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ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF SPINACH, ONION AND RADISH LEAVES

Neeraj Mishra*, Rani Rai and Chirag E Hayat

Department of Biotechnology, Saroj Institute of Technology and Management, Arjunganj, Lucknow, Uttar Pradesh, India

Abstract:

The aim of study is to assess the antimicrobial activity and antioxidant activity of aqueous and alcohol extracts that were prepared from green leaves of Spinacia oleraecea, Allium cepa, Raphanus sativus. The antimicrobial activities of the extract were tested bacteria against E.coli, P.aurignosa, S.aureus, and B.subtilis by the use of agar well diffusion method. Antioxidant activities were tested by Reducing power assay method, Superoxide anion scavenging activity, Total antioxidant capacity.

Key words: *Antimicrobial activity, Antioxidant activity, leaves of Spinacia oleraecea, Allium cepa, Raphanus sativus.*

Corresponding author:

Neeraj Mishra,

Department of Biotechnology,
Saroj Institute of Technology and
Management, Arjunganj, Lucknow,
Uttar Pradesh, India
E- mail:segneeraj@gmail.com

QR code

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INTRODUCTION:

In traditional societies nutrition and healthcare are strongly inter-connected and many plants have been consumed both as food and for medicinal purpose [1]. Free radicals are reactive compounds, they are chemical species associated with and odd or unpaired electron and can be formed when oxygen interacts with certain molecules they are neutral, short lived, unstable, highly reactive to pair with the odd electron and finally achieve stable configuration. Once formed these highly reactive radicals can start a chain reaction they are capable of attacking the healthy cells of the body causing them to lose their structure and function. Cells may function poorly or die if this occurs [2].

Nearly 1000 species of plants with edible leaves are known. Leafy vegetables most often come from short lived herbaceous plants such as lettuce and spinach. Woody plants whose leaves can also be eaten as leafy vrgetable viz. Amarthaus tri color L, Centella asiatica L, hibiscus sabdariffa L, Moringa oleifera Lam, Sesbania grandiflora (L) Poir, and solanum trilobatum L. Where investigated for the antioxidant and antimicrobial activities. The presence of carotenes, phenolic compounds such as flavanoids, coumarins, alkaloids, etc where also investigated [3].

Plants are wealthy sources of antimicribial agent as they contain a different variety of phytoconstituents. The past few decades have seen increasing scientific interest in the both growth of plat tissue culture and commercial developement of this technology as means of producing that valuable phytochemical [4]. These types of natural drugs are always a better substitute of sythetic drugs. Thus numerous drugs have entered the international pharmacopoeia through enthnobotany and traditional medicine [5].

Spinacea oleracea Linn (family chenopodiaceae), is commonly known as “spinach”. It is an erect herb with about 30 to 60 cm height. It is native to south-west asia and cultivated throghout world as vegetables. Several parts of these plants are used in traditional indian medicine for numerous therapeutic effects [6].

Dental caries and periodontal diseases are considered to be the most common chronic diseases all over the world. Dental caries is defined as an infectious disease with microbial origin that results in destruction of the classified tissues of the teat. One group of the bacteria attributed to dental caries in streptococcus mutans. All eight S. Mutans serotypes are both acidogenic and aciduric and are strongly stimulated by sucrose [7]. Pioneering animal model studies, as well as human epidemiologic researches, have strongly implicated streptococcus mutans as the main etiological agents in human dental caries [8]. Streptococcus sanguinis is recognized not only for its assosiation with bacterial endocarditis but also because of its known antagonist role in dental caries. This microorganism competes with streptococcus mutans for colonisation sites on tooth surfaces [9].

Infectious diseases are serious problem worldwide [10]. Although pharmaceutical industries have produce number of new antibiotics in last few decades, resistance of these drugs by microorganism as increased. In general microorganisms have the genetic ability to transmit and acquire resistance to drugs, which are utilised as therapeutic agents [11]. Historically, plants have provided a good source of anti infective agents; emetine, quinine, bervine etc. Remain highly effective instruments to combate against microbial infections [12]. Plants have a great potential for producing new drugs of great benefit to mankind. There are many approaches to the search for new biologically active principles in higher plants [13].

