis of methanolic extracts assured the existence of flavonoids and tannins etc. Moreover, it contains mild antibacterial and antioxidant activity where IC_{50} of the extract is 1424.30µg/ml. It also contains slightly analgesic and antidiabetic activity.

Conclusion: Our results recommend that presence of flavonoids and tannins render Brassica oleracea L. var. italic with therapeutic potential for oxidative stress and inflammation associated disorders.

Keywords: Brassica oleracea L. var. italica, antidiabetic, analgesic, antioxidant and antimicrobial activity.

ABBREVIATIONS

DPPH= 2,2-diphenyl-1-picrylhydrazyl , IC_{50} = Concentration of an inhibitor

ICDDR, B=International Centre for Diarrheal Disease and Research, Bangladesh.

1. INTRODUCTION

The role of medicinal plants in healing of diseases is increasing because of the presence of versatile compounds that have the flexibility to cure a spread of diseases and serving to physicians to influence increasing quantitative relation of ailments recently [1]. Medicinal plants

contain different bioactive compounds with the ability to heal. Phyto-chemicals like saponins, tannins, essential oils, flavonoids, alkaloids and other bioactive compounds found as secondary metabolites in plants [2]. Plants are rich of secondary metabolites are good measure for the most potential supply of novel drugs like antibiotics, insecticides, herbicides and potential health advantages akin to antioxidant, anti-aging, anti-atherosclerotic, antimicrobial and anti-inflammatory activities [3]. Regular intakes of plant products rich in phenolics are reportable to decrease extent the risks of developing chronic diseases similar to cancer, heart diseases and diabetes [4].

Diabetes is evolving in concert of the foremost fatal diseases endeavor humanity right behind cancer and cardiovascular diseases. Existing databases indicate its high prevalence, morbidity and mortality rate [5-6]. About 4 % population worldwide is dying by this deadly malady and this toll is probably going to swell by 5.4 % in the year 2025 [7]. Poor management of blood glucose levels is that the key conducive issue to the associated complications and treatment of hyperglycemia is thus, the most targets within the interference of those diabetes connected complications [8-9]. Hyperglycemia plays a crucial role in the development and progression of diabetic complications by various mechanisms together with exaggerated oxidative stress, minimized nitric oxide bioavailability, glucose autoxidation and non-enzymatic protein glycation [10]. The global exponential growth of diabetes has led to a synchronous rise within the usage of herbal remedies to treat diabetes due to their natural origin, free accessibility and lesser side effects [11].It is also well renowned that oxidative stress develops once reactive oxygen-derived free radical production exceeds the antioxidant defense mechanism of the cell [12]. Antimicrobial properties are rumored a lot of times during a wide selection of plant extracts and essential oils and natural products in a trial to discover new chemical categories of antifungal and antibacterial drugs that might resolve strains expressing resistance to the obtainable antifungal and antibacterial drugs [13-14]. Brassica oleracea L. var. italica (roots, leaves and fruits) is utilized in anti cancer especially prostate cancer, anti aging, management of diabetes, preventing anemia, protects against ultraviolet radiation and reducing the chance of heart disease cholesterol and high pressure [15-16].

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Identification

Fruits and shoots of Brassica oleracea L. var. italica were collected from Savar area district of Dhaka and were identified by the expert Mohammad Omar Faruk, department of Botany, University of Chittagong and preserved in the herbarium (Acc. No: CU/DP/PS/2015600321) department of pharmacy, University of Chittagong.

2.2 Trial registration:

For experimental study on animal trial registration and permission was issued from departmental clinical ethical review committee, department of pharmacy, university of Chittagong. The trail registration reference number is ERC/DP/CU/2015/0014

2.3 Extraction of plant material

Dried, ground fruits and shoots of Brassica oleracea L. var. italica (900 g) was taken in a clean flat bottomed glass container and soaked in 2L of methanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by clean, white cotton then followed by a filtration through Whatmann filter paper. The filtrate was allowed to keep for 7 days to evaporate the solvent followed by vacuum desiccation. Finally a blackish crude extract was obtained. The % yield was 5.5%.

2.4 Phytochemical screening

All of the crude extracts were qualitatively analyzed for the presence of different chemical groups, such as Alkaloids, Glycosides, Tannins, Flavonoids and Saponins [17-18].

