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Screening of *In-Vitro* Antioxidant Activity of Poly Herbal Methanolic Extract

Ganta Radhika Reddy*, Dr. P Mani Chandrika, K. Shailaja, K. Priyanka, D. Ratna Kumari, B. Sandhya, T. Sarika, P. Shalini

Bojjam Narasimhulu Pharmacy College for Women, Vinay Nagar, Saidabad, Hyderabad, Telangana, India.

A B S T R A C T

The present study was carried out to evaluate antioxidant activity of poly herbal methanolic extract of peels of *Beta vulgaris (*beetroot*), Luffinae aegyptiaca (*ridge gourd*), Cucumis sativus (*cucumber), and flower buds of *Syzygium aromaticum (*clove*).* The plant parts i.e the fruits were collected from the local market and the peels were removed, they were shade dried and grinded to get the coarse powdered material. The coarse powder was subjected for extraction in soxhlet extraction apparatus using methanol as solvent and concentrated using Rota vapour. The product was subjected to lyophillization process to get fine powder. The qualitative screening of the poly herbal extract revealed the presence of alkaloids, glycosides, saponins, phenolics and flavonoids. The collected fine powder was used to evaluate the *in-vitro* antioxidant activity by DPPH free radical scavenging method and Hydrogen peroxide radical scavenging (H_2O_2) assay. IC_{50} Value of standard ascorbic acid was found to be 190 and poly herbal extract was found to be 197 by DPPH free radical scavenging method. IC_{50} Value of standard ascorbic acid was found to be 190 and poly herbal extract was found to be 193 by Hydrogen peroxide radical scavenging (H_2O_2) assay. The IC_{50} value of the poly herbal extract was found to be near to the standard ascorbic acid value.

Keywords: Antioxidant activity, Ascorbic acid, .*Beta vulgaris*, *Cucumis sativus*, *Luffinae aegyptia*, *Syzygium aromaticum*,

A R T I C L E I N F O

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***Corresponding Author**
Ganta Radhika Reddy
Bojjam Narasimhulu Pharmacy College for Women, Vinay Nagar, Saidabad, Hyderabad, Telangana, India.
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1. Introduction

Numerous scientific investigations point at consecutive rich sources of antimicrobials, especially among fruits and vegetables, but only few of them involve waste parts of fruits, i.e. seeds and peels [1]. Many of the fruits and vegetables skins are thrown in the garbage or fed to livestock. Fruits and vegetables wastes and by-products, which are formed in great amounts during industrial processing, represent a serious problem, as they exert an influence on environment and need to be managed and/or utilized. On the other hand, they are very rich in bioactive components, which are considered to have a beneficial effect on health. Since last decade, efforts have been made to improve methods and ways of reusing fruits and vegetables wastes. The important purpose is the vaporization of the bio components in by-products from fruit and vegetable industries.

Plant waste is prone to microbial spoilage; therefore drying is necessary before further exploitation. Till now, agro industrial waste often is utilized as feed or fertilizer. But using this agro waste therapeutically is a new idea which is slowly gaining popularity [2]. They are high value products and their recovery will be economically attractive. These are novel, natural, eco friendly and economic sources of antimicrobials, which can be used in the prevention of diseases caused by pathogenic microbes and also reduce pollution. In the, present investigation, reported the antioxidant property and antimicrobial property of peels of different fruits and vegetables, that are commonly available and readily consumed in India, and to indicate which of them can become a new source of natural antimicrobials and natural antioxidants for pharmaceutical industries [1].

2. Materials and Methods

Plant material:

*Beta vulgaris (*beetroot*), Luffinae aegyptiaca (*ridge gourd*), Cucumis sativus (*cucumber), and flower buds of *Syzygium aromaticum (*clove*)* were collected from the local market. The peels were identified and authenticated by Dr. Venkata Ramana, Botanist, Osmania University, Hyderabad.

Preparation of extract: [3]

The peels were removed they kept for drying under sunlight. After complete drying the peels were subjected to grinding so as to get the coarse powdered material. The coarse powder was subjected to extraction in soxhlet extraction apparatus using methanol as solvent. The powdered material was taken around 250 gms, so that each raw material should contain 50 gms approximately. The extraction process was carried out up to completion of 8×3 cycles using 500 ml of methanol. After the complete extraction the extract was collected and subjected for evaporation by using Rota evaporator. The product was subjected to lyophillization process to get fine powder. The collected fine powder was used to test the antioxidant activity.