Scientific experiments on the antimicrobial properties of the plants compounds were first documented in the late 19th century [14]. Plants are which in awide variety of secondary metabolites such as tannins, terpenoides, alkaloides and flavonoid which have been found in-vitro to have antimicrobial properties [15]. Extracts of many plants are known to exhibit antimicrobial activity. Different plant extract have been evaluated for their antimicrobial properties by Mahmoud (1993), Digrak et al (1999), Bowers and Locke (2000) [16-18].

Raphanus sativus L. Belongd to family Brassicaceae and its common name are Radish, Japanese radish, Leafy Daikon, Daikon and Fodder Radish. It flowers from June to august, and the seeds ripen from July to septembert. Radishes have long been grown as a food crop, but thy also have various medicinal action. The root stimulate the appetite and digestion, having a tonic and laxative effect upon the intestine and indirectly stimulating the flow of bile.consuming Radish generally results in improved digestion in, but some people are sensitive to its acridity and robust action [19].

MATERIALS AND METHODS:

Antimicrobial Activity

Materials and Methods for antimicrobial activity

Vegetable leaves sample and extract preparation

Onion leaves (Allium cepa), Radish leaves (Raphanus sativus), Spinach leaves (Spinacia oleraecea), were brought from the local market of Lucknow. Aqueous and alcoholic extracts were prepared by Clarkson and Bibby, 1969[20].

Aqueous extract

The vegetable leaves are cut and crushed till it attains

a roughage state; 0.5gm of ground/crushed fresh vegetable leaves is mixed with 100ml of distilled water in a soxhlet. Extraction apparatus for 4 hrs at 100°C. Water extracts is prepared after running the extracts in soxhlet.

Alcoholic extract

The vegetable leaves are cut and crushed, and 5g of ground/crushed fresh vegetable leaves is mixed with 100ml of absolute alcohol. The mixture was agitated at room temperature for 8 hrs at room wrist action shaker. The mixture is allowed to stand for 12 hrs and alcohol is evaporated without heat. The residue is then mixed with 100ml of distilled water at 80°C. Alcohol extract is prepared.

Test microorganisms

The following microorganisms: E.coli, P.aeruginosa, S.aureus, B.subtilis were used for evaluating antimicrobial activity.

The bacterial stock cultures were incubated for 24 hrs at 37°C on nutrient agar.

Material and Methods

Antioxidant Activity

Distilled water, 2M phosphate buffer 1%, Potassium ferricynide, Trichloro acetic acid, Pyrogallol, 6M sulphuric acid, 28mM sodium phosphate, 4mM ammonium molybdate, HCl, 0.1% $FeCl_3$, Eppendorf tube, micropipette, Pipette tips and test tubes.

Determination of antimicrobial activity

Antimicrobial activity of the extracts was determined by Agar well diffusion method by Arora and Kaur, 1999 [21].

Determination of antioxidant activity

Reducing power assay

The reducing power assay of the extract was determined as described by Oyaizu 1986[22]. The suspension of prepared extract in 1ml of distilled water was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes subsiquently, 2.5 ml of trichloroacetic acid was added and the mixture was then centrifused at 3000 rpm for 10 minutes. A 2.5 ml aliquot of the upper layer was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1 % $FeCl_3$, and the absorbance of the mixture was taken at 700nm. A higher absorbance indicates a higher reducing power. In the assay, the color of test solution changed to various shapes of green and blue, depending on the reducing power of each extract.

Super Oxide Ion Scavenging Activity

Method of Markulund et al (1974) [23] modified by Ekanayake et al (2004) was used in this test. The method is based on inhibition of the autoxidation of pyrogallol by phenolic compounds. To the assay mixture composed of a phasphat buffer solution (2.6 ml, 50mM on water, pH 8.22+0.03) with the analytical prepared extract (0.3 ml) was added a freshly prepared solution of pyrogallol (0.1ml of a 3mM solution of pyrogallol in 0.010M HCl(37.5%)). The autoxidation reaction rate of pyrogallol was determined at 400nm by monitoring the absorbance every 30 seconds for total periods of 10 min. corresponding to the end of the reaction.

