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Phenol Content and Antioxidant Capacity of Selected Ethnic Plant Foods: Alleviation of Experimental Depression

Zannatul Ferdous Miftah[1], Jyosna Khanam[1], Sheikh Faisal Assadullah Mahadi[2], and Sheikh Nazrul Islam[1]*

[1]*Institute of Nutrition and Food Science, University of Dhaka, Dhaka 1000, Bangladesh.*

[2]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh.*

***Correspondence:**
Sheikh Nazrul Islam, Institute of Nutrition and Food Science, University of Dhaka, Dhaka 1000, Bangladesh, Tel: +880 2 9661900-73 ext 8418; cell: +880 01817522414, Fax: +880 2 9667222.

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ABSTRACT

This article describes the antioxidant capacity (AC) and total phenol content (TPC) of selected ethnic plant foods and their therapeutic uses in alleviating experimental stress-induced depression in rat model. Antioxidant capacity was determined by radical scavenging activity and TPC was estimated by Folin-Ciocalteau method. Depression was induced by chemical stressor- reserpine. Alleviation of depression by the ethnic foods was evaluated by behavioral changes in Forced Swim Test (FST) and Tail Suspension Test (TST), and by analysis of oxidative stress marker Malondialdehyde (MDA), fasting blood glucose (FBG) and change of weight of adrenal gland and brain. Antioxidant capacity was determined by IC_{50} value, which was ranged from 74.814 µg/ml to 411.895 µg/ml. The lowest IC_{50} value indicates the strongest antioxidant activity. Hence, the strongest AC was found in Gondhovatali followed by Sabarang and Titbegun. TPC ranged from 85.5 ± 5.51 to 650 ± 2.75 mgGAE/100g. Jaamalu was found to have the highest TPC value followed by Gondhovatali, Titbegun and Khudemanik. The difference among experimental and control groups was found to be significant in the weight of adrenal gland and brain. Biochemical stress indicators (MDA, FBG) and behavioral tests (TST, FST) showed significant differences among plant extract fed groups compared to that of depressed control group, but was found to be almost similar to antidepressant clomipramine treated and baseline control groups. The data indicated that the selected ethnic plant foods containing higher TPC and lower IC_{50} values significantly alleviated depression symptoms in the rats.

Keywords

Ethnic plant foods, Total phenol, Antioxidant capacity, Depression alleviation.

Introduction

Depression is said to be a leading cause of morbidity [1], which affects function and quality of life [2]. It has been suggested that depression is associated with increased oxidative stress [3], which has been linked to many chronic diseases including neurological disorder [4], cardiovascular disease, inflammation and aging [5]. Brain is susceptible to oxidative stress [6] because of its high energy demand, oxygen consumption, and auto-oxidizable neurotransmitters [7]. The adrenal gland, a stress-responsive organ, changes in stress conditions [8]. Stress marker- malondialdehyde level is declined in oxidative stress.

Antioxidant defense includes enzymatic and non-enzymatic antioxidants. Dietary intake of plant antioxidants such as phenolic compounds, vitamin E, vitamin C, beta carotene present fruits and vegetables are essential along with natural antioxidant mechanism in the body to fight against reactive oxygen species [9]. It has been reported that some plant products have antidepressant activity on experimentally stress-induced animal model [10,11].

The present study investigated total phenols and antioxidant capacity of some ethnic plant foods, and their potentials in alleviation of stress-induced depression.

Methods and materials

Chemicals and regents

Methanol (HPLC grade), 2,2-diphenyl-1-picrylhydrazzyl (DPPH), Gallic acid, Folin-Ciocalteu reagent (FCR), reserpine, clomipramine, thiobarbituric acid (TBA), tricholoroacetic acid (TCA), ascorbic acid, hydrochloric acid, and formaldehyde were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Sample collection and processing

Seven ethnic plant foods included in this study are presented in the Table 1 and Figure 1.