Phytochemical screening: [4]

Preliminary Phytochemical screening of the extract was subjected for the presence of Phytosterols, glycosides, Carbohydrates, Flavonoids, Alkaloids, Tannins, Proteins, Saponins Terpenoids ,Steroids ,Phenols ,amino acids and proteins.

Antioxidant activity: [5,6,7]

Biological combustion involved in various processes produces harmful products or intermediates called reactive oxygen species or free radicals. Excess of free radicals in living beings has been known to cause various problems like asthma, cancer, cardiovascular diseases, liver diseases, muscular degeneration, and other inflammatory processes, resulting in the so-called oxidative stress[5]. Oxidative stress is defined as imbalance between oxidants and antioxidants and causes damage in all types of bio molecules like protein, nucleic acid, DNA, and RNA. Hence, the balance between reactive species or free radicals and antioxidants is believed to be a critical concept for maintaining a good biological system[6]. Antioxidants act as free radical scavengers, reducing agents, quenchers of singlet oxygen molecule, and activators for antioxidative enzyme to suppress the damage induced by free radicals in biological system [7].

Drugs and Chemicals:

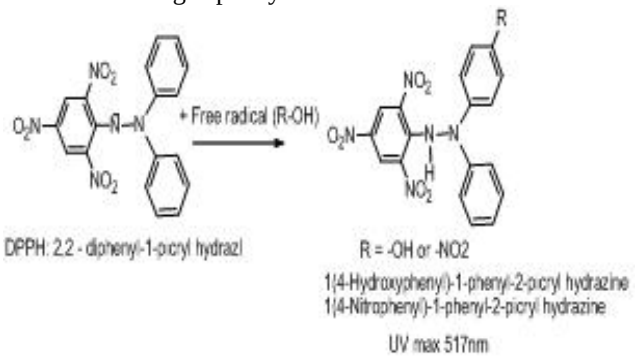
DPPH, H_2O_2, Methanol, Alcohol is used from Qualigens Fine Chemicals Pvt., Ltd and analytical reagents are obtained from KP labs (Kothapet,Hyd,Telangana,India)

Methodology:

Antioxidant activity of poly herbal methanol extract of peels of *Beta vulgaris (*beetroot*), Luffinae aegyptiaca (*ridge gourd*), Cucumis sativus (*cucumber), and flower buds of *Syzygium aromaticum (*clove*)* is evaluated by DPPH free radical scavenging method and Hydrogen peroxide radical scavenging (H_2O_2) assay method

DPPH radical scavenging method: [8,9,10,11]

Principle: DPPH is a nitrogen-centered free radical, stable at room temperature and produces a purple solution in methanol. In its radical form, DPPH shows an absorbance maximum at 517 nm which disappears upon reduction by an antioxidant. This is visualized as a discoloration from purple to yellow in a spectrophotometer. The fast electron-transfer from the phenoxide anions of the phenolic compounds to the DPPH radicals is a possible mechanism for their reducing capacity.



Procedure

Preparation of DPPH solution

4 mg of DPPH was taken and dissolved in 10ml of methanol. The solution was kept in dark place for 30 minutes.

Preparation of extract solution

4 mg of extract were taken and dissolved in 40 ml of methanol. The concentration of the solution is 100µg/ml.

Preparation of standard solution

Ascorbic acid is taken as standard. 4mg of ascorbic acid is dissolved in 40ml of methanol and kept the concentration at solution is 100 µg/ml.

Procedure

Take 1ml of extract or standard solution from the stock in different test tubes and 4ml of methanol was added to make 5 ml solution. The concentration of the solution is 20µg/ml. Then 2 ml of stock solution was added to other test tubes and 3 ml of methanol was added to the test tubes. The concentration of the solution is 40µg/ml. then 3ml, 4ml, 5ml of stock solution was mixed with 2ml, 1ml and 0ml of methanol to make concentration of 60, 80, 100 100µg/ml. 5 ml of methanol was taken in a test tube as blank. Then 100µl of DPPH solution was added to each test tube. The test tubes were kept in dark place for 20 minute. After that, the absorbance was taken at 517 nm [9].

