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MEDICINAL VALUE OF SOME EDIBLE LEAFY VEGETABLES FROM BANGLADESH.

Maksuda Khatun[1], Shariful haque[2*], Md. Abul Hassan[3], Sheikh Nazrul Islam[4]

[1,3]Department of Botany, University of Dhaka, Dhaka-1100, Bangladesh.

[2]Department of Pharmacy, Pabna University of Science and Technology, Pabna-6600, Bangladesh.

[4]Institute of Nutrition and Food Science, University of Dhaka, Dhaka-1100, Bangladesh.

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***Correspondence for Author**
Shariful Haque
Department of Pharmacy, Pabna University of Science and Technology, Pabna-6600, Bangladesh.

ABSTRACT

The present study was aimed to evaluate the antibacterial activity and the anti-hyperglycemic effect of fourteen edible leafy vegetables from Bangladesh. The anti-hyperglycemic effect of fourteen edible leafy vegetables from Bangladesh was studied on 60 healthy rabbits, submitted weekly to subcutaneous glucose tolerance tests after gastric administration of water, glipizide or methanol extract of leafy vegetables. Glucose oxidase method was used to determine the serum glucose level of normal mice. SPSS software package (version 11.5 Inc. Chicago USA.) was used to analysis the data. Among fourteen plants tested for hypoglycemic property, ten showed significant ($p<0.05$) hypoglycemic action at some point of treatment. They lowered the serum glucose level at different rate and nearly made it normal within 28 days of treatment, however, almost all of the nutraceuticals made the blood glucose normal by 7 day to 14 day treatment. Seven species showed more antidiabetic properties than positive control (Dimerol) indicating highly significant for Diabetes. These are: *Chenopodium album, Hewittia sublobata, Hygrophila auriculata, Ipomoea batatus, Malva verticillata, Piper longum* and *Portulaca quadrifida*. Also the methanol extract of fourteen edible leafy vegetables were evaluated for antimicrobial activity against six gram positive and six gram negative bacterial strains.The in-vitro antimicrobial activity was performed by agar well diffusion method on Nutrient agar media and Muller Hinton agar media. The Methanol extract of *Leucas aspera* showed moderate antimicrobial activity against only gram positive bacteria, the methanolic extract of *Enhydra fluctuans* showed mild antimicrobial activity

against only gram positive bacteria and the methanolic extrac of *Hewittia sublobata* showed very small action against only gram positive bacteria. The methanol extract of all used plants did not show any antimicrobial activity against gram negative bacteria.

KEYWORDS: Gliclazide, Antihyperglycemic effect, Antibacterial activity, *Hewittia sublobata, Enhydra fluctuans, Chenopodium album, Hewittia sublobata, Hygrophila auriculata.*

INTRODUCTION

The global prevalence of type 2 diabetes has shown a trend of rapid growth over the past few decades. More than 40 per cent of U.S. adults have diabetes or pre-diabetes.[1] Two individuals develop diabetes every 10 sec worldwide, and two individuals die of diabetes-related conditions every 10 sec worldwide.[2] Diabetes therefore has become a very serious public health problem with a heavy socio-economic burden to each country. Asia is the world's most populated area and over 56 per cent of the world's population lives in the continent.[3] Nutraceuticals are medical foods that are used in prevention and treatment of many ailments.[4, 5] Nutraceuticals have been reported to have a versatile spectrum of medicinal properties against viruses[6] to cardiovascular disorders.[8] They have strong antimicrobial action, even effective against many drug resistant strains.[9] Some nutraceuticals have, even, been claimed to be effective in prevention and/or management of HIV/AIDS.[10] Although, some nutraceuticals have long been traditionally used in the treatment of diabetes, a very few scientific reports on their hypoglycemic activity is available. This study has, therefore, investigated the hypoglycemic activity of fourteen nutraceuticals on an alloxan induced diabetic mice model. Green leafy vegetables have been used as medicine since ancient times and have been playing a very important role in our diet and nutrition. They are the most readily available sources of carbohydrates, fats, important proteins, vitamins, minerals, essential amino acids, and fibers.[11] Their bioactive substances have a wide range of biological functions, including antioxidant and antimicrobial activities[12–15] and can be helpful in management of oxidative stress and age related human ailments..[16] They are rich source of carotene, ascorbic acid, riboflavin, folic acids and minerals like calcium, iron and phosphorus.[17] Being a photosynthetic tissue, leafy vegetables have higher levels of vitamin K when compared with other fruits and vegetables due to direct involvement of vitamin K (phylloquinone) in photosynthesis process. Vegetables as medicinal plants contain none or less toxic effects,[18-19] and have the ability to synthesize several secondary metabolites of