In order to be representative, the food samples were collected from the local tribal markets of Rangamati, Bandarbans and Mymensingh, and were identified by a taxonomist of the Department of Botany, University of Dhaka. Samples were brought to the laboratory of the Institute of Nutrition and Food Sciences, University of Dhaka, cleaned with tap water followed by distilled water and air dried. The edible portions of the samples were cut into small pieces to increase the surface area to facilitate the drying process. After drying, samples were powdered with a grinder and sieved through a strainer to separate fined particle of the powdered sample, which were kept in crucibles and stored in desiccators.

Preparation of plant extract

Approximately 2 g powdered sample from each sample was taken in a conical flask, 42.5 ml methanol and 7.5 ml 1N HCl were added. The mixture was allowed to soak in the solvent at room temperature for 24 hours with intermittent shaking. Extracts were filtered through Whatman No. 1 filter paper, and the filtrate was separated and evaporated by a rotary evaporator (Heidolph, Germany) to concentrate the extracts.

Determination of total phenol content in plant extracts

Total phenol content (TPC) was determined by Folin-Ciocalteu method as described elsewhere [12]. A 500 µl of each of the extract (1 mg/ml equivalent) was mixed with 5 ml FCR (1:10 v / v distilled water) and 4 ml of sodium carbonate (75 g per l) and the mixture was then vortexed for 15 second. The mixture was allowed to stand for 30 minutes at 40 °C for color development. Absorbance

Table 1: Plant food investigated.

	Ethnic name	English name	Scientific/Botanical name	Family	Collection area
1	Khudemanik	Indian pennywort	*Centella asiatica*	Apiaceae	Rangamati, Bandarbans, and Mymensingh
2	Sabarang	Curry leaf	*Murraya koenigii*	Rutaceae	
3	Gondhvatali	Stinkvine	*Paederia foetida*	Rubiaceae	
4	Jolpaipata	Olive leaves	*Olea europa*	Oleaceae	
5	Holudpata	Turmeric leaves	*Curcuma longa*	Zingiberaceae	
6	Titbegun	Black night shade	*Solanum nigrum*	Solanaceae	
7	Jammalu	Purplish potato	*Solanum tuberosum*	Solanaceae	

Figure 1: Selected plant foods

was read against blank at 765 nm using a double beam UV-visible spectrophotometer. A standard calibration curve was constructed with gallic acid (R^2 = 0.989) for calculation of the phenol content expressed as mg GAE/100g dry weight of the plant material.

Estimation of antioxidant activity of plant extracts

The antioxidant activity of methanolic extracts was estimated by DPPH assay with little modification [13]. Stock ascorbic acid solution (1.0 g/ml) was made and diluted into 200 µg, 400 µg, 600 µg and 800 µg per ml. Two ml of the diluted solution of every concentration was taken and 2.0 ml of 0.1 ml mM methanolic DPPH solution was then added. The mixture was stirred for 15 seconds and kept in dark for 30 minutes. Stock solution (approximately 1.0 mg per ml) of each of the plant extracts was prepared, which was also diluted into 200 µg, 400 µg, 600 g and 800 µg/ml. Similarly, 2.0 ml of the 0.1 mM methanolic DPPH solution was added to 2.0 ml of the serially diluted extract, and was stirred vigorously for 25 seconds. The solution was allowed to stand in dark for 30 minutes at room temperature. Absorbance was read against blank at 517 nm with a spectrophotometer (UV-1201 UV-VIS, Shimadzu, Japan). The percent of DPPH radical scavenging activity of the extract was calculated using the following formula

$$\%\ of\ Inhibition = \left(\frac{Ao - A}{Ao}\right) \times 100$$

Here, A_0 is the absorbance of the control (solution without extract) and A is the absorbance of the DPPH solution containing plant sample extract.

The DPPH radical scavenging activity % was plotted against the extract concentration (µg/ml) to determine the concentration of the extract required to decrease DPPH radical scavenging by 50% known as IC_{50} (concentration required to scavenge 50% free radical). IC_{50} value of each extract was estimated by Sigmoid non-linear regression using sigma plot.