The scavenging activity of superoxide anion (O_2) was calculated by the following formula (Sun et al. 2001):

$$S= (K_0-K_1)/K_0*100$$

Where,

K^0 and k^1 are autoxidation rates of the pyrogallol without and with the extract respectively.

Total Antioxidant Activity

Total antioxidant activity was determined by (Shirwaikar et al., 2006)[24].

RESULT AND DISCUSSION:

1) Antimicrobial activity

Aqueous extract of Radish leaves shows higher inhibition zone (table 2) as compared to Onion and Spinach leaves whereas alcoholic extract of onion and radish leaves show higher inhibition zone (table 1).

Table 1: alcoholic extract of vegetable leaves

S.No.	Leaves of vegetables	E. coli (Zone of inhibition in mm)	P.aurignosa (Zone of inhibition in mm)	B.subtilis (Zone of inhibition in mm)	S.aureus (Zone of inhibition in mm)
1	Onion	11mm	14mm	12mm	14mm
2	Radish	14mm	13mm	14mm	13mm
3	Spinach	11mm	13mm	12mm	14mm

Table 2: aqueous extract of vegetables leaves

S.No.	Leaves of vegetables	E. coli (Zone of inhibition in mm)	P.aurignosa (Zone of inhibition in mm)	B.subtilis (Zone of inhibition in mm)	S.aureus (Zone of inhibition in mm)
1	Onion	8mm	10mm	10mm	10mm
2	Radish	12mm	11mm	11mm	14mm
3	Spinach	8mm	11mm	10mm	11mm

