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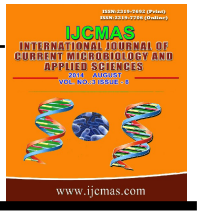

Original Research Article

Antioxidant Activity and Growth Influence of *Lactobacillus bulgaricus* by Selected Herbal Extracts

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Keywords

Lactobacillus bulgaricus, Herbal extracts, DPPH assay, Antioxidant activity, *Ocium sanctum.*

A B S T R A C T

Antioxidant capacity of selected herbal extracts and Lactobacillus bulgaricus were studied with DPPH assay. The growth pattern analysis was done for *Lactobacillus bulgaricus* individually and with herbal extracts. Based on the different reaction mechanisms such as (herbal extract and DPPH), (herbal extract, DPPH andH_2O_2), (herbal extract, DPPH, H_2O_2 and culture), (herbal extract, DPPH and culture) the inhibition percentage values for each of these reaction mechanism were calculated and the ANOVA Single Factor test is done and tabulated. The P value obtained for herbal extract + DPPH% inhibition and herbal extract, DPPH, H_2O_2 % inhibition values are significant. It was concluded that out of the five herbal extracts *Ocium sanctum* (Tulsi) is said to be significant suggesting that it has the highest antioxidant activity followed by *Mentha arvensis* (Mint), *Murraya koenigii* (Curry), *Azadirachta indica* (Neem) and *Emblica officinalis* Gaertn (Indian Gooseberry) which is the least significant. For the herbal extract, DPPH, H_2O_2, culture treatment the values obtained are *Ocium sanctum* (Tulsi) is significant suggesting that it has high antioxidant activity followed by *Murraya koenigii* (Curry), *Emblica officinalis* Gaertn (Indian Gooseberry), *Azadirachta indica* (Neem) and *Mehntha arvensis* (Mint) which is the least significant.

Introduction

Antioxidants have become synonymous with good health. Antioxidants are class of compounds thought to prevent certain types of chemical damage caused by a surplus of free radicals, charged molecules that are engender by a variety of sources including smoking, pesticides and exhaust fumes. Destroying free radicals may help heart disease and stroke, fight cancer, researchers believe.

The role of free radicals in many ailments has been well established. Various biochemical reactions in our body system generate reactive oxygen species and these are capable of damaging crucial biomolecules. If they are not completely scavenged by cellular constituents, they may lead to several diseases (Halliwell 1994). Much research into free radicals has confirmed that foods or plants rich in antioxidants play an important role in the prevention of free radical related diseases (Kris-Etherton 2002; Di Matteo 2003). A wide range of antioxidants of synthetic origin such as Butylated Hydroxy Toluene (BHT) has been reported for treatment of various free radicals related diseases (Jayaprakash, 2003; Lee 2004), but it has been proven that these compounds also show toxic effects like mutagenesis and liver damage (Grice 1986). Hence, nowadays search for sources of natural antioxidants is gaining much importance. High antioxidant potential observed in many herbs or tropical plants is obviously part of their natural defense mechanism against noxious events causing oxidant damage for e.g. microbial infections.

Although, *Ocium sanctum* (Tulsi), *Mentha arvensis* (Mint), *Azadirachta indica* (Neem), *Murraya koenigii* (Curry) and *Emblica officinalis* Gaertn is reported for different folk medicine use the present work was carried out to explore the *in vitro* antioxidant potential of this herbs on *Lactobacillus bulgaricus.*

The human body is incapable of producing its own concentration of antioxidants. For this reason, we solely depend on our diet in order to get the store of antioxidants we need to combat diseases. Antioxidants belong to a class of compounds used to retard oxidation of chemicals in foods. “Free radicals” are fatal substances, chemical compounds and toxins found within the body. Herbal antioxidants help in clearing the body from these toxins and help people to live healthier lives. As an important part of almost every function within our body, antioxidants also play a crucial role in digestive function. Helping the body run at its optimal cellular level, antioxidants can help your digestive tract avoid becoming upset and developing various diseases so it keeps working at a healthy level.