Evaluation of stress reducing capacity of the plant extracts

Experimental design

In order to evaluate stress reducing properties of the plant foods, an in vivo animal model was used [14]. Thirty healthy Wistar Albino rats of 125g to 160 g were collected from the animal house of the Faculty of Pharmacy, Jahangirnagar University, Savar. The animals were acclimatized in the animal house at the Institute of Nutrition and Food Science, University of Dhaka for 14 days, and were then divided into six groups named A, B, C, D, E, and F groups, each containing five rats, which were fed with basal diet for 21 consecutive days. Except the baseline control group, chemical stressor reserpine (0.38mg/kg body weight) was given to all rats to induce the stress (Teixeira et al., 2008). The experimental A, B, and C group were respectively orally given 200 mg/kg/day Gondhovatali, Sabarang and Titbegun extract. Group D received antidepressant clomipramine (12.65 mg/kg/day), which was treated as positive control. Negative control E group received the reserpine (0.38mg/kg/day). The F group rats receiving the basal diet was treated as baseline control.

Ethical permission was obtained from the ethical board of the Faculty of Biological Sciences, University of Dhaka, Bangladesh.

Analysis of behavioral tests (FST and TST)

On the 22nd day, Forced Swim Test (FST) and Tail Suspension Test (TST) of all rats were performed to assess the behavioral changes. Rats were forced to swim individually in a cylinder of 40 cm height and 15 cm diameter containing fresh water up to 30 cm height for a period of 5 minutes as described by Porsolt, Bertin, & Jalfre (1977). The tail suspension test (TST) as described by Steru, Chermat, Thierry, & Simon, 1985) involved suspending the rat on the edge of a table 50 cm above the ground by adhesive tape placed 1 cm from the tip of the tail. The total period of immobility was recorded for 5 minutes.

Collection of blood, adrenal gland and brain

All the rats were anaesthetized with 30% chloroform to sacrifice them on the 23rd day. Approximately 3 ml blood sample was collected from every rat heart puncture using sterile syringes. One drop of blood was used for estimation of fasting blood glucose with a glucometer and the rest blood was processed for serum to be used MDA analysis.

Brain and one adrenal gland were extracted from each rat, which were washed in saline, wiped in tissue paper, air dried for few minutes and then weighted by electric balance (Mettler Toledo, Switzerland) for their weights.

Analysis of malondialdehyde (MDA)

MDA level was analyzed by thiobarbituric acid assay as described by Buege, & Aust [15]. In brief, 15% w/v trichloroacetic acid, 0.375% w/v thiobarbituric acid and 0.25 N hydrochloric acid was used to prepare TCA-TBA-HCl reagent. One ml serum was combined with 2.0 ml of TCA-TBA-HCl and mixed thoroughly and boiled in water bath for 15 minutes and then cooled at room temperature. After centrifugation, clear supernatant was read for absorbance at 535 nm against a blank. Serum MDA was measured by using the following formula-

$$\text{MDA concentration (mol/L)} = \text{Absorbance}/(b \times 1.56 \times 105)$$

Width of tube, b = 1 cm

Statistical analysis

The assays were performed in triplicate and the results were expressed as mean ± standard deviation (SD). Data analysis was be done by Microsoft Excel 2013 and SPSS version 22. ANOVA and Tukey post hoc test were done to analyze the data.

Results and discussion

The moisture of the plant foods was determined and it ranged 80% to 93% (Table 2). This value corresponds to those of the reported range (70% to 91%) in plant foods [16].

Total phenol content and antioxidant activity

Total phenol content ranged from 85.5 ± 5.51 to 650 ± 2.75 mg GAE/100g fresh dried weight (Table 2), which was in the order

Jaamalu>Gondhovatali > Titbegun > Khudemanik > Holudpata > Sabarang > Jolpaipata. Jaamalu was found to be containing the highest TPC value whereas Jolpaipata contained the least. To some extent, it was like some other data [17].