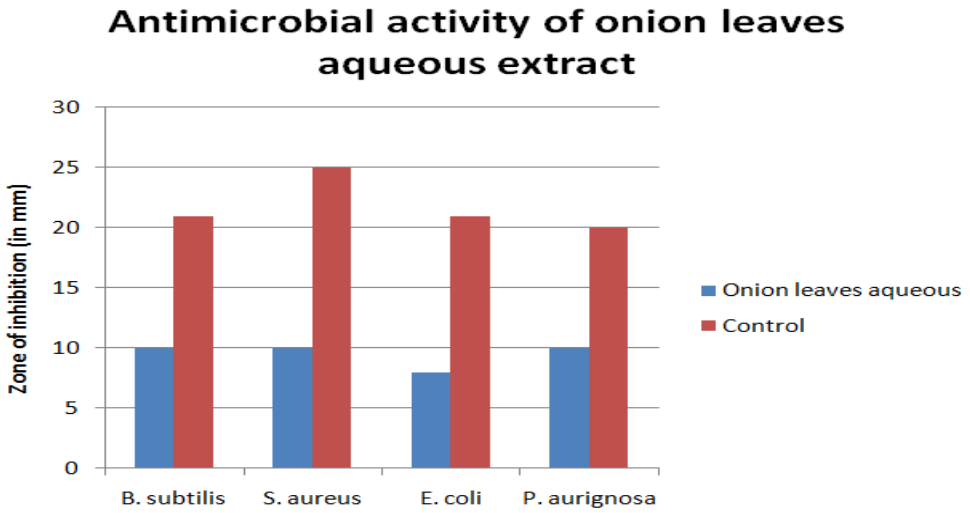

Fig.1: Antimicrobial activity of onion leaves aqueous extract

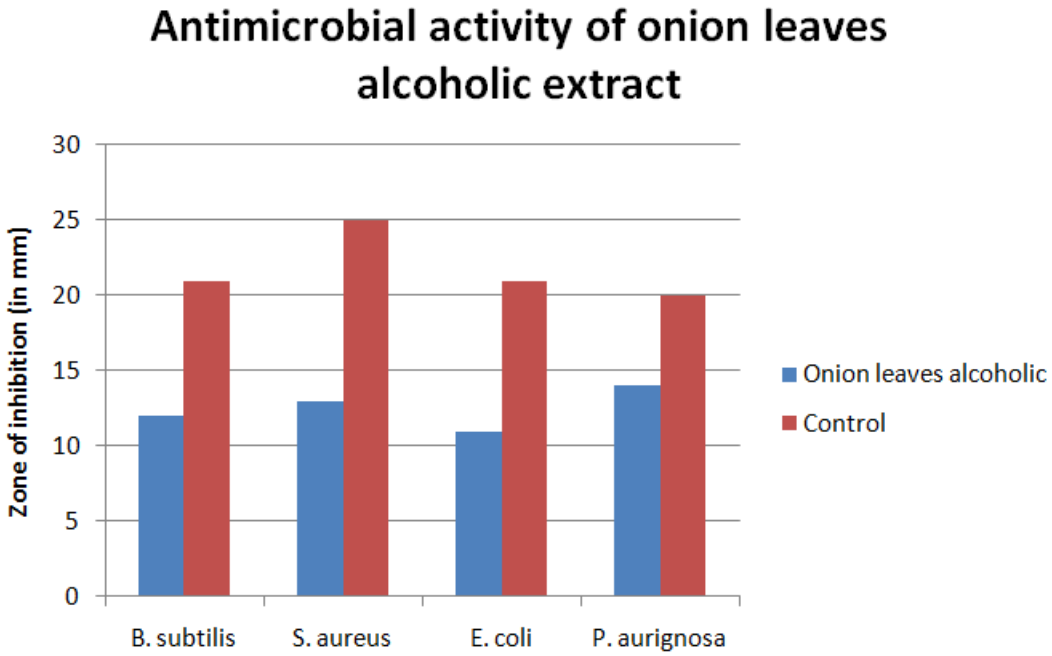

Fig.2: Antimicrobial activity of onion leaves alcoholic extract

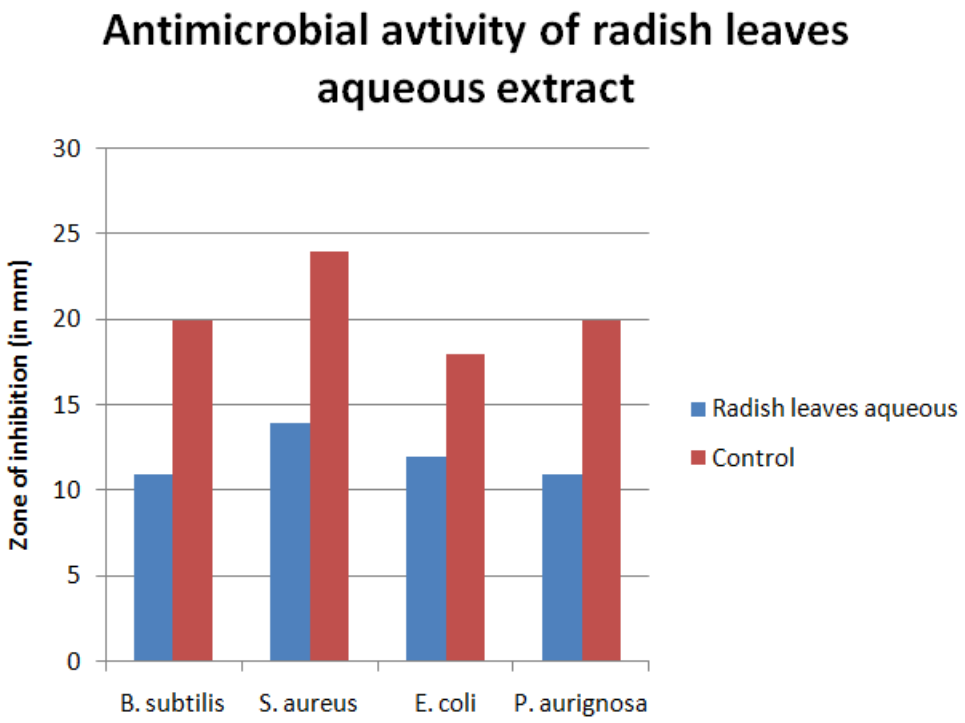

Fig.3: Antimicrobial activity of radish leaves aqueous extract

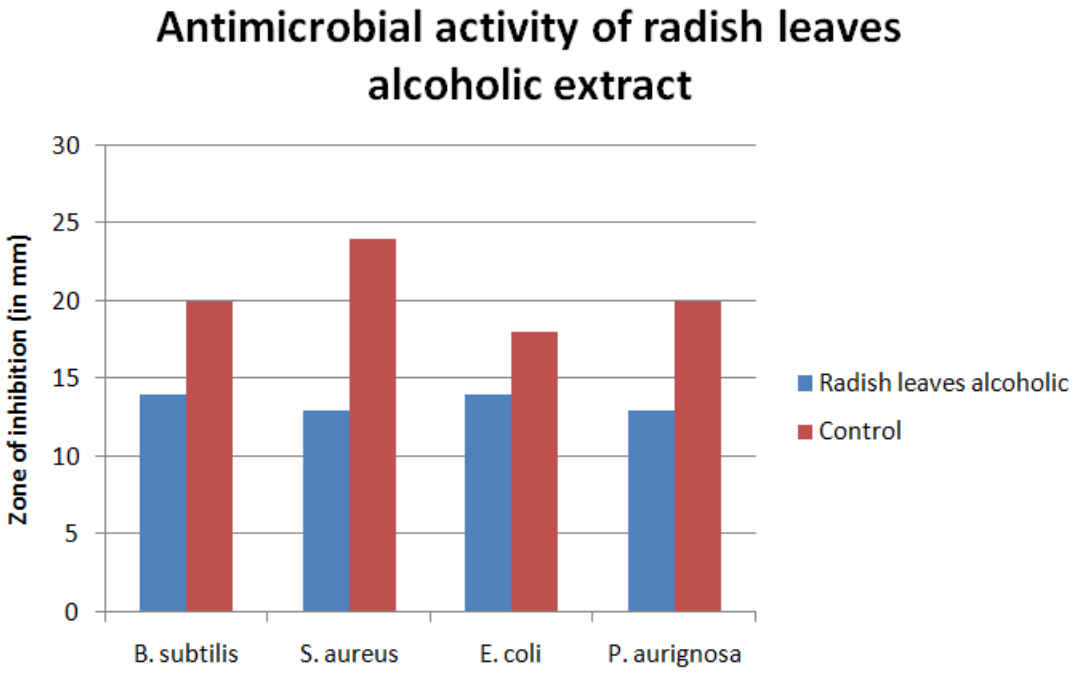

Fig.4: Antimicrobial activity of radish leaves alcoholic extract