2.5 Antioxidant Activity

Antioxidant activity of the methanolic extract of Brassica oleracea L. var. italica was determined by DPPH free radical scavenging activity on the basis of the modified method of Gupta [19]. Stock solutions (10 mg/ml) of the plant extracts were prepared in ethanol from which serial dilutions were carried out to obtain concentrations of 1, 5, 10, 50, 100 and 500 μg/ml. Diluted solutions (2 ml) were added to 2 ml of a 0.004% ethanol solution of DPPH, mixed and allowed to stand for 30 min for reaction to occur. The absorbance was determined at 517 nm using a double beam UV-visible spectrophotometer and from these values corresponding percentage of inhibitions were calculated. Then % inhibitions were plotted against log concentration and from the graph IC_{50} was calculated. The experiment was performed in triplicate and average absorption was noted for each concentration. Ascorbic acid was used as positive control. Free

Radical scavenging activity was expressed as the inhibition percentage (I%) and calculated as per the following equation:

% Inhibition(I) = [(Blank absorbance - Sample absorbance)/Blank absorbance] × 100

2.6 Antibacterial Activity

Antibacterial activity of the methanolic extract of Brassica oleracea L. var. italica was assessed by the disc diffusion method according to the previously described method [20-21]. Bacteria used as test organisms for the antibacterial activity test is listed in table 5.

2.7 Experimental Animals

Young Swiss-albino mice aged 4-5 weeks old and average weight 20-25 g was employed for the experiment. The mice were purchased from the Animal Research Branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B). They were kept in standard environmental condition (RH 55% to 60%, room temperature 25± 2^{o}C and 12 h light/ dark cycle) for one week for adaptation after their purchase and fed ICDDRB formulated rodent food and water. The experimental study was performed under the guidelines of Institutional Animal Ethics Committee [22].

2.8 Chemicals and Drugs

The standard drug, Metformin hydrochloride was the generous gift samples from Beximco Pharmaceuticals Ltd of Bangladesh. Alloxan monohydrate was purchased from Loba Chemie, India. Carrageenan was purchased from Otto Chemika, India. Blood samples analyzed for blood glucose content by using OK meter Match glucose test meter (Hsinchu, Taiwan). Acetic acid was collected from laboratory of Bangladesh University. The standard drug Diclofenac-Na was purchased from Square Pharmaceuticals Limited of Bangladesh

2.9 Experimental induction of diabetes

Experimental induction of diabetes in mice, freshly prepared solution of alloxan monohydrate in normal saline at a dose of 120 mg/kg body weight, were injected to mice intraperitoneally. Alloxan can produce fatal hypoglycemia as a result of massive pancreatic insulin release mice were treated with 20% glucose solution (5 - 10 ml) orally after 6 h. The mice were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycemia. After 1

week, mice with moderate diabetes that exhibited glycosuria and hyperglycemia (i.e. blood glucose concentration >200 mg/dL) were taken for the experiment [22].

2.10 Experimental design for antidiabetic activity study

Fifteen mice were divided in to five groups as Group I: normal rats received only distilled water during the experimental period, Group II: diabetic control rats received only distilled water during the experimental period, Group III: diabetic mice administered 500 mg/kg sample, Group IV: diabetic mice administered 250 mg/kg sample, Group V: diabetic mice administered 0.25 mg/kg glibenclamide.

Treatment was continued for a period of 6 hours following oral administration to the experimental animals by gastric intubation, using a force - feeding needle. Blood samples were collected from tail vein prior to dosing (0 hour) and then after 1st hour, 3rd hour and 5th hour respectively from all groups of mice, after administration of sample. Blood glucose was estimated on withdrawing blood samples. Fixed amount of rat chow and fluid was given to each rat and replenished the next [23-25].

2.11 Acetic acid-induced writhing test for Analgesic activity

The analgesic activity of the samples was also studied using acetic acid-induced writhing model in mice. Test samples and vehicle were administered orally 30 munities before intra-peritoneal administration of 1% acetic acid but Diclofenac-Na was administered intraperitonially before 15 mins, the mice were observed for specific contraction of body referred to as “writhing” for the next 10 munities [26-27].

2.12 Statistical analysis

Results were expressed as mean ± SEM. One-way ANOVA was used for analysis of data followed by Dunnet’s multiple comparisons. Differences were considered significant at $P \leq 0.05$.

3. RESULT

3.1 Phytochemical Screening:

Phytochemical screening of methanolic extract indicates the presence of tannins and flavonoids. Alkaloids and glycosides are absent in extract.