The Gondhovatali was found to have the highest antioxidant capacity (IC_{50} 74.814). The higher the IC_{50} value, the higher the antioxidant capacity. The varying total phenol and antioxidant value with the wild or local plant foods have also been reported elsewhere [18]. Chanda, Sarethy, De, & Singh [19] reported a promising ethno-medicinal use of Gondhovatali (*Paederia foetida*) ethnic plant.

Effect on behavioral change

Table 3 and Figure 3 show the period of immobility in TST, and climbing, swimming and immobility in FST for the rats subjected to different treatments. In tail suspension test, the extract of Gondhovatali, Sabarang, not the Titbegun showed lower immobility period than negative control, but it was almost similar to those of positive control. ANOVA indicated a significant difference among them. In forced swim test, the climbing, swimming and immobility had significant changes. However, swimming and immobility were found higher as compared to the negative and almost similar to the antidepressant clomipramine. Sabarang had higher climbing and swimming period, but a little less immobility.

Table 2: Moisture content, total phenol and IC_{50} value of ethnic plant extract.

Plant food	Moisture	Phenol content	% Inhibition at different concentration				IC_{50} µg/ml
	%	mg GAE/100 mg	200 µg/ml	400 µg/ml	600 µg/ml	800 µg/ml	
Khudemanik	93	351.50 ± 5.27	47.59 ± 2.10	61.26 ± 0.82	67.85 ± 1.43	73.25 ± 0.28	201.076
Sabarang	90	323 ± 7.39	53.38 ± 0.57	59.79 ± 0.09	67.41 ± 0.89	77.29 ± 1.89	135.264
Gondhovatali	91	622 ± 5.94	55.74 ± 0.67	61.48 ± 1.44	72.62 ± 0.43	79.02 ± 0.23	74.814
Jolpaipata	90	85.5 ± 5.51	38.97 ± 1.81	51.44 ± 2.60	56.82 ± 0.31	70.24 ± 1.94	411.895
Holudpata	90	332.5 ± 6.92	44.04 ± 0.26	53.93 ± 0.49	62.27 ± 1.92	75.96 ± 0.97	325.816
Titbegun	92	441.75 ± 6.7	51.82 ± 2.11	60.57 ± .91	65.70 ± 1.34	73.99 ± 0.17	136.592
Jaamalu	80	650 ± 2.75	42.53 ± 0.27	50.09 ± 0.86	67.24 ± 0.66	73.19 ± 0.28	348.352

Table 3: Behavioral changes of different groups of rats

Groups	Tail Suspension Immobility (s)	Forced Swim Test (s)		
		Climbing	Swimming	Immobility
Gondhovatali (A)	28.76 ± 2.94	121.93 ± 4.72	86.62 ± 2.53	27.44 ± 3.02
Sabarang (B)	29.08 ± 3.30	124.33 ± 5.27	87.12 ± 3.26	29.30 ± 3.98
Titbegun (C)	30.08 ± 3.30	122.21 ± 5.75	85.34 ± 3.70	28.90 ± 4.53
Positive control (D)	31.44 ± 3.51	125.48 ± 3.48	85.24 ± 3.56	27.08 ± 3.54
Negative control (E)	34.32 ± 3.25	115.54 ± 3.76	82.63 ± 2.36	36.20 ± 2.95
Baseline (F)	25.92 ± 2.66	135.58 ± 4.34	90.52 ± 2.69	22.98 ± 3.05
ANOVA	F (29) = 7.37 P=0.0001	F (29) =18.64 P = 0.0001	F (29) = 3.65 P = 0.014	F (29) = 7.91 P = 0.0001
Tukey test	D vs A, B, C; p=0.43, 0.87, 0.93	D vs A, B, C; p=0.56, 1.00, 0.66	D vs A, B, C; p=1.00, 0.92, 1.00	D vs A, B, C; p=1.00, 0.92, 0.96