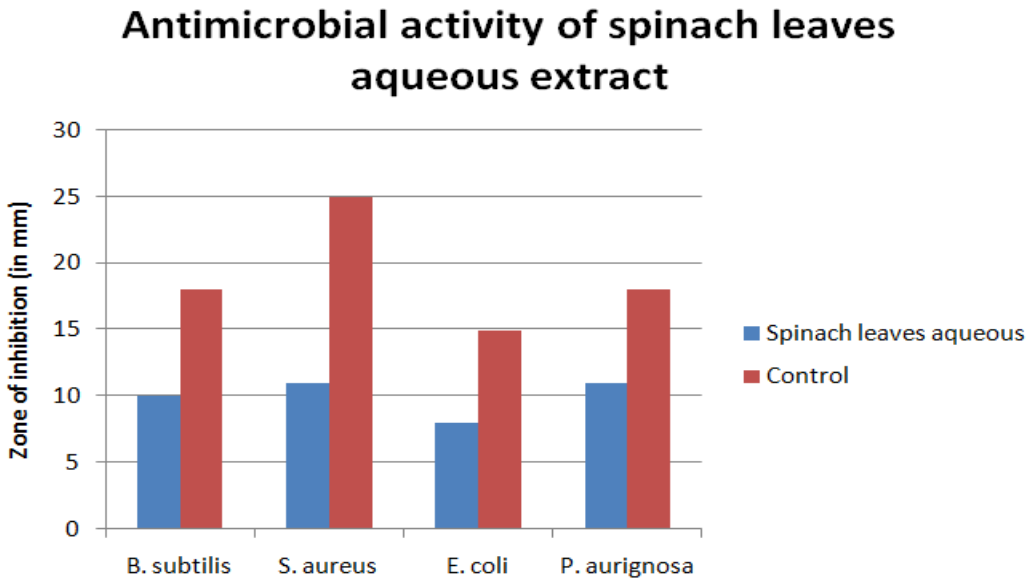

Fig.5: Antimicrobial activity of spinach leaves aqueous extract

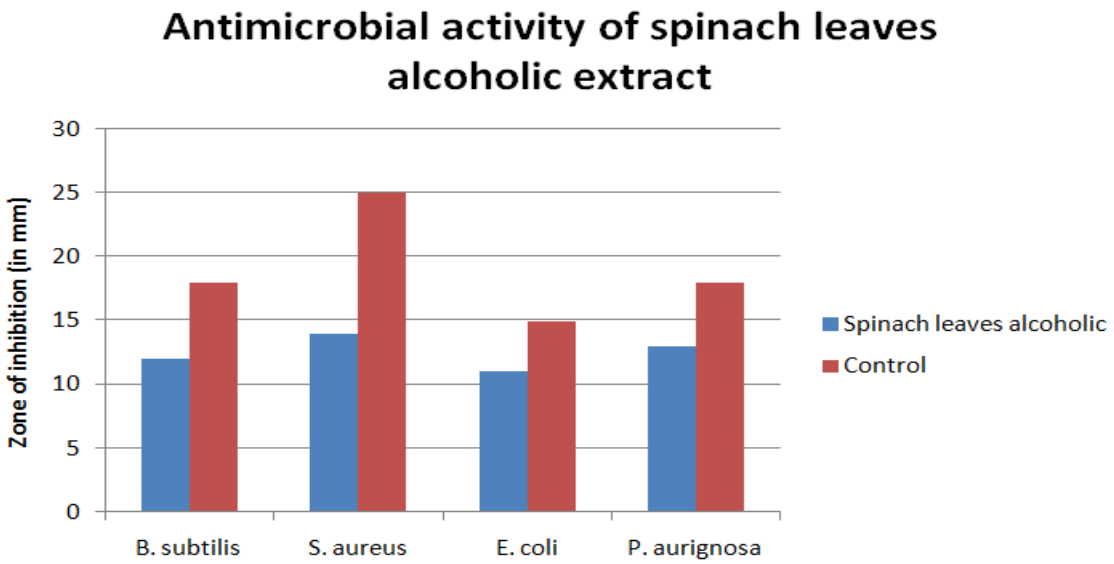

Fig.6: Antimicrobial activity of spinach leaves alcoholic extract

Alcoholic extract of Onion and Radish leaves show maximum zone of inhibition against 14 against E.coli and B.subtilis, where as aqueous extract of Radish leaves shows maximum zone of inhibition14mm.

Result and discussion of Antioxidant Activity

Reducing power assay

In accordance to Lobo et al., 2010 in the reducing power assay, the presence of antioxidant in the sample would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's Prussian blue at 700nm.. The aqueous extract of spinach leaves show maximum reducing activity of 2.7. Whereas the aqueous extract of Radish leaves shows minimum reducing activity of 0.22.