3.2 Antioxidant activity

Antioxidant activity of Brassica oleracea L. var. italica was determined on the basis of its ability to scavenge DPPH free radicals. Methanolic extracts of Brassica oleracea L. var. italica showed potential DPPH free radical scavenging activity where the IC_{50} was 1424.30μg/ml compared to that of ascorbic acid, used as standard, where the IC_{50} was 9.48 μg/ml results are summarized as Table 1 & Figure1.

Table -1 Antioxidant activity of Brassica oleracea L. var. italica

Sample	**Conc. (μg/ml)**	**% inhibition**	**IC_{50} (μg/ml)**
MeOH extract of Brassica oleracea L. var. italica	1	1.20 ± 0.023	**1424.30**
	5	3.20 ± 0.032	
	10	5.28 ± 0.025	
	50	16.20 ± 0.015	
	100	21.23 ± 0.023	
	500	54.11 ± 0.017	
Ascorbic acid	1	3.56±0.011	**9.48**
	5	31.00±0.024	
	10	73.37±0.034	
	50	84.66±0.014	
	100	89.69±0.023	
	500	96.20±0.031	

Values are expressed as mean ± S.D

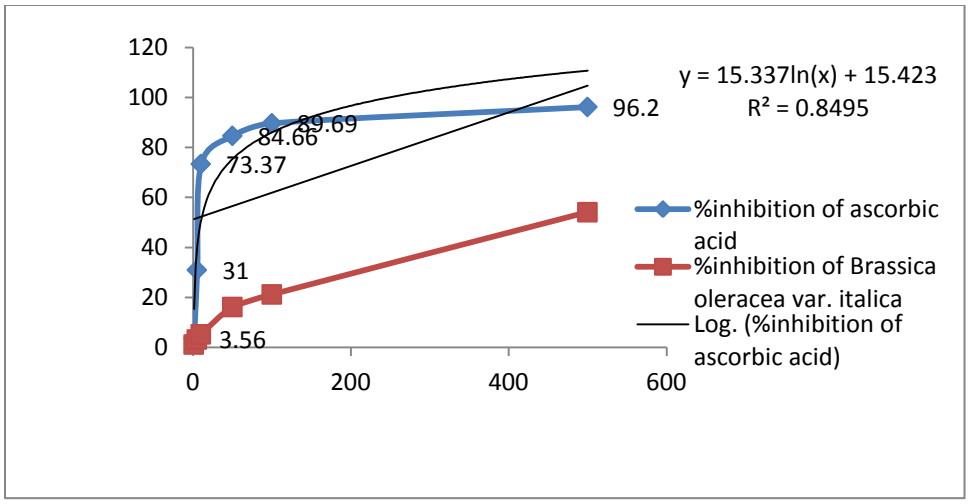


Figure 1: Antioxidant effect of the methanolic extract of Brassica oleracea L. var. italica.

3.3 Anti-diabetic activity

Oral glucose tolerance test was performed to determine the anti-diabetic activity. Results are summarized as Table 2 & Figure 2.

Table-2 Effect of the methanolic extract on Anti-diabetic activity in diabetic mice:

Time	Normal Group	Control Group	Standard Group	Extract Group (250 mg/kg)	Extract Group (500mg/kg)
0 min	5.8±0.36	18.5±0.26	25.6±0.20	11.1±0.43	12.1±0.36
30 min	5.7±0.32	21.3±0.47	18.5±0.25	18.0±0.31	24.6±0.25
90 min	5.8±0.52	19.7±0.21	16.3±0.35	16.3±0.25	17.1±0.40
60min	5.7±0.25	16.6±0.27	12.8±0.45	11.3±0.27	13.3±0.28
120 min	5.7±0.22	15.2±0.45	05.2±0.33	09.5±0.56	09.3±0.58