Calculation of % inhibition

The radical scavenging activity was expressed as the percentage inhibition which was calculated by using the following formula:

$$\text{Percentage Inhibition} = \frac{(\text{Blank Absorbance} - \text{Sample Absorbance})}{\text{Blank Absorbance}} \times 100$$

Table 1: Apparatus and Reagents used in DPPH Test

Methanol	**Beaker**
Distilled Water	Pipette both 10 & 2 ml
UV–Vis Spectrophotometer	Pumper
Test Tubes	Funnel
Micropipette	Spatula
Screw Cap Test Tubes	

Hydrogen peroxide radical scavenging (H2O2) assay

Procedure:

Plant extract prepared in various concentrations (20-100µg/ml) was mixed with 0.6 ml of 4 mM H2O2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm against blank solution containing the H2O2 without plant extract [10]. All the analyses were performed in triplicate and results were averaged and ascorbic acid used as a positive control treated in the same way with H_2O_2 solution. The percentage inhibition was measured by comparing the absorbance of control and test [11, 12].

$$H_2O_2 \text{ scavenging activity} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where,

$A_{control}$ = Absorbance of control reaction and

A_{test} = Absorbance in the presence of the samples of extract

Statistical Analysis

The entire assay was done in triplicate for all the treated polyhedral methanolic extract. The data obtained is subjected to statistical analysis.

3. Results and Discussion

The percentage yield of the polyherbal extract was found to be 17.5% and the color was identified as reddish brown with semisolid nature. Preliminary Phytochemical screening of the polyherbal extract has shown following results

Table 1: Results for phytochemical preliminary tests.

Constituents	**Poly herbal extract**
Alkaloids	+
Saponins	++
Terpenoids	+
Steroids	--
Glycosides	++
Tannins	--
Phenols	++
Flavonoids	+++
Amino acids and proteins	+
Carbohydrates	+

- Not present, + present, ++ and +++ significantly present

Based on preliminary phytochemical screening, the polyherbal extract was found to be rich in phenols, saponins, flavonoids, and glycosides. The qualitative analysis also revealed the presence of alkaloids, terpenoids, aminoacids and proteins, carbohydrates

DPPH free Radical Scavenging Assay: [14]

DPPH is a nitrogen-centred free radical, stable at room temperature and produces a purple solution in methanol. In its radical form, DPPH shows an absorbance maximum at 517 nm which disappears upon reduction by an antioxidant. This is visualized as a discoloration from purple to yellow in a spectrophotometer. The fast electron-transfer from the peroxide anions of the phenolic compounds to the DPPH radicals is a possible mechanism for their reducing capacity [14].

$$\text{Percentage Inhibition} = \frac{(\text{Blank Absorbance} - \text{Sample Absorbance})}{\text{Blank Absorbance}} \times 100$$

Table 2: Absorbance and percentage inhibition by DPPH for test sample

Concentration (µg/ml)	**Absorbance (nm)**	**Percentage inhibition**	**X value (IC50)**
20	0.107	71.314±0.02	193
40	0.14	62.466±0.02	
60	0.168	54.959±0.01	
80	0.179	52.011±0.02	
100	0.199	46.649±0.01	

The test sample was evaluated for antioxidant activity. The percentage inhibition by DPPH assay was measured using U.V Spectrophotometer and depicted in Fig.10 and table.6 The concentrations of test sample were 20, 40, 60, 80, 100 µg/ml with their percentage inhibitions were 71.314, 62.466, 54.959, 52.011, and 46.649 respectively. The IC_{50} Value of test sample was found to be 193.

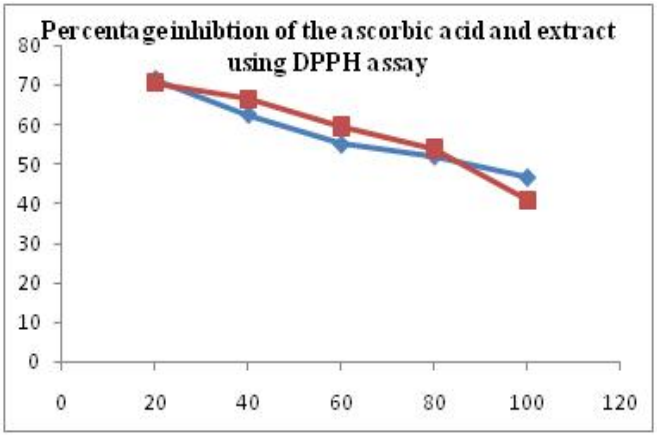


Figure 1: Graph showing percentage inhibition of the standard and extract using DPPH assay