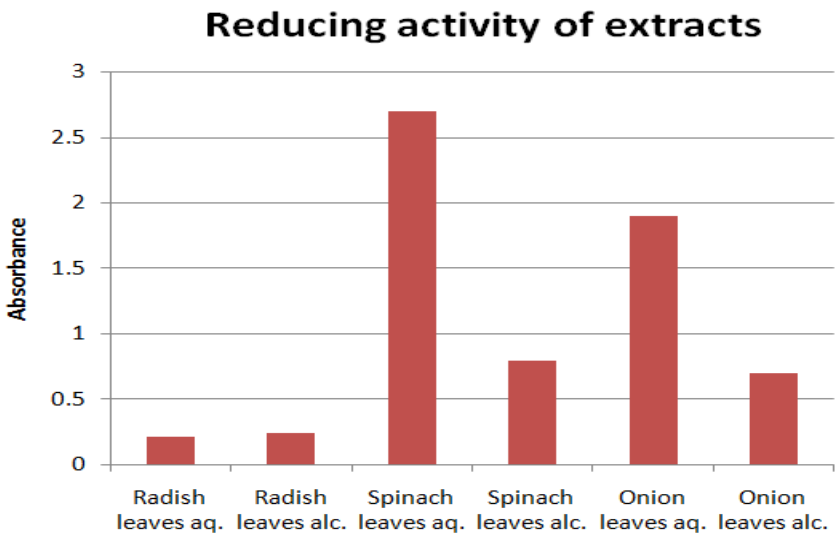

Fig.7: Reducing activity of extracts

Table: 3: Reducing activity of Vegetable leaves

Sample	Reducing activity
Radish leaves aq.	0.22
Radish leaves alc.	0.25
Spinach leaves aq.	2.7
Spinach leaves alc.	0.8
Onion leaves aq.	1.9
Onion leaves alc.	0.7

Super anion scavenging activity

Superoxide radical a biologically quite toxic oxygen molecules with one paired electron and deployed by the immune system to kill the invalding microorganism but also deleterious to cellular macromolecules on the other hands. Although superoxide anion was a weak oxidant, it gives rise to generation of powerfull and dangerous and hydroxyl radicals as well as singlet oxygen, both of which contribute to the oxidative stress and needs to genesis of several chronic diseases in human being. In superoxide scavenging method Di oxygen from superoxide anion O_2 by a single electron transfer during the pyrogallol autoxidation in basic solution. The superoxide anions are scavenge by antioxidant and consequently, decrease the rate of pyrogallol autoxidation or even inhibit it. The result showed that aq and alc extract of spinach, radish and onion leaves. Alcoholic and aqueous extract of Radish leaves shows maximum scavenging activity of extract concentration of 0.2% and 0.4% i.e 2.081 and 2.041 respectively.

Table 4: Superoxide anion scavenging activity of vegetable leaves

SAMPLE	EXTRACT CONCENTRATION(.2)%	EXTRACT CONCENTRATION (.4%)
Radish leaves aq.	**2.081**	**2.041**
Radish leaves alc.	**0.82**	**0.76**
Spinach leaves aq.	**0.67**	**0.34**
Spinach leaves alc.	**0.78**	**0.56**
Onion leaves aq.	**0.34**	**0.341**
Onion leaves alc.	**1.34**	**1.23**