relatively complex structures possessing antimicrobial activities.[20–22] Green leafy vegetables are also rich in compounds having anti-diabetic,[23] anti-histaminic,[24] anti-carcinogenic[25] and hypolipidemic[26] properties and possess preventive or curative properties against cardiovascular disease, ageing, obesity, hypertension, insomnia and ageing.[27–29] Leafy vegetables are natural source of antioxidants and rich in phytochemicals.[30-31] Also in recent years, multiple drug resistance in human pathogenic microorganism has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of various diseases.[32] The present work was therefore designed to investigate the antibacterial effects of abovementioned fourteen leafy vegetables.

MATERIALS AND METHODS

Plant Materials

The fresh leaves of fourteen nutraceuticals which are locally consumed as leafy vegetables were included in this study. They were collected from natural habitat and vegetable markets of different areas in Bangladesh. The plant species were brought to the laboratory in clean poly bags and were processed. The leaves were botanically authenticated and a voucher specimen has been preserved for future reference.

Table (1): List of Plants tested.

Sl. No.	Name of the Plant	Plant Family	Part(s) used	Vernacular Name
1.	*Chnopodium album L.*	Chenopodiaceae	Leaves	Bathua shak
2.	*Enhydra fluctuans Lour*	Asteraceae	Leaves	Helencha shak
3.	*Hewittia sublobata(L.f)*	Convolvulaceae	Leaves	Dhudla shak
4.	*Hygrophila schulli (Buch-Ham) M.R. & S.N.Almeida*	Acanthaceae	Leaves	Surmadani Shak
5.	*Ipomoea batatus Lamk.*	Convolvulaceae	Leaves	Mist-alu
6.	*Leucas aspera (Wild) Link*	Lamiaceae	Leaves	Swetodrone
7.	*Malva verticillata L.*	Malvaceae	Leaves	Napa shak
8.	*Moringa olifera Lamk*	Moringaceae	Leaves	Sajna pata
9.	*Piper longum Roxb*	Piperaceae	Leaves	Pipul shak
10.	*Portulaca quadrifida L*	Portulacaceae	Whole Plant	Chotto nunia shak
11.	*Premna esculenta Roxb*	Verbenaceae	Leaves	Lelom pata
12.	*Spilanthes Calva D.C*	Asteraceae	Leaves	Marfati-tiga (Marma)
13.	*Syndrella nodiflora*	Asteraceae	Leaves	Humfui (Marma, chakma)
14.	*Trigonella foenum-graecum*	Fabaceae	Leaves	Mthi Shak

Preparation of extract for anti-hyperglycemic test

The leaves were separated from the stalk and washed by distilled water followed by sun dry. Dried leaves were crushed and made into powder using mortar and pestle. About 200 gram

powdered leaves of each was subjected to cold extraction using 300-400 ml of methanol for at least 7days with occasional shaking everyday at room temperature. Then, it was filtered with a fine cloth. The methanol extract was then concentrated under reduces pressure at 40^0C and stored at -20^0C until use for testing for hypoglycemic activity on mice model.

Chemical and antidiabetic agent for anti-hyperglycemic test

Alloxan was purchased from Sigma Chemicals (St. Louis, Mo, USA). Oral hypoglycemic drug- Gliclazide BP 80m tablet of Square Pharmaceutical Ltd, Bangladesh was purchased from a drug store.

Animal experimentation for anti-hyperglycemic test

Sixty white albino mice (22-30gm) of both sexes were procured from the Animal House of International Centre for Diarrheal Disease and Research, Bangladesh (ICDDRB); Dhaka, Bangladesh. They were housed at standard environmental conditions of temperature and dark/light cycle, and were fed with commercial pellet diet and drinking water. The animal were fasted for 12 hours before the experiments, but had free access to water. Ethical guidelines in animal handling and use were adequately maintained during the study.