Values are expressed as mean ± S.D

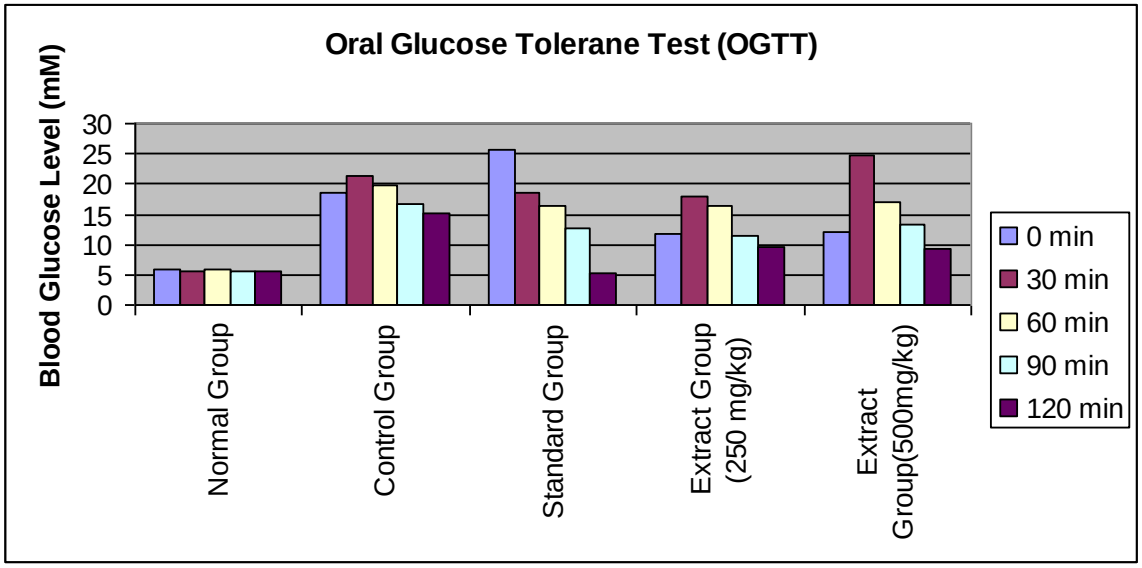


Figure 2: Effect of the methanolic extract on oral glucose tolerance test in diabetic mice.

3.4 Acetic acid-induced writhing in mice:

The analgesic effect of Brassica oleracea L. var. italica methanolic extract on acetic acid-induced writhing in mice test result were summarized as table 3 and figure 3 & 4.

Table-3 Acetic acid-induced writhing in mice

Animal Group	Writhing Counting (Mean ± SEM)	Writhing Inhibition (%)
Control Group	92.75±0.66	-
Standard Group	35.00±0.38	62.26
Extract Group (250mg/kg)	85.50±0.56	07.82
Extract Group (500 mg/kg)	75.75±0.32	18.32

Figure 3: Effects of the methanolic extract on acetic acid–induced writhing mice

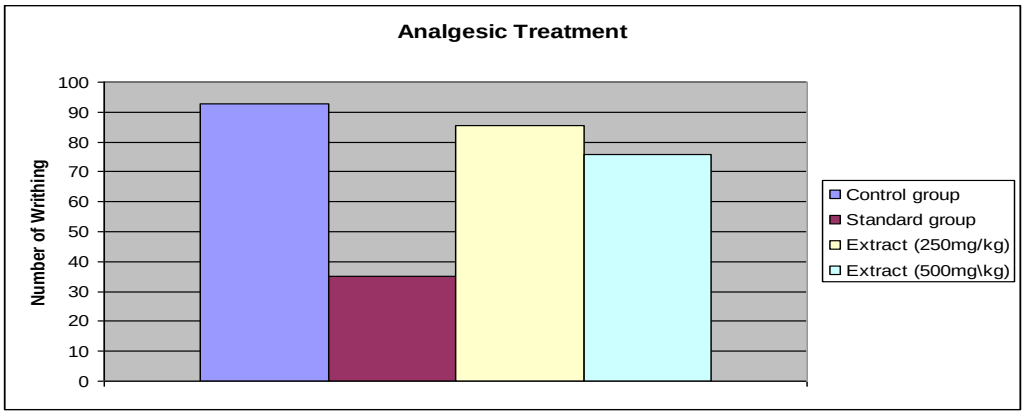
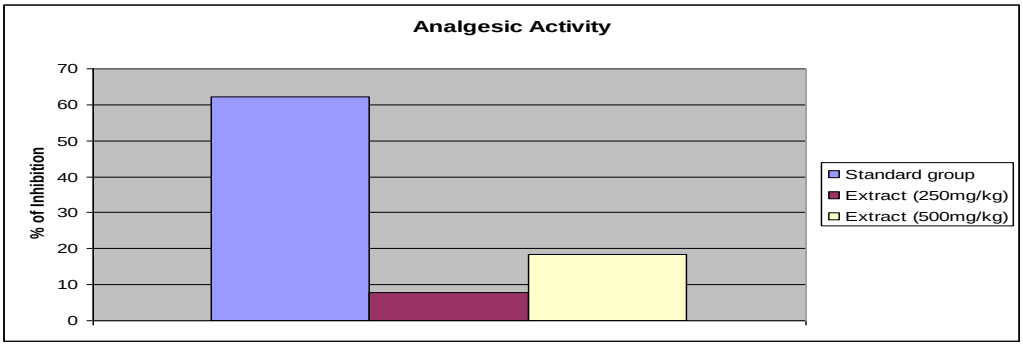


Figure 4: Percent of inhibition effects of the methanolic extract on acetic acid-induced writhing in mice.