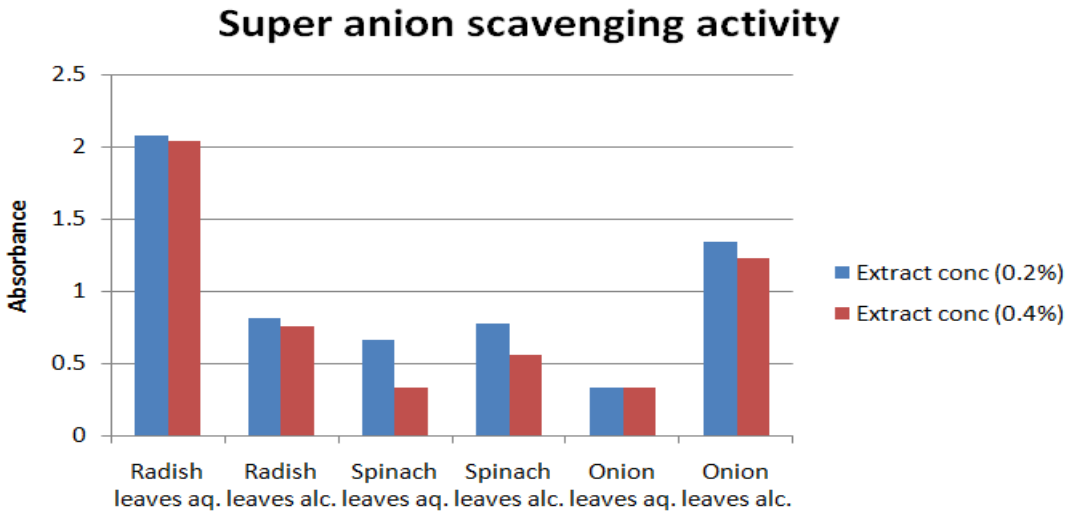

Fig.8: Super anion scavenging activity

Total antioxidant activity–

Total antioxidant capacity is show in Table.5 The extracts demonstrated electron donating capacity and thus they may act as radical chain terminators, transformating reactive free radical species. Alcoholic extract of radish leaves shows maximum total antioxidant activity i.e 2.47

Table.5 Total antioxidant activity of vegetable leaves

NAME OF EXTRACT	TOTAL ANTIOXIDANT CAPACITY
Radish leaves alc.	**2.47±1.69**
Radish leaves aq.	**2.56±1.69**
Spinach leaves alc.	**1.63±1.69**
Spinach leaves aq.	**1.54±1.69**
Onion leaves alc.	**1.42±1.69**
Onion leaves aq.	**1.65±1.69**

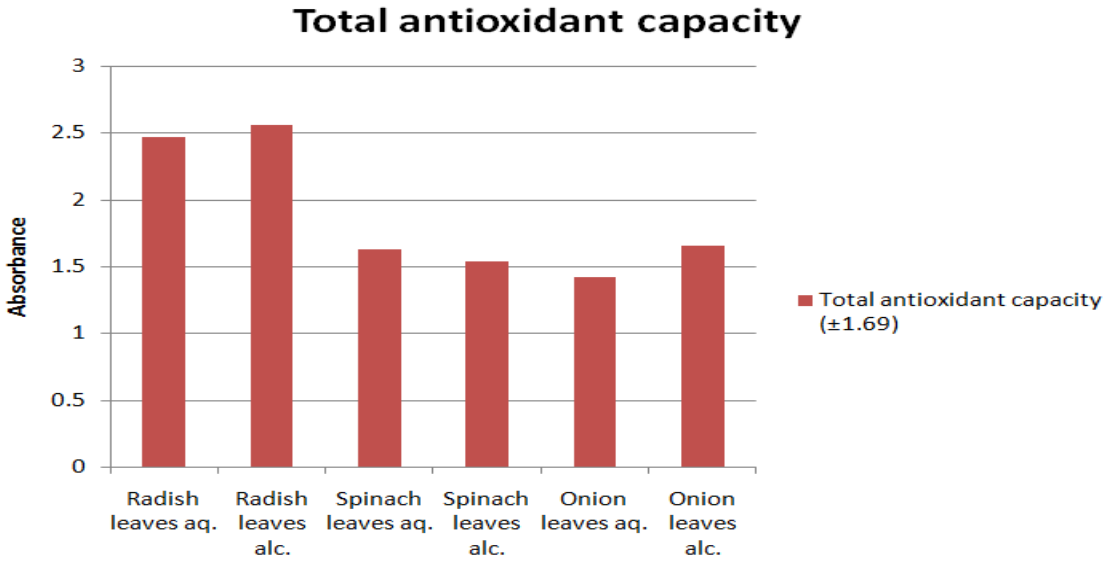

Fig.9: Total antioxidant capacity

CONCLUSION:

It is known that green leafy vegetables are important source of minerals, organic acid, dietary fibre that have wide range of action which includes antimicrobial, antiviral, antimicrobial, cardio protective and ant mutagenic activity. The study focuses on the possibility of using vegetable leaves as a source of natural antimicrobial and antioxidant activity.

The hazardous effect of synthetic antioxidant and the emergence of antibiotic resistant strains have revived the search for antioxidant and antimicrobial agents from natural sources. Different studies conducted on vegetables leaves, it had been found vegetables leaves holds a tremendous potential to serve as a source of effective, safer and better antimicrobial and antioxidant agent.

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