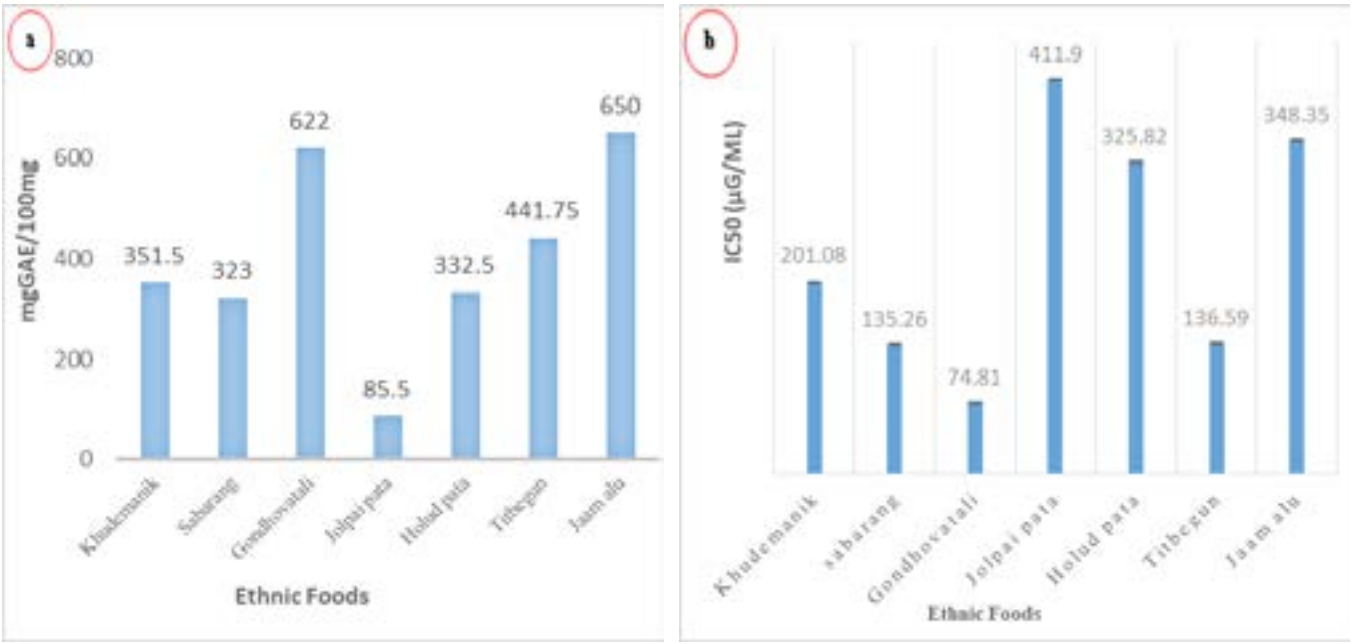

Figure 2: Total phenol (a) and IC_{50}values (b) of different ethnic foods.

Table 4: Percent change of body weight, adrenal gland and brain in different group of rats

Groups	Body weight (g)		Body wt change %	Adrenal gland wt(mg)	Brain wt(g)
	Initial	Final			
Gondhovatali (A)	161.20 ± 2.23	189.20 ± 2.20	17.75 ± 4.41	13.40 ± 2.97	1.52 ± 0.10
Sabarang (B)	162.80 ± 2.68	194.00 ± 1.01	19.11 ± 4.38	15.40 ± 2.88	1.56 ± 0.09
Titbegun (C)	161.20 ± 1.19	188.80 ± 1.27	17.18 ± 2.23	13.00 ± 2.55	1.64 ± 0.01
Positive control (D)	161.20 ± 1.29	192.40 ± 1.75	19.27 ± 1.92	14.20 ± 4.02	1.58 ± 0.07
Negative control (E)	161.20 ± 2.71	184.00 ± 2.69	14.57 ± 4.54	19.00 ± 2.35	1.49 ± 0.03
Baseline (F)	162.80 ± 2.91	200.40 ± 2.40	24.11 ± 7.19	10.40 ± 0.89	1.60 ± 0.05
		ANOVA	F=2.51, P=0.058	F=5.34, P=0.002	F=3.58, P=0.015
		Tukay test	D vs A, B, C: p=0.87, 0.60, 0.94	D vs A, B, C: p=0.10,1.00, 1.00	D vs A, B, C: p=1.00, 0.14, 0.02

