Antidiabetic, Analgesic, Antioxidant And Antimicrobial Potentials of Methanol Extracts of Fruits and Shoots of oleraceaL. Var. Italic.

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Authors' contributions

We are a research group members from different universities. All authors have equal contribution on this work. SMMH contributed in the conception and design of study; works in drafting and revising the manuscript. MMI, JI, MSH, FA, AKA & RTT were involved in experimental works. TSN and RAS helped during 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 phytoanalysis of methanol 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 renders *Brassica oleracea* L. var. italic with therapeutic potential for oxidative stress and inflammation associated disorders.

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

ABBREVIATIONS

DPPH=2,2-diphenyl-1-picrylhydrazyl , IC_{50} =The half maximal inhibitory concentration

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 to antioxidant, anti-aging, anti-atherosclerotic, antimicrobial and anti-inflammatory activities [3].Regular intakes of plant products rich in phenolics are reportable to decrease risks of developing chronic diseases similar to cancer, heart diseases and diabetes mellitus[4].

Diabetes mellitus 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 important 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, minimize 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 cells[12]. Antimicrobial properties are rumoreda 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 cancer therapy especially prostate cancer, anti-aging, management of diabetes, preventing anemia, protecting against ultraviolet radiation and reducing the chance of coronary heart disease 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 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

2.3Extraction 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 with yield of 5.5%.

2.4Phytochemical 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].

2.5Antioxidantactivity

Antioxidant activity of the methanol extract 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 to react. 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, the half maximal inhibitory concentration (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 following equation:

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

2.6Antibacterial activity

Antibacterial activity of the methanol extract 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 (vide infra).

2.7Experimental 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 and fed ICDDRB formulated rodent food and water ad libitum. The experimental study was performed under the guidelines of Institutional Animal Ethics Committee [22].

2.8Chemicals and drugs

The standard drug, metformin hydrochloride was the generous gift 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 by using OK meter Match glucose test meter (Hsinchu, Taiwan). Acetic acid was prepared from laboratory of Bangladesh University. The standard drug diclofenac-Na was purchased from Square Pharmaceuticals Limited of Bangladesh.

2.9Experimental induction of diabetes mellitus

Experimental induction of diabetes mellitus in mice, freshly prepared solution of alloxan monohydrate in normal saline solution at a dose of 120 mg/kg body weight injected to mice intraperitoneally. Alloxan can produce initially 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 mellitus that exhibited

glycosuria and hyperglycemia (i.e. blood glucose concentration >200 mg/dL) were taken for the experiment[22].

2.10Experimental 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 1^{st} hour, 3^{rd} hour and 5^{th} hour respectively from all groups of mice after treatment. 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].

2.11Acetic 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 or mean ± SD. One-wayANOVA 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 Phytochemicalscreening

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

3.2 Antioxidant activity

Antioxidant activity of 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 its$IC_{50}$was 9.48μg/ml results are summarized as Table 1&Figure1.

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

Sample	Conc. (μg/ml)	% inhibition	IC_{50}(μg/ml)
Metanol extract	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.

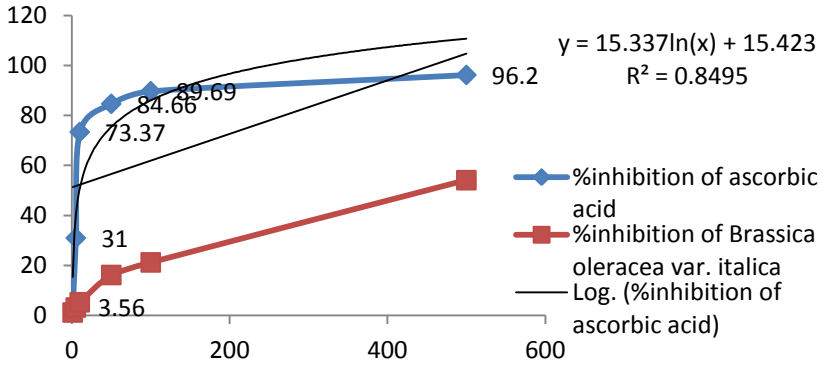


Figure 1: Antioxidant effect of the methanol extract of *Brassica oleracea* L. var. italica

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-2Effect of the methanol extract of *Brassica oleracea* L. var. italica 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.