Experimental procedure for anti-hyperglycemic test

Diabetes was induced in 60 fasting mice with a single dose of intraperitoneal injection of alloxan (120mg/kg body weight). The diabetes was confirmed by estimating fasting blood glucose level is of the alloxan injected mice. Fasting blood glucose level~ 3.5-5.5mmol/L. Diabetes mellitus is characterized by fasting blood glucose level ≥7.0 mmol/L. The diabetes mice were divided into 12 groups each comprising 5 mice. Group I-X were used as experimental sets receiving respective plant extracts, group XI was used as positive control receiving Gliclazide and group XII was used as negative control taking only normal diet. The diabetes mice in experimental groups orally received each of the respective 10 plant extracts with 300mg extract per kg body weight daily for 28 days. The mice of group XI received Gliclazide (2.5mg/kg body weight) daily for the same period.

Estimation of serum glucose level for anti-hyperglycemic test

Fasting blood samples of normal and diabetes mice were drawn by tail bleeding at different time intervals at 0 day, 7^{th} day, 14^{th} day, 21^{st} day and 28^{th} day for estimation of serum glucose level. After taking the blood sample into eppendorf tube, it was centrifuged at 3000 rpm for

10-15minutes. Serum glucose level was estimated by Glucose oxidase method [33] using commercial kit (Human, Germany with the help of ELISA plate reader (Labsystem, Finland).

Statistical analysis for anti-hyperglycemic test

SPSS software package (version 11.5 Inc. Chicago USA.) was used to analysis the data. Descriptive statistics were calculated for all variables. Values were expressed as mean ± SD. Comparison of the serum glucose levels in the seven day interval was performed by independent sample t-test (i, e. unpaired 't' test) and p values less than 0.05 were considered significant. Repeated measures ANOVA was then used to assess for significant differences between the various time points in the subjects of both groups independently. The significance level was set at $p<0.05$.

Preparation of Plant Extract for anti-bacterial test

Fresh leaves of plants were washed with tap water followed by rinsing with deionized distilled water and immediately air-dried for overnight and then sun-dried for 7days. Dried leaves were crushed and made powder using mortar and pestle. A 200 gm powdered leaf of each species was subjected to cool extraction for 7 days using 70% methanol. It was then filtered to collect the extract. This extraction was repeated twice to collect the maximum amount of extract. The plant extracts (extracted twice) were combined together and then, filtered through a Whatman filter paper(#1) and concentrated to remove the solvent using a Rotary evaporator under vacuum at 40˚C. The concentrated extracts were kept in refrigerator until use.

Disc preparation for anti-bacterial test

The concentrated plant extract was diluted with chloroform to 500 mg/ml. The dry weight of extract was obtained by allowing the solvent to evaporate.[24] Sterile blank disc was impregnated with 15 µl of diluted chloroform extract to have approximately 7.5 mg plant extract/disc. The discs were then subjected to evaporation of solvent.

Antibacterial Activity

The freshly autoclaved agar medium was transferred into sterile petridishes under aseptic condition & allowed to solidify. The freshly grown (24 hr culture) bacteria were suspended in sterile saline to have 10^6 cells/ml is aseptic condition. The plates were the inoculated with the bacterial suspensions by using sterile cotton swabs. The sample disc impregnated with the extract and the standard antibiotic disc were placed in the agar plates inoculated with test

organism. The discs were pressed slightly on the medium. The plates were then kept in refrigerator for 2 hours so that the test materials could be diffused in the medium. The plate was then incubated at 37˚C for 24 hours. After incubation, the antibacterial activity of the materials was determined by measuring the diameter of zone of inhibition in millimeter.

RESULTS AND DISCUSSION

Anti-hyperglycemic test: Glucose oxidase method was used to determine the serum glucose level of normal mice. Baseline serum glucose level were 5.19 to 5.85 mmol/L for normal mice, and glucose level for the alloxan induced diabetes mice were mice 7.86 to 11.87mmol/L. Hypoglycemic activity of the nutraceuticals was investigated on alloxan induced diabetic mice. Gliclazide (2.5mg/kg body weight) was used as (+)ve control and normal saline as (-)ve negative control.

It was noted that 10 plants showed significant ($p<0.05$) hypoglycemic action at some point of treatment. They lowered the serum glucose level at different rate and nearly made it normal within 28 days of treatment, however, almost all of the nutraceuticals made the blood glucose normal by 7day to 14 day treatment. The finding is elaborated in the table 2.

It was found that positive control did it faster than *Piper longum* and *Hewittia sublobata.* Negative control did not show any effect (table 2, figure 1).