Table 5: Changes in fasting blood glucose and malondialdehyde in different groups of rats.

Groups	Fasting Blood Glucose (mmol/L)	MDA Concentration (nmol/mL)
Gondhovatali (A)	4.4 ± 0.62	1.39 ± 0.24
Sabarang (B)	4.42 ± 0.22	1.29 ± 0.39
Titbegun (C)	4.42 ± 0.16	1.78 ± 0.41
Positive control (D)	4.5 ± 0.51	1.67 ± 0.20
Negative control (E)	4.9 ± 0.20	2.26 ± 0.21
Baseline (F)	3.54 ± 0.36	1.28 ± 0.17
ANOVA	F (29) = 6.68 P = 0.001	F (29) = 8.71 P = 0.0001
Tukey test	D vs A, B, C; p=1.00	D vs A, B, C; p=0.64, 0.32, 1.00

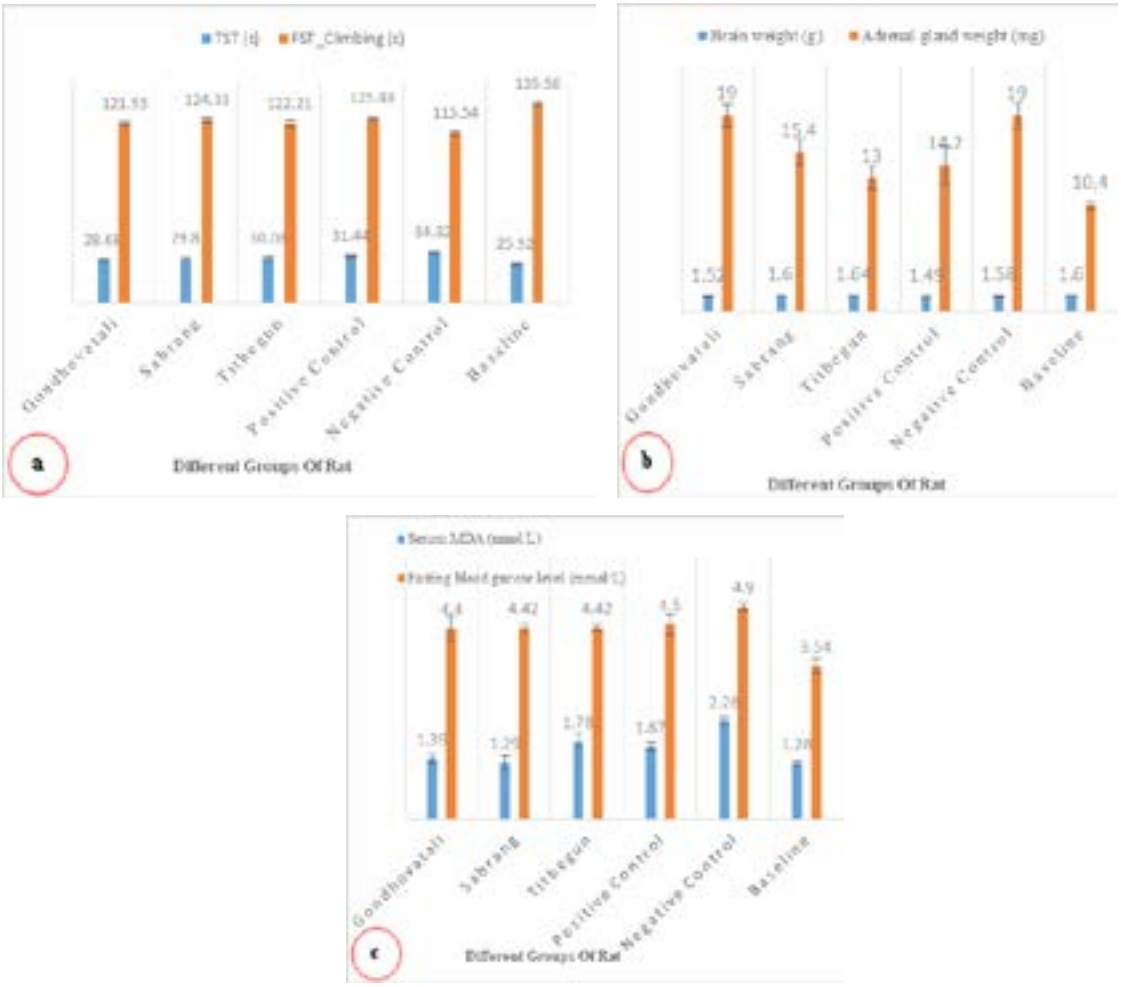

Figure 3: (a) Behavior change, (b) change in brain and adrenal gland) and (c) change in MDA and fasting blood glucose level of different groups of rats

Effect on adrenal gland and brain function

The weight of adrenal gland and brain (mean ± sd) of the animals subjected to different treatments are shown in Table 4 and Figure 3. A decrease in the weight of adrenal gland and an increase in the brain weight was observed apparently in the groups compared to the negative control. Sabarang had change in adrenal and brain weight like the antidepressant clomipramine. A significant variation among different groups was observed.

Effect on fasting blood glucose and malondialdehyde level

Treatments with food extracts made insignificant effect on fasting blood glucose levels, which was observed similar that of positive control but significant decrease was found in baseline compared with that of negative control group (Table 5 and Figure 3). Decreased serum malondialdehyde level was found in the groups given extract of Gondhovatali and Sabarang, which were shown to be more effective on serum MDA compare to Titbegun. ANOVA analysis showed significant difference on fasting blood glucose level and serum MDA level among the groups.