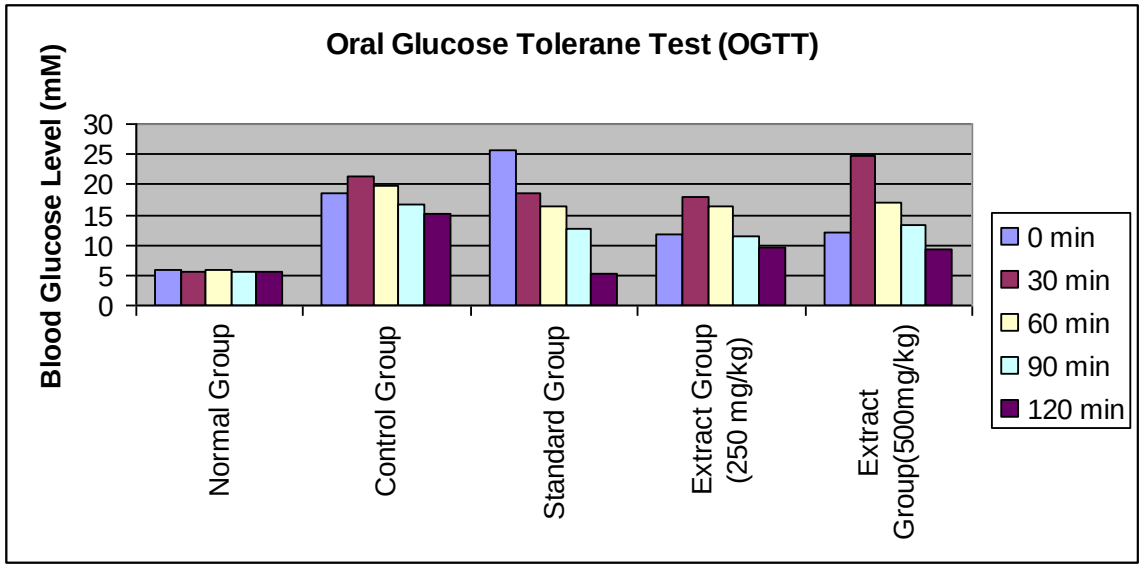


Figure2: Effect of the methanol extract of *Brassica oleracea* L. var. italica on glucose tolerance test in diabetic mice

3.4Acetic acid-induced writhing in mice:

The analgesic effect of *Brassica oleracea* L. var. italica methanol 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 treated with methanol extract of *Brassica oleracea* L. var. italica

Animal Group	Writhing Counting	Writhing Inhibition (%)
Control Group	92.75±0.66	-
Standard Group	35.00±0.38	62.26
Extract Group (250mg/kg)	85.50±0.56	7.82
Extract Group (500 mg/kg)	75.75±0.32	18.32

Values are expressed as mean ± S.E.M.

Figure 3: Effects of the methanol extract of *Brassica oleracea* L. var. italica on acetic acid–induced writhing mice

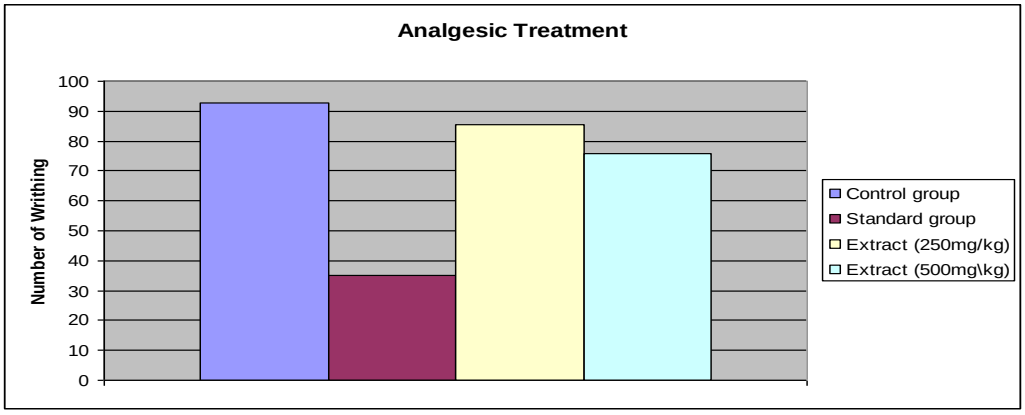
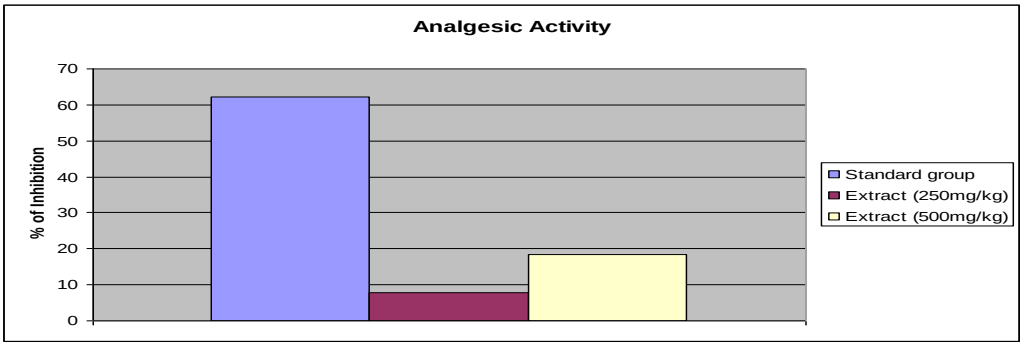


Figure 4: Percent of inhibition effects of the methanol extract of *Brassica oleracea* L. var. italic on acetic acid-induced writhing in mice



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*, *Pseudomonas 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 aeruginosa	5.23 ± 0.25	32.06 ± 0.36
E. coli	6.14 ± 0.23	35.04 ± 0.34

Values are expressed as mean ± S.E.M or S.D.

4. RESULT AND DISCUSSION

The present experimental research work was undertaken to determine the anti-diabetic, analgesic, antimicrobial and antioxidant effects of the methanol 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 methanol extract was satisfactory and considerable 500 mg/kg showed mild decrease (from 12.1 mM ± S.D. to 9.3 mM ± S.D.) 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 ± S.D. to 5.2 mM ± S.D.).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 decreasein blood glucose and liver glycogen at 14^{th} and 21^{st} day.

The methanol extract of *Brassica oleracea* L. var. italicahas minor antioxidant 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 methanol extract of *Brassica oleracea* L. var. italicahas minor antimicrobial activity. It showed mild antibacterial activity against *Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosaand 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 methanol extract derived from *Brassica oleracea* L. var. italica possesses noticeable anti-diabetic, 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 have no competing interests.

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