3.5 Antibacterial activity

Table 4 showed the antibacterial activity of Brassica oleracea L. var. italica relative to that of the standard drug Ciprofloxacin. It showed mild antibacterial activity against *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonus aeruginosa* and *E.coli* where the zone of inhibition was about 6 mm.

Table 4: Antibacterial activity of Brassica oleracea L. var. italica

Bacteria	Zone of inhibition (mm)	
	Methanol extract (500 µg/disk)	**Ciprofloxacin (30 µg/disk)**
Gram Positive		
Bacillus subtilis	7.02 ± 0.21	31.01 ± 0.31
Bacillus cereus	6.11 ± 0.22	33.21 ± 0.33
Gram Negative		
Pseudomonas aeruginosae	5.23 ± 0.25	32.06 ± 0.36
E. coli	6.14 ± 0.23	35.04 ± 0.34

4. DISCUSSION

The present experimental research work was undertaken to determine the anti-diabetic, analgesic, antimicrobial and antioxidant effects of the methanolic extract of Brassica oleracea L. var. italica leaf on white albino mice (male).

The present study illustrates about the hypoglycemic effect of Brassica oleracea L. var. italica methanolic extract was satisfactory and considerable 500 mg/kg showed mild decrease (from 12.1 mM ± SD to 9.3 mM ± SD) and 250 mg/kg showed mild decrease (from 11.1 mM ± SD to 9.5 mM ± SD) compared to standard drug metformin (from 25.6 mM ± SD to 5.2 mM ± SD). Previous study also supports the antidiabetic activity of Brassica oleracea L. var. italica. through treatment of streptozotocin induced diabetic rats with dose of 100 mg/kg and 200 mg/kg body weight broccoli sprouts aqueous extract. The experimental result proves the significant decrease in blood glucose and liver glycogen at 14^{th} and 21^{st} day.

The methanolic extract of Brassica oleracea L. var. italica has minor anti oxidant activity. The IC_{50} of the extraction is 1424.30µg/ml, whereas IC_{50} of Ascorbic Acid is 9.48 µg/ml. The previous experiment proves that the ethanolic extract has higher antioxidant activity in DPPH radical and superoxide anion scavenging activity of aqueous extract. Furthermore, 3 day old Brassica oleracea L. var. italica (broccoli) seedlings showed the highest antioxidative activity than mature plant when tested for antioxidative activity using DPPH radical method.

The methanolic extract of Brassica oleracea L. var. italica has minor antimicrobial activity. It showed mild antibacterial activity against *Bacillus subtilis, Bacillus cereus, Pseudomonus aeruginosa* and *E.coli* where the zone of inhibition was around 6 mm. Ethyl acetate and chloroform extracts of broccoli florets were found to be effective against *B. cereus* and *B. subtilis*, respectively. Ethyl acetate and ethanol extracts were highly active against *E. coli*. Additionally, ethyl acetate and chloroform extracts showed high activity against *Candida albicans*.

Significant analgesic effect was monitored in dose 500 mg/kg of extract inhibited 17.2 % and dose 250 mg/kg of extract of Brassica oleracea L. var. italica inhibited 6.81 % of writhing movements compared to control group where as standard drug diclofenac showed 62.26 % of inhibition. This experimental result proves the analgesic activity of the extract [27].

5. CONCLUSION

We concluded from the current work that methanolic extract derived from Brassica oleracea L. var. italica possesses noticeable antidiabetic, analgesic, antioxidant and antimicrobial potentials. The usefulness of this plant should be confirmed through further phytochemical and toxicity analyses.

Competing interests

The authors declare that they do not have any competing interests.