Table 2: Serum glucose level of normal, diabetic and nutraceutical treated mice at different time intervals

S. no	Nutraceutical	Baseline[a]	0 day[b]	7day[c]	14 day[d]	21 day[e]	28 day[f]
1	*Ipomoea batatus*[1]	6.20±0.19	11.2±0.93	9.64±1.03	8.25±0.54	7.28±1.27	7.04±0.37
2	*Trigonella foenum-graecum*[2]	5.04±1.66	10.20±0.73	9.21±1.81	8.90±0.58	7.46±0.44	6.91±0.48
3	*Enhydra fluctuans*[3]	5.68±0.54	11.87±1.39	8.50±0.37	8.25±0.69	7.71±0.62	7.06±0.59
4	*Chenopodium album*[4]	5.85±1.05	10.96±0.87	8.53±1.27	7.76±1.28	6.59±0.93	6.18±0.24
5	*Hygrophilla auriculata*[5]	5.77±1.22	11.68±1.91	9.22±2.02	6.97±0.73	6.62±0.43	6.64±0.50
6	*Malva verticillata*[6]	4.56±0.29	10.81±1.21	7.88±0.49	6.71±0.75	6.30±0.47	6.03±0.57
7	*Moringa oliefera*[7]	5.57±0.15	9.92±2.26	7.36±0.30	6.88±0.40	6.24±0.44	5.98±0.29
8	*Portulaca quadrifida*[8]	5.18±0.65	7.86±0.74	8.04±0.59	7.85±0.91	6.65±1.06	5.95±0.85
9	*Piper longum*[9]	5.39±0.82	9.61±1.23	6.29±0.22	6.05±0.13	5.35±0.21	5.06±0.38
10	*Hewittia sublobata*[10]	5.32±0.73	8.91±0.59	7.25±1.35	6.52±0.47	6.37±0.30	5.75±0.52
11	Gliclazide (+ve control)	5.51±0.34	8.22±1.09	7.67±0.39	7.35±0.60	6.68±0.45	5.78±0.48
12	Normal saline (-ve control)	5.22±0.16	7.75±0.70	7.70±0.78	7.38±0.25	7.05±0.24	7.04±0.17

*values were expressed in mean±sd Level of significance ($p<0.05$) was expressed by *student t test.*

1ab: p<0.00	1bc:p<0.03	1bd:p<0.001	1be:p<0.001	1bf:p<0.001	1cf;p<0.07	1df;p<0.046
2ab:p<0.00		2bd: p<0.014	2be: p<0.001	2bf: p<0.017	2df:p>0.046	2ef:p>0.095
3ab: p<0.00	3bc<0.002	3bd: p<0.001	3be: p<0.001	3bf: p<0.001	3cf: p<0.096	
4ab p<0.00	4bc<0.008	4bd: p<0.002	4be: p<0.001	4bf: p<0.001		
5ab p<0.00	5bc<0.095	5bd: p<0.001	5be: p<0.001	5bf: p<0.001		
6ab p<0.00	6bc<0.001	6bd: p<0.002	6be: p<0.001	5bf: p<0.001	6cf: p<0.059	
7ab p<0.00		7bd: p<0.086	7be: p<0.054	7bf: p<0.04	7cf: p<0.059	
8ab p<0.00		8bd: p<0.042	8cf: p<0.036	8df: p<0.042		
9ab p<0.00	9bc<0.001	9bd: p<0.001	9be: p<0.001	9bf: p<0.001		
10ab p<0.00	10bc<0.036	10bd: p<0.001	10be: p<0.016	10bf: p<0.001	10ce p<0.025	10 cf: p<0.036
Gliclazide		Gbe: p<0.04	Gbf: p<0.006	Gdf: p<0.034		

Fasting blood glucose level~ 3.5-5.5mmol/L Diabetes mellitus is characterized by fasting blood glucose level ≥7.0 mmol/L.