Previous studies have demonstrated that reserpine at a certain dose induces oxidative stress through dopamine metabolism [20], which causes chronic disorders including neurodegeneration like depression, which is characterized by changes of muscle tone, stress marker [21], and weight change of brain and adrenal gland [22]. The present study showed that oxidative stress was associated with changes in behaviour, stress maker, biochemicals and weight of brain and adrenal gland. Therapeutic uses of antioxidant rich plant foods made a decrease of the depression symptoms. Accordingly, the use of antioxidant rich foods may be a dietary approach for alleviation of oxidative stress induced depression.

Further, it was shown that Gondhovatali, Sabarang and Titbegun contained a rich amount of phenol having significant antioxidant capacity. They also had a remarkable depression alleviation potential. Sabarang, Gondhovatali, and Titbegun along with total phenol content. Gondhovatali and Sabarang extracts significantly reduced the plasma malondialdehyde level compared to negative control and antidepressant clomipramine. In our previous study conducted with nutraceuticals- omega-3 fatty acid, vitamin C, zinc also reported a significant relieve of depression in experimental stress model [23]. Some other studies also reported alleviation of experimental depression by plant products [10,11,24].

Conclusion

The ethnic vegetables- Gondhovatati, Sabarang, and Titbegun contain a rich amount of phenols and have significant antioxidant capacity, and antidepressant potential. They can be used to relieve or alleviate stress-induced depression.

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Authors' contribution

ZFM conducted the experiment, analysed and interpreted data, SA involved in interpreting results and drafting manuscript, SFAM has given input in result interpretation and drafting; JK assisted in conducting the research, and SNI planned, designed, facilitated and supervised the work.

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