Foods high in antioxidants include specific fruits and vegetables - green leafy vegetables such as spinach and kale delivering significant amounts of the antioxidant lutein; tomatoes and tomato products carrying the antioxidant lycopene; fruits such as blueberries and other berries, kiwi, prunes, and pink grapefruit; vegetables rich in the antioxidant beta-carotene including carrots and sweet potatoes; and foods high in antioxidants such as Vitamins A, C, and E. Other delicious tasting and helpful antioxidant foods would include foods such as watermelon, spinach, apricots, pumpkin, carrots, sweet potatoes and nuts. The antioxidant activity of fruits and vegetables is mainly correlated with their contents of polyphenols, carotenoids and vitamins C and E.

The gut flora is the human flora of microorganisms that normally live in the digestive tract and can perform a number of effective functions for their hosts. There are 17 *Lactobacillus* species that are associated with the human GIT.

According to the recent studies *Lactobacillus* species are described as real (autochthonous) inhabitants. Species such as *L. brevis, L. casei, L. paracasei, L. acidophilus, L. rhamnosus, L. delbrueckii, L. plantarum, L. johnsonii* and *L. fermentum* have, so far, not have been

reported to form stable populations in the gut. Most of these species are commonly present in fermented foods, and they are very common inhabitants of oral cavity.

Bacteria which are present in the gut fulfil a host of useful functions for humans, including digestion of unutilized energy substrates stimulating cell growth, repressing the growth of harmful micro organisms, training the immune system to respond only to pathogens, and defending against some diseases.

L. plantarum is the commercial starter most frequently used in the fermentation of vegetable food products. However, only a limited number of studies have been made to study the influence of phenolic compounds on the growth and viability of *L. plantarum* strains. The role of quinate and shikimate in the metabolism of lactobacilli was studied by (Whiting and Coggins 1969). They described that *L. plantarum* reduced quinate and shikimate under anaerobic conditions in the presence of suitable hydrogen donors. The effect of hydroxycinnamic acids their quinic esters and quinic acid (a non-phenolic acid) on the growth of *L. plantarum* studied by (Salih *et al*., 2000). From compounds assayed, the results showed that bacterial growth was only affected by the hydroxycinnamic acid at concentrations used (up to 3 mM).

A lower inhibitory effect was shown from ferulic acid to p-coumaric acid and caffeic acid. Biomass production was not affected, and only the probable growth rate was affected in *L. plantarum*. (Marsilio and Lanza 1998) have been reported that *L. plantarum* growth was significantly reduced in the presence of 1 g/l p-coumaric acid and the inhibitory activity increased in the presence of NaCl. Growth was particularly low when p-coumaric acid was combined with 40 g/l NaCl and negligible in the presence of 60 g/l (Sodium chloride) NaCl. Lower concentrations (0.5 g/l) of p-coumaric acid did not show the inhibitory activity against *L. plantarum*. (Landete *et al*., 2007) analyzed the antimicrobial activity of ten wine phenolic compounds against *Lactobacillus plantarum* strains.

Materials and Methods

Preparation of plant materials and herbal extract

The *Ocimum sanctum* (Tulsi), *Mentha arvensis* (Mint), *Azadirachta indica* (Neem), *Murraya koenigii* (Curry) is collected from local area and *Emblica officinalis* Gaertn is collected from GKVK Bangalore.

The leaves are collected from the plants and washed and soaked in distilled water over night next day it is air dried and 1gram of specific leaves is taken and by using PBS medium, 10% extract is prepared by homogenizing with the help of mortar and pestle.

Preparation of PBS medium for the preparation of herbal extract

PBS medium

Nacl	8.0g
KCl	0.20g
Na_2HPO_4	1.44g
KH_2PO_4	0.24g
PH	7.4
Distilled water	1000ml

After crushing the extract is filtered with the help of cheese cloth, the filtrate is collected in clean conical flask and labeled and stored in dark. The herbal extract can be stored only for 2days**.**

Preparation of the medium for *Lactobacillus Bulgaricus*

Yeast Glucose Broth

Peptone	10.0g
Meat/Beef extract	10.og
NaCl	5.0g
D-Glucose/Dextrose	5.0g
Yeast extract	3.0g
PH	7.0
Distilled water	1000ml

For sub culturing 10ml of media is prepared and 0.1ml of culture is inoculated in 37˚c for 24-48hrs

Growth curve analysis of *Lactobacillus Bulgaricus*

The Yeast Glucose Broth is prepared, 50ml in each conical flask is added and it is labeled as medium + culture, medium + culture+ herbal extract, medium + culture + herbal extract + H_2O_2. To each respective flask 500µlculture is inoculated, 500µl herbal extract is added and 50µl H_2O is added and kept for incubation in 37 ^{o}C from zeroth minute to 35th hour each hour the turbidity is measured at 550nm the absorbance is noted for each hour .Time verses absorbance graph is plotted, the growth curve is assayed and compared.