REFERENCES:

1. Petrovska BB. Historical review of medicinal plants' usage. *Pharmacogn Rev*. 2012; 6(11):1-5.
2. Pavarini DP, Pavarinib SP, Niehuesa M, Lopesa NP. Exogenous influences on plant secondary metabolite levels. *Ani Feed Sci Tech*. 2012; 176:5-16.
3. Mulaudzi RB, Ndhlala AR, Kulkarni MG, Van Staden J. Pharmacological properties and protein binding capacity of phenolic extracts of some Venda medicinal plants used against cough and fever. *J Ethnopharma*. 2012; 143:185-19.
4. Zhu F, Cai Y, Sun M, Corke H. Effect of phytochemical extracts on the pasting, thermal, and gelling properties of wheat starch. *Food Chem*. 2009; 112: 919-23.
5. Kannel WB, McGee DL. Diabetes and cardiovascular disease: the Framingham study. *JAMA*. 1979; 241(19):2035-8.
6. King H, Aubert RE, Herman WH. Global burden of diabetes, 1995–2025: prevalence, numerical estimates, and projections. *Diabetes Care*. 1998; 21(9):1414-1431.
7. Kim SH, Hynn SH, Choung SY. Antidiabetic effect of cinnamon extract on blood glucose in db/db mice. *J Ehthnopharma.* 2006; 104(1-2):119-123.
8. Stettler C, Allemann S, Juni P, Cull CA, Holman RR, Egger M et al.. Glycemic control and macrovascular disease in types 1 and 2 diabetes mellitus: Meta-analysis of randomized trials. *Am Heart J*. 2006; 152(1):27-38.
9. Liu CT, Sheen LY, Lii CK. Does garlic have a role as an antidiabetic agent? *Mol Nutri Food Res*. 2007; 51(11):1353-64.
10. Rahimi R, Nikfar S, Larijani B, Abdollahi M. A review on the role of antioxidants in the management of diabetes and its complications. *Biomed Pharmacother*. 2005; 59(7):365-73.
11. Modak M, Dixit P, Londhe J, Ghaskadbi S, Devasagayam TPA. Indian herbs and herbal drugs used for the treatment of diabetes. *J Clin Biochem Nutr*. 2007; 40(3):163-73.

12. Huang CS, Yin MC, Chiu LC. Antihyperglycemic and antioxidative potential of Psidium guajava fruit in streptozotocin-induced diabetic rats. *Food Chem Toxicol*. 2011; 49(9):2189-95.
13. Ali NA, Julich WD, Kusnick C, Lindequist U. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. *J. Ethnopharmacol*. 2001; 74(2):173-9
14. Angioni A, Barra A, Coroneo V, Dessi S, Cabras P. Chemical composition, seasonal variability, and antifungal activity of Lavandula stoechas L. ssp. stoechas essential oils from stem/leaves and flowers. *Agric. Food Chem*. 2006; 54(12):4364-70.
15. Santegra USA Catalog - Herbal Remedies Catalog.
16. Source: USDA National Nutrient data base.
17. Evans WC. *Trease and Evan's Text book of Pharmacognosy.* 13th ed. University Press, Cambridge;1989. pp.546.
18. Hossen SMM, Hossain MS, Islam J, Pinto MN, Jannat N, Ahmed F. *Der Pharma Chemica*, 2014; 6 (3):332-338.
19. Gupta M, Mazumdar UK, Sivahkumar T, Vamis MLM, Karki S, Sambathkumar R, Manikandan L. Antioxidant and anti-inflammatory activities of *Acalypha fruticosa*. *Nig. J. Nat. Prod. Med.* 2003; 7: 25-29.
20. Ahmed F, Das PK, Islam MA, Rahman KM, Rahman MM, Selim MST. Antibacterial activity of *Cordyline terminalis*. Kunth. leaves. *J. Med. Sci.* 2003; 3(5-6): 418-422.
21. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Path.* 1966; 45 (4): 493-496.
22. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* 1983;16(2):109-110.
23. Hossain MS, Islam J, Sarkar R, Hossen SMM. *IJP, 2014;* 1(7): 449-453.
24. Rajan M, Kishor Kumar V, Satheesh Kumar P, Thiruvenkatasubramaniyam R, Senthil Kumar N. Antidiabetic activity of ethanolic extract on Albizia odoratissima (l.f) benth in alloxan induced diabetic rats. *Int.J.Ph.Sci.* 2010; 2(3): 786-791.
25. Jithendra CH, Muralidharan P. Anti-hyperglycemic and antioxidant activity of the Ayurvedic drus Nisha kathakathadhi churnam in alloxan-induced hyperglycemic rats. *International Journal of Green Pharmacy*. 2009: 66-69.

26. Turner RA. *Screening methods in pharmacology*. New York: Academic Press; 1971. pp. 100–113.
27. Vogel HG. *Drug discovery and evaluation, pharmacological assay*. 2nd ed. New York: Springer; 2002. pp. 670.