Table 3: Absorbance and Percentage inhibition by DPPH for standard ascorbic acid

Concentration (µg/ml)	Absorbance (nm)	Percentage inhibition	X value (IC50)
20	0.11	69.509±0.01	190
40	0.125	65.488±0.02	
60	0.151	58.517±0.02	
80	0.172	52.887±0.01	
100	0.22	40.019±0.03	

The test sample was evaluated for antioxidant activity. The percentage inhibition was measured using U.V Spectrophotometer. The concentrations of standard sample were 20, 40, 60, 80 and 100 µg/ml with their percentage inhibition were 69.509, 65.488, 58.517, 52.887, and 40.019 respectively. The IC_{50} Value of standard ascorbic acid was found to be 190.

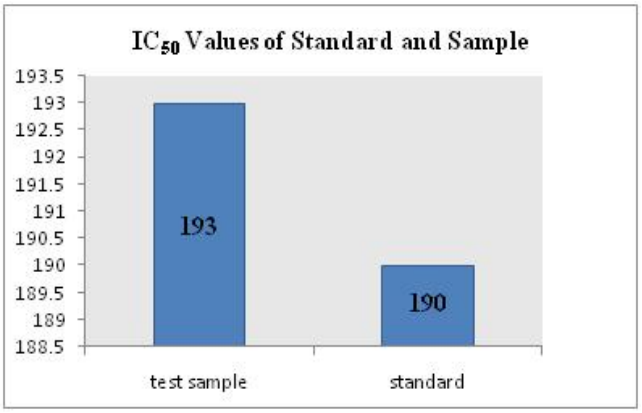


Figure 2 : Graph showing IC_{50} values of standard and sample by DPPH assay

From the tables, it was evident that percentage scavenging activity of extract and the ascorbic acid at different concentration was observed and it was found that the polyherbal extract has near IC_{50} value to standard .The IC_{50} of extract is 190 and the IC_{50} value of standard is 193. The evaluation of the poly herbal extract has proved antioxidant activity near to the standard. It may be, due to the presence of polyphenolic compounds such as glycosides, flavonoids and tannins in the extract of the peels.

Hydrogen peroxide radical scavenging (H_2O_2) assay [15-17]

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually the oxidation of essential thiol (-SH) groups. The scavenging activity of hydrogen peroxide can be evaluated using standard ascorbic acid by U.V spectrophotometry [15]. The percentage inhibition of the extract and the standard were determined by H_2O_2 assay using U.V Spectrophotometer [17] and depicted in Fig.12 and table8, 9. The concentrations of test sample were 20, 40, 60, 80, 100 µg/ml with their percentage inhibitions were 71.314, 62.466, 54.959, 52.011, and 46.649 respectively. The IC_{50} Value of test sample was found to be 197.

Table 4: Absorbance and percentage inhibition by H_2O_2 assay for test sample

Concentration (µg/ml)	Absorbance (nm)	Percentage inhibition	X value (IC50)
20	0.107	71.509±0.01	197
40	0.14	68.488±0.02	
60	0.168	61.517±0.01	
80	0.179	55.887±0.03	
100	0.199	42.019±0.01	

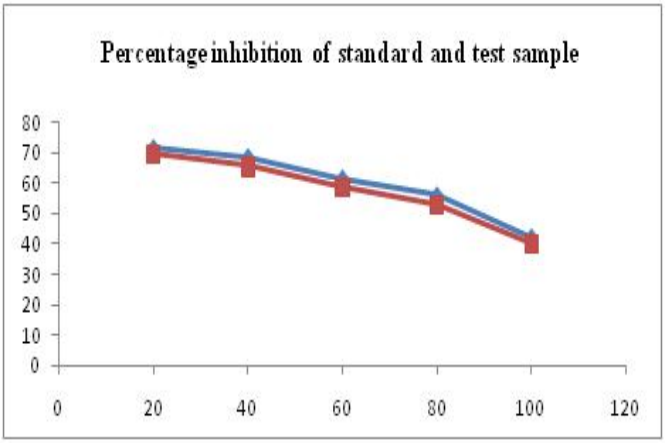


Figure 3: Graph showing Percentage inhibition of extract and sample by H_2O_2 assay