DPPH Assay

The antioxidant activity was tested by the 2, 2-diphenly-1-picryl hydrazyl (DPPH) method.

A solution of 100µM DPPH dissolved in methanol which is freshly prepared and the absorbance was monitored until it reached 1.030-1.035. DPPH solution (1.5ml) was mixed with 0.25ml of methanol as a negative control, or with treatments that included Quercetin and ascrobate as positive controls. After 30 mins the absorbance was measured at 517nm. For the comparison between treatment and control absorbance values using the following formula,

% inhibition = (negative control A_{517} – treatment A_{517}) X 100 / negative control A_{517}

The concentration was taken as 50% and the change in its absorbance was determined by the control value. This was determined using the inhibition of the rate of DPPH reduction versus plant extract concentration.

Results and Discussion

Several methods have been used to determine antioxidant activity of plants. To date there is enough evidence that supports antimicrobial activity of antioxidants; however, research on this possible stimulatory role of antioxidants on beneficial intestinal bacteria growth is scarce. Therefore our study involved the study of DPPH antiradical scavenging activity of five different herbal extracts (*Ocium sanctum* (Tulsi), *Mentha arvensis* (Mint), *Azadirachta indica* (Neem), *Murraya koenigii* (Curry) and *Emblica officinalis* Gaertn (Indian Gooseberry).The results obtained from our analysis are considered below.

DPPH assay:

The results of the determination of antioxidant capacity of an herbal extract depend greatly on the methodology used. The model DPPH provides a method to evaluate antioxidant activity in relatively short time compared to other methods; thus, we used the DPPH assay to achieve at our results. Radical scavenging activity of plant extracts against stable DPPH$^{\cdot}$ (2, 2-diphenly-1-picryl hydrazyl, Sigma-Aldrich Chemie, Steinheim, Germany) was determined colorimetrically.

Table.1 Herbal Extract + DPPH

Time (mins)	% inhibition Neem leaves	% inhibition Tulsi leaves	% inhibition Mint leaves	% inhibition Curry leaves	% inhibition *Gooseberry* leaves
0	10.6	15.9	14.8	12.7	9.5
5	12.7	21.2	19.1	14.8	9.5
10	13.8	23.4	20.2	14.8	11.7
20	14.8	24.4	23.4	15.9	13.8
30	14.8	28.7	24.4	17.0	14.8
40	14.8	30.8	34.2	17.0	14.8

P value = 0.034721

Table.2 Herbal Extract + DPPH + H_2O_2

Time (mins)	% inhibition Neem leaves	% inhibition Tulsi leaves	% inhibition Mint leaves	% inhibition Curry	% inhibition *Gooseberry* leaves
0	1.0	14.8	5.3	8.5	8.5
5	4.2	13.8	9.5	8.5	7.4
10	8.5	14.8	3.1	10.6	10.6
20	6.3	12.7	2.1	10.6	6.3
30	17.0	13.8	6.3	8.5	6.3
40	17.0	14.8	6.3	10.6	14.8

P value = 0.054859

Table.3 Herbal Extract + DPPH + Culture

Time (mins)	% inhibition Neem leaves	% inhibition Tulsi leaves	% inhibition Mint leaves	% inhibition Curry leaves	% inhibition *Gooseberry* leaves
0	40.7	42.5	45.7	34.0	30.1
5	31.9	34.0	35.1	40.4	35.1
10	32.9	35.1	35.1	39.3	31.9
20	36.1	36.1	39.3	40.4	36.1
30	41.4	40.4	43.6	41.4	38.2
40	43,6	43.6	45.7	43.6	41.4

P value = 0.980413

Table.4 Herbal Extract + DPPH +H_2O_2 + Culture

Time (mins)	% inhibition Neem leaves	% inhibition Tulsi leaves	% inhibition Mint leaves	% inhibition Curry leaves	% inhibition *Gooseberry* leaves
0	42.5	70.2	51.0	56.3	56.3
5	37.2	59.5	41.4	59.5	58.5
10	37.2	58.5	41.4	57.4	58.5
20	40.4	57.4	42.5	57.4	56.3
30	42.6	58.5	45.7	56.3	56.3
40	52.6	60.6	48.9	56.3	56.3