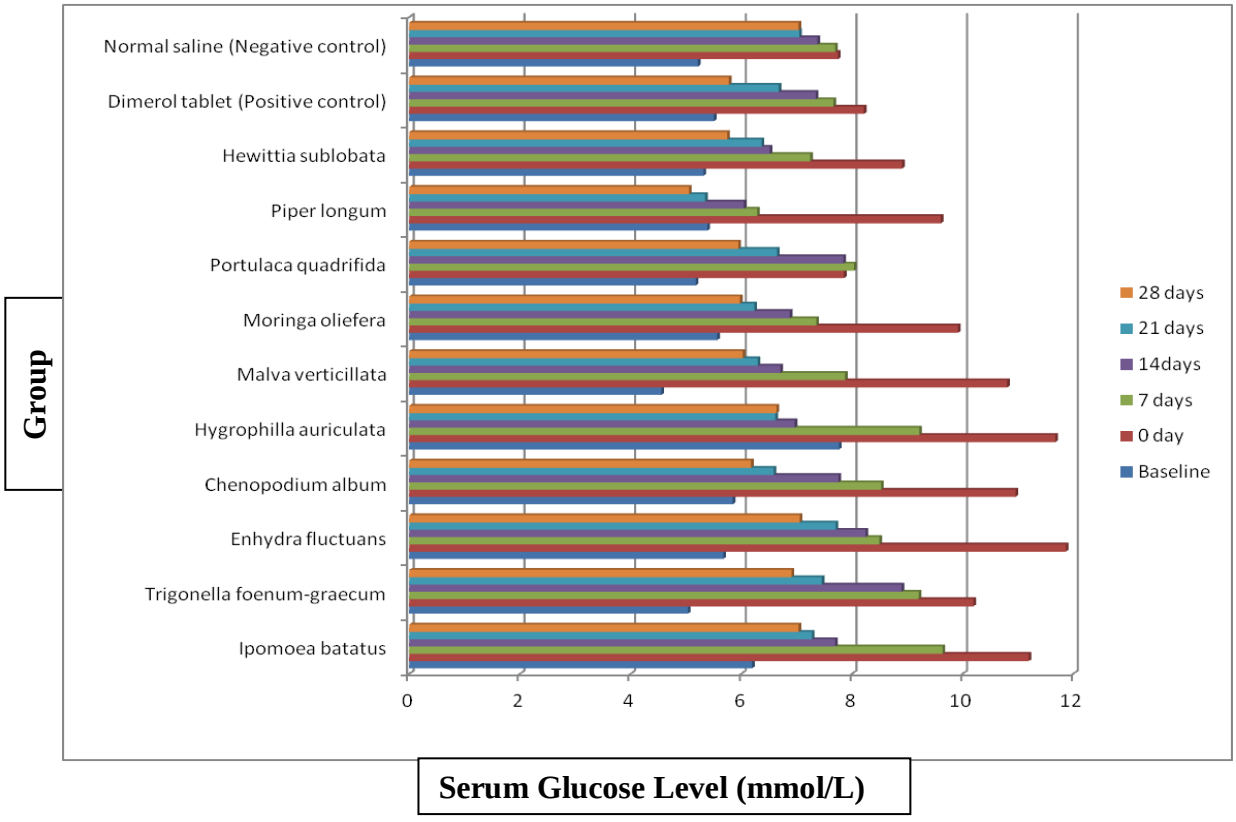

Figure 1. Comparison of different groups of antidiabetic effect based on different time.

Anti-bacterial test

Table3: Inhibitory zone in mm of methanol extract of fourteen leafy vegetables from Bangladesh.

Name of bacteria (test bacteria)	Strain No / Plant species	Inhibitory zone in mm													
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Bacillus subtilis(+)	QL 40	-	-	9	-	-	-	-	-	-	6	-	-	12	-
Bacillus megaterium(+)	QL 32	-	-	10	-	-	-	-	-	-	5	-	-	14	-
Bacillus cereus (+)	QL 29	-	-	9	-	-	-	-	-	5	5	-	-	15	-
Sarcina lutea (+)	QL 166	-	-	10	-	-	-	-	-	-	8	-	-	17	-
Staphylococcus aureus (+)	QL 102	-	-	7	6	-	-	-	-	-	-	-	-	-	-
Salmonella typhi (-)	QL 147	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Shigella flexneri (-)	Al 17316	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Shigella sonnei(-)	Al 16958	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Shigella boydii (-)	Al 7303 B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Shigella dysenteriae type-1 (-)	T1683/3551	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa (-)	BTCC13	-	-	-	-	-	-	-	-	-	-	-	-	-	-

-: No inhibitory zone

Plant species: 1= *Ipomoea batatus*, **2=** *Trigonella foenum-graecum*, **3=***Enhydra fluctuans*, **4=** Chenopodium album, **5=** *Hygrophila schulli*, **6=***Malva verticillata*, **7=** *Moringa oleifera*, **8=** *Portulaca quadrifida*, **9=** *Piper longum*, **10=** *Hewittia sublobata*, **11=** *Synedrella nodiflora*, **12=** *Premna esculenta*, **13=** *Leucas aspera*, **14=** *Spilanthes calva.*

Fourteen leafy vegetables which were traditionally used for the treatment of different infection were selected for sensitivity screening against the 12 bacteria. It was found that 5 plants exhibited antibacterial activity against 4 to 5 gram positive bacterial strains (Tab-1).