Table 5: Absorbance and percentage inhibition by H_2O_2 assay for standard ascorbic acid

Concentration (µg/ml)	Absorbance (nm)	Percentage inhibition	X value (IC50)
20	0.107	69.509±0.01	190
40	0.14	65.488±0.02	
60	0.168	58.517±0.02	
80	0.179	52.887±0.01	
100	0.199	40.019±0.03	

The test sample was evaluated for its *In-vitro* antioxidant activity. The percentage inhibition by DPPH assay was measured using U.V Spectrophotometer[15] and depicted in Fig.13 and table.9. The concentrations of ascorbic acid

were 20, 40, 60, 80, 100 µg/ml with their percentage inhibition were 69.509, 65.488, 58.517, 52.887, and 40.019 respectively and the IC_{50} value was found to be 190.

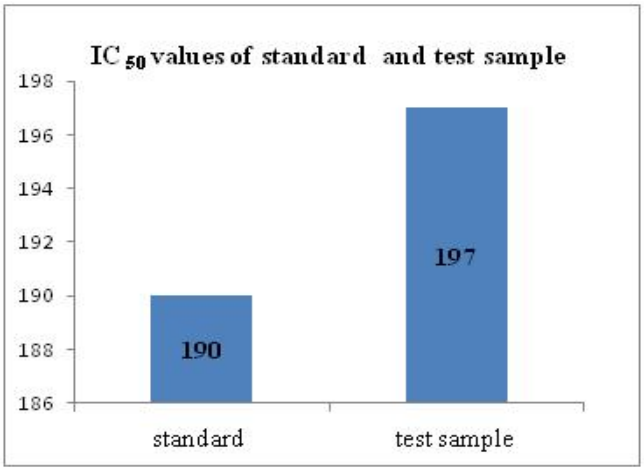


Figure 4: Graph showing IC_{50} values of standard and test sample by H_2O_2 assay

From the tables, it was evident that percentage scavenging activity of extract and the ascorbic acid at different concentration was observed and the polyherbal extract was found to have nearest anti oxidant value to the standard which may be further investigated. The IC_{50} of extract is 197 and the IC_{50} value of standard is 190. The evaluation of the poly herbal extract has proved antioxidant activity near to the standard. It may be, due to the presence of polyphenolic compounds such as glycosides, flavonoids and tannins in the extract of the plant.

Discussion:

The peels are considered as the agro economical wastes with economical and medicinal value. Hence the utilization of plant parts i.e., peels may result in good pharmacological activity. The *In-vitro* antioxidant activity can be evaluated on a polyherbal extract consisting of peels of ridge gourd, cucumber, beet root and flower buds of clove. The present study is to investigate the antioxidant activity of polyherbal extract containing peels of ridge gourd, cucumber, beet root and flower buds of clove. The peels were collected and are shade dried for 5 days and are extracted by Soxhlet apparatus for 24 cycles for 2 days using methanol as solvent and later concentrated using Rotavapour. The poly herbal extract was reddish brown semisolid in nature and the percentage yield of the extract was found to be 17.5%. The qualitative analysis of the extract revealed the presence of glycosides, flavonoids, alkaloids, terpinoids, phenols and saponins. The poly herbal extract was further evaluated for its *In-vitro* anti oxidant activity using DPPH free radical scavenging assay and H_2O_2 free radical scavenging assay. The evaluation of the poly herbal extract has proved that the IC_{50} value which determines the *In- vitro* antioxidant activity of the poly herbal extract by both the assays was found to be near to the standard ascorbic acid value. It may be, due to the presence of polyphenolic compounds such as glycosides, flavonoids and tannins in the extract of the plant. The *In-vitro* antioxidant activity was evaluated which proved that this poly herbal extract if further investigated for its *in vivo* activity may prove this as an potent anti oxidant for the treatment for stroke and neurodegenerative diseases.

4. Conclusion

The present study was carried out to evaluate antioxidant activity of poly herbal methanolic extract of peels of *Beta vulgaris (*beetroot*), Luffinae aegyptiaca (*ridge gourd*), Cucumis sativus (*cucumber), and flower buds of *Syzygium aromaticum (*clove*).* The qualitative screening of the poly herbal extract revealed the presence of alkaloids, glycosides, saponins, phenolics and flavonoids. The collected fine powder was used to evaluate the *in-vitro* antioxidant activity by DPPH free radical scavenging method and Hydrogen peroxide radical scavenging (H_2O_2) assay. The IC_{50} value of the poly herbal extract was found to be near to the standard ascorbic acid value.