P value = 0.429468

Table: 5. The growth of *Lactobacillus bulgaricus* in experimental condition

S.No	Hours	Optical density (550 nm)										
				Culture + Herbal extract (1%)					Culture + Herbal extract + H_2O_2 (0.015%)				
		Culture	Culture + H_2O_2 (0.015%)	*Ocimum sanctum* (tulsi)	*Mentha arvensis* (mint)	Azadirachta *indica* (neem)	*Murraya koenigii* (curry)	*Emblica officinalis (Gooseberry)*	*Ocimum sanctum* (tulsi)	*Mentha arvensis* (mint)	Azadirachta indica (neem)	*Murraya koenigii* (curry)	*Emblica officinalis (Gooseberry)*
1	1	0.26	0.01	0.23	0.19	0.34	0.22	0.05	0.27	0.20	0.32	0.27	0.01
2	2	0.25	0.01	0.24	0.20	0.34	0.24	0.05	0.27	0.22	0.34	0.28	0.02
3	3	0.25	0.02	0.26	0.21	0.36	0.24	0.06	0.30	0.24	0.35	0.30	0.04
4	4	0.21	0.04	0.32	0.26	0.40	0.27	0.25	0.33	0.28	0.39	0.32	0.26
5	5	0.20	0.01	0.35	0.32	0.42	0.30	0.26	0.36	0.30	0.40	0.35	0.30

6	6	0.20	0.01	0.37	0.34	0.45	0.34	0.27	0.40	0.30	0.44	0.37	0.34
7	7	0.19	0.01	0.38	0.37	0.46	0.36	0.27	0.39	0.34	0.43	0.38	0.35
8	8	0.22	0.09	0.26	0.27	0.33	0.25	0.35	0.27	0.24	0.31	0.29	0.39
9	9	0.26	0.10	0.27	0.31	0.34	0.29	0.37	0.27	0.27	0.33	0.29	0.35
10	10	0.26	0.17	0.58	0.64	0.43	0.60	0.44	0.54	0.55	0.40	0.55	0.40
11	11	0.30	0.28	0.65	0.67	0.44	0.72	0.47	0.57	0.57	0.46	0.57	0.55
12	12	0.53	0.37	0.70	0.69	0.57	0.68	0.80	0.67	0.58	0.59	0.60	0.67
13	13	0.64	0.43	0.70	0.73	0.57	0.72	0.93	0.60	0.60	0.54	0.63	0.69
14	14	0.68	0.52	0.70	0.81	0.53	0.75	0.85	0.52	0.60	0.42	0.66	0.78
15	15	0.75	0.59	0.73	0.88	0.56	0.81	0.89	0.55	0.64	0.45	0.69	0.80
16	16	0.84	0.67	0.74	0.94	0.56	0.86	0.89	0.55	0.63	0.47	0.69	0.84
17	17	0.87	0.70	0.71	0.90	0.55	0.84	0.94	0.51	0.63	0.47	0.72	0.85
18	18	0.86	0.72	0.72	0.92	0.56	0.86	1.00	0.53	0.63	0.48	0.72	0.79
19	19	0.90	0.77	0.73	0.94	0.57	0.87	1.03	0.57	0.63	0.50	0.72	0.95
20	20	0.74	0.77	0.71	0.87	0.52	0.82	1.04	0.53	0.55	0.47	0.68	0.95
21	21	0.73	0.77	0.68	0.87	0.53	0.83	1.05	0.51	0.57	0.46	0.69	1.00
22	22	0.70	0.84	0.71	0.88	0.53	0.86	1.11	0.51	0.54	0.49	0.72	1.13
23	23	0.70	0.89	0.75	0.86	0.59	0.92	1.15	0.53	0.55	0.48	0.68	1.19
24	24	0.74	0.90	0.95	1.25	.90	0.97	0.86	0.69	1.23	0.75	1.13	0.81
25	25	1.14	0.90	1.02	1.28	1.08	1.03	0.84	0.71	1.26	0.84	1.18	0.86
26	26	1.44	0.90	1.00	1.28	0.99	1.06	0.88	0.71	1.22	0.81	1.17	0.85
27	27	1.00	0.88	1.00	1.28	1.03	1.05	0.88	0.69	1.23	0.82	1.17	0.90
28	28	0.91	0.86	1.00	1.25	1.09	1.03	0.87	0.67	10.23	0.83	1.17	0.88
29	29	0.86	0.88	0.97	1.24	1.04	1.01	0.87	0.64	1.20	0.80	1.17	0.83
30	30	0.64	0.87	0.98	1.25	1.06	1.00	0.87	0.66	1.18	0.74	1.16	0.83
31	31	0.77	0.82	0.99	1.23	0.99	1.03	0.88	0.65	1.18	0.73	1.17	0.83
32	32	0.53	0.87	0.96	1.26	0.97	0.99	0.89	0.66	1.18	0.77	1.18	0.92
33	33	0.83	0.89	0.95	1.27	0.97	0.98	0.87	0.66	1.18	0.75	1.17	0.89
34	34	0.83	0.89	1.00	0.90	1.30	1.03	0.92	0.92	0.76	1.22	0.99	0.92
35	35	0.71	0.88	1.02	0.93	1.37	1.09	0.97	0.88	0.86	1.22	1.02	0.95
STANDARD DEVIATION		0.3122	0.3593	0.2710	0.3756	0.3034	0.2994	0.3342	0.1660	1.6522	0.2332	0.3303	0.3159
STANDARD ERROR		0.0527	0.0607	0.0458	0.0635	0.0512	0.0506	0.0565	0.0280	0.2792	0.0394	0.0558	0.0533