Out of 5 gram positive bacteria investigated, *Leucas aspera* showed moderate (12-17 mm in diameter zone of inhibition) antibacterial activity against the four gram positive organism except *Staphylococcus aureus. Enhydra fluctuans* showed mild activity (7-10 mm in diameter zone of inhibition) against all of gram positive bacteria tested. *Hewittia sublobata* showed very small (5-8 mm diameter zone of inhibition) antibacterial activity against all gram positive organism except *Staphylococcus aureus. Chenopodium album* gave poor activity (6 mm diameter zone of inhibition) against *Staphylococcus aureus. Piper longum showed very mild action* (5 mm diameter zone of inhibition) only against *Bacillus cereus.* The largest zone

of inhibition (17 mm in diameter) was recorded against *Sarcina Lutea* with the leaf of *Leucas aspera.*

CONCLUSION

In the present study, we attempted to evaluate the magnitude of hypoglycemic effect of fourteen nutraceuticals (leafy vegetables) on alloxen induced diabetic mice. Diabetic mice (n=5) were treated with the methanolic extract of *Ipomoea batatus, Trigonella foenum-graecum, Enhydra fluctuans, Chenopodium album, Hygrophila auriculata, Malva verticillata, Moringa oliefera, Portulaca quadrifida, Piper longum* and *Hewittia sublobata* a dose of 25 µl daily for 28 days. It reduced serum glucose level in the experimental animal significantly ($p<0.05$) at different time intervals. But rate of reduction was not same at different time intervals. Some others reports have also been documented on the hypoglycemic property of these samples. Among fouteen species, Seven species showed more antidiabetic properties than positive control (Dimerol) indicating highly significant for Diabetes. These are: *Chenopodium album, Hewittia sublobata, Hygrophila auriculata, Ipomoea batatus, Malva verticillata, Piper longum* and *Portulaca quadrifida*. The antidiabetic properties were determined in these seven species for the first time in Bangladesh. The anti-diabetic principle of *Chenopodium album, Hewittia sublobata, Hygrophila auriculata, Ipomoea batatus, Malva verticillata, Piper longum* and *Portulaca quadrifida* would be of indicative for effective use of nutraceuticals in the management of diabetes. Long term treatment with large number of animals should be conducted before taking any attempt of clinical trial. Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay.[34] The various leave extracts showed varied antimicrobial activity to the test organism which was species dependent. Gram-positive bacterial strains were more susceptible to the extracts when compared to Gram negative bacteria. Gram negative bacteria are surrounded by the cell wall which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering. The absence of this barrier in Gram positive bacteria allows the direct contact of the essential oil's hydrophobic constituents with the phospholipids bilayer of the cell membrane, causing either an increase of ion permeability and leakage of vital intracellular constituents, or impairment of the bacterial enzyme systems.[35] Also two groups of bacteria differ in their structure of cell wall. Ability of tannin to disintegrate bacterial colonies is hinder with bacterial cell wall.[36] Medicinal plants which are rich in tannins are used to treat inflamed or ulcerated tissues.[37]

Bioactive compounds are normally accumulated as secondary metabolites in all plant cells but their concentration varies according to the plant parts, seasons, climates and particular growth phases. Leaves are one of the highest sources of accumulation and are highly beneficial.[38-39]

Most of the secondary metabolite identified in the test samples like flavonoids, saponins, tannin, steroids and alkaloids are phytoprotectants and are important for cell growth, replacement, and body building.[40] Their medicinal value is due to presence of some chemical substances that can produce a defined physiological action on human body with antioxidant, antibacterial, anti-inflammatory, antiviral, immune system stimulant and detoxification activities.[41]

Green leafy vegetables contain various pharmacologically active compounds. On the whole the present investigation confirmed the traditional uses of the studied vegetables in the treatment of bacterial infections.

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