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6. References

1. Irda Fidrianny, Monika Harnovi, M Insanu, Evaluation Of Antioxidant Activities From Various Extracts Of Sweet Orange Peels Using Dpph, Frap Assays And Correlation With Phenolic, Flavonoid, Carotenoid Content , Asian J Pharm Clin Res., **2014**, 7(3): 186-190.
2. Bushra Sultana, Farooq Anwar, Muhammad Rafique Asi and Shahzad Ali Shahid Chatha Antioxidant potential of extracts from different agro wastes. **2008**, pp. 205-217.
3. A.E. Hegazy and M.I. Ibrahium, Antioxidant Activities of Peel Extracts, World Applied Sciences Journal. **2012**, 18(5): 684-688.
4. Ajayi, I. A.; Ajibade, O.; Oderinde, R. A., Preliminary Phytochemical Analysis of Some Plant Seeds. Research Journal of Chemical Sciences, **2011**, 1(3): 58-62.
5. Bandita Deo, Monalisha Nath, Pretham Kumar Nayak, Yogamaya Dhal Evaluation of antioxidant acitivity of *ocimum tenurifolium*, an important medicical herb. IJPAES, **2013**, 3(2): 150–154.
6. Nooman A. Khalaf, Ashok K. Shakya, Atif Al-othman, Zaha El-agbar, Husni Farah, Antioxidant Activity of Some Common Plants , Turk. J. Biol., **2008**, 32: 51-55**.**
7. Nagavani V, Rao TR (2010) Evaluation of antioxidant potential and qualitative analysis of major polyphenols by RP-HPTLC. International Journal of Pharmacy and Pharmaceutical Sciences. **2010**, 2(4): 98-104.
8. Mensor LL, Meneze FS, Leitao et al., Screening of Brazilian plant extracts for Antioxidant activity by the use of DPPH free radical method. Phytother. Res., **2001**, 15: 127-130.

9. Nithya Narayanaswamy and K P Balakrishnan, Evaluation of some Medicinal Plants for their Antioxidant Properties, International Journal of Pharm. Tech Research, 3(1): 381-385
10. Sylwester Ślusarczyk, Michał Hajnos, Krystyna Skalicka-Woźniak Adam Matkowski Antioxidant activity of polyphenols from *Lycopus lucidus* Turcz, **2009**, 113(1): 134–138
11. Edak A. Uyoh, Peter N. Chukwurah, Imabasi A. David, Anna C. Bassey , Evaluation of Antioxidant Capacity of Two *Ocimum* Species Consumed Locally as Spices in Nigeria as a Justification for Increased Domestication, American Journal of Plant Sciences, **2013**, 4: 221-229.
12. Hazra, B; Biswas, S.; Mandal N., Antioxidant and free radical scavenging activity BMC Complimentary Alternative Medicine. **2008**, pp. 1-10.
13. Floriano-Sánchez E, Villanueva C, Medina-Campos ON, Rocha D, Sánchez-González DJ, Cárdenas-Rodríguez N, Pedraza-Chaverrí. J. Nordihydro guaiaretic acid is a potent *in-vitro* scavenger of peroxynitrite, singlet oxygen, hydroxyl radical, superoxide anion, andhypochlorous acid and prevents in vivo tyrosine nitration in lung. Free Radic Res., **2006**, 40: 523–533
14. S. Ghosh, P.Bhateja, A. Saini, J. Rani *In-vitro* evaluation of antioxidant activity International journal of medical science and clinical Invention. **2014**, 1(3) 2014: 89-96
15. Herborn, J.B., Phytochemical methods. A guide to modern techniques of plant analysis. Use of a free radical method to evaluate antioxidant activity. **1998**, pp. 2:5-11.
16. Oldschmidt, S., and Renn, K.,Chem. Ber., Antioxidant Determinations by the Use of a Stable Free Radical. 1922, pp. 55, 628.
17. Rong Tsao, Zeyuan Deng Separation procedures for naturally occurring antioxidant phytochemicals. **2004**, 812(1–2): 85–99.