When DPPH reacts with an antioxidant compound, which can give hydrogen, it is reduced. The change in the colour from deep-violet to light-yellow were measured at 520nm using a colorimeter. Over a period of 5, 10, 20, 30 and 40mins of incubation we see a decreasing OD value and the colour change from violet to yellow using the OD values we calculated the inhibition percentage.

Based on the different reaction mechanisms such as (herbal extract and DPPH), (herbal extract, DPPH andH_2O_2), (herbal extract, DPPH, H_2O_2 and culture), (herbal extract, DPPH and culture) the inhibition percentage values for each of these reaction mechanism were calculated and the ANOVA Single Factor test is done and tabulated. P value obtained for herbal extract + DPPH% inhibition and herbal extract, DPPH, H_2O_2 % inhibition values are significant, and hence the CD analysis for the above two treatments is done.

By CD analysis of herbal extract, DPPH we arrive at a conclusion that out of the five herbal extracts we considered *Ocium sanctum* (Tulsi) is said to be significant suggesting that it has the highest antioxidant activity which is also supported by (Somashekar Shetty 2008) followed by *Mentha arvensis* (Mint), *Murraya koenigii* (Curry), *Azadirachta indica* (Neem) and *Emblica officinalis* Gaertn (Indian Gooseberry) which is the least significant.

By CD analysis of herbal extract, DPPH, H_2O_2 we arrive at a conclusion that out of the five herbal extracts we considered *Ocium sanctum* (Tulsi) is said to be significant suggesting that it has the highest antioxidant activity followed by *Murraya koenigii* (Curry), and *Azadirachta indica* (Neem) and *Emblica officinalis* Gaertn (Indian Gooseberry) which are in the same range and *Ocium sanctum* (Mint) which is least on significant.

From the non significant values obtained from the ANOVA single factor test, the DMRT test (Dunchun Multiple Range Test) is done to know the significant value of the other two treatments i.e., herbal extract, DPPH, H_2O_2 and culture and herbal extract, DPPH and culture. The DMRT analysis is done to find out the significant value from the non significant treatments values.

For the herbal extract, DPPH, H_2O_2 , culture treatment the values obtained are *Ocium sanctum* (Tulsi) is significant suggesting that it has high antioxidant activity followed by *Murraya koenigii (*Curry), *Emblica officinalis* Gaertn (Indian Gooseberry), *Azadirachta indica (*Neem) and *Mehntha arvensis* (Mint) which is the least significant.

And for the herbal extract, DPPH, culture treatment the values obtained are *Emblica officinalis* Gaertn (Indian Gooseberry) is significant suggesting that it has high antioxidant activity followed by *Azadirachta indica (*Neem), *Murraya koenigii (*Curry), *Mentha arvensis* (Mint) and *Ocium sanctum* (Tulsi) which is the least significant.

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