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EVALUATION OF ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACTS OF *SALVADORA PERSICA*

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ABSTRACT

Much effort has been focused on examining the antioxidant and inhibitory effect of *Salvadora persica* (miswak) on oral microorganisms, but information related antioxidant and antibacterial activity against other human pathogens, particularly multidrug resistant (MDR) isolates, is very rare. The main objective of this study was to evaluate antibacterial and antioxidant activity of ethanolic extracts of leaves and stem of *Salvadora persica*. Antimicrobial activity of ethanolic extracts was also evaluated and MIC values were calculated by broth dilution method. Extracts prevented the growth of gram positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis* and gram negative bacteria such as *E.coli* and *Pseudomonas aeruginosa*, the MIC values of ethanolic extract of the stems were higher than those of the leaves extract. Antioxidant activity was assayed by the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, nitroblue tetrazolium (NBT) and ferric reducing power (FRAP). In all the assays, leaves extract exhibited stronger antioxidant activity than that of stem extract. The present results showed antibacterial and antioxidant activity of the extracts was found to be positively associated with the total phenolic and flavonoid content of the extracts.

KEYWORDS: Antibacterial Activity, Antioxidant Activity, *Salvadora Persica*, 2, 2-iphenyl-1-picrylhydrazyl (DPPH), Ferric Reducing Power (FRAP).

1. INTRODUCTION

Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.[1] Plants have to adapt to the changing environmental conditions for their survival of existence. The oxidative environment presents a range of free radicals including superoxide, hydroxyl radical, nitric oxide and peroxynitrite, for living organisms to deal with. Many evidences are exists to explain the role of free radicals in the development of various diseases including cancer, neurodegeneration and some inflammatory diseases.[2,3,4] Antioxidants have therefore gained importance for their capacity to neutralize free radicals. In this context, the antibacterial and antioxidant properties of various medicinal plants are being investigated throughout the world because of the toxicological concerns associated with the synthetic antioxidants and preservatives.[5] The toothbrush tree, *Salvadora persica* is also called miswak, belonging to the Salvadoraceae family, is one of the most important ones among 182 species of plants being used as chewing sticks. It has been widely used in many Asian, African, and Middle Eastern countries. The roots, twigs, and stems of this plant have been used for oral hygiene and small miswak sticks have been used as toothpicks for maintaining oral hygiene.[6,7] It has been reported that the aqueous and methanol extracts of miswak possess various biological properties against organisms considered important for the development of dental plaque and periodontitis.[8]

Previous in vitro studies have reported the antibacterial and antifungal effects of miswak on cariogenic bacteria and periodontal pathogens including *Streptococcus mutans, Streptococcus faecalis, Streptococcus pyogenes, Lactobacillus acidophilus, Porphyromonas gingivalis, Haemophilus influenzae* and Candida albicans.[9,10,11] Moreover, data from controlled clinical studies showed that Salvadora persica extract is also an effective antimicrobial agent when utilized clinically as an irrigant in the endodontic treatment of teeth with necrotic pulps.[12,13] Much effort has focused on examining the inhibitory effect of *Salvadora persica* on oral organisms, but information concerning the antibacterial activity of *Salvadora persica* against other human pathogens, particularly MDR isolates, is scarce.[14,15] Therefore, the main objective of the present study was to determine the total phenolic and flavonoid content and to evaluate the antioxidant and antibacterial activity of ethanolic extracts of leaves and stem of *Salvadora persica.*

2. MATERIALS AND METHODS

2.1. Chemicals 1,1-Diphenyl-2-picrylhydrazyl (DPPH), potassium ferricyanide, trichloroacetic acid, gallic acid (GA), rutin (RU), nitroblue tetrazolium (NBT) and Folin–Ciocalteu's reagent, were purchased from Sigma–Aldrich (St. Louis, MO, USA).

Aluminium chloride, Sodium carbonate, Mueller Hinton media were purchased from SR Scientifics (Tirupathi, India). All other chemical reagents used were of analytical grade.

2.2. Collection of plant material

The fresh leaves and stem of *Salvadora persica* were collected from the Western Ghats of Karnataka, India, during winter season. Plant parts were packed immediately after picking and kept in cold (-20^0C) dark storage until processed. The plant specimen was identified with the help of an expert, Prof. C.Sudhakar (Department of Botany, S.K University, India).

2.3. Preparation of extracts

Flowers and stems of the plant were collected and dried under shade at room temperature. The plant material was then chopped and ground to fine powder using a mechanical blender. 20gm of powder of leaves and stem of *Salvadora persica* was taken into conical flask. The phytoconstituents were extracted by adding 100ml of ethanol to the powder. The flask was incubated in orbital shaker for 48 hrs. The extract was filtered through five layers of Muslin cloth. The process was repeated twice. The collected extract was pooled and concentrated by evaporation.[16] The extract was preserved and stored at 4^0C in airtight bottles for further study.

Fig 3.1 Stems and leaves of extract of *Salvadora persica*

2.4. Determination of total phenolic content

Total phenolic content of ethanolic extracts flowers and stem of *Salvadora persica* was measured using the Folin–Ciocalteu reagent method as described earlier.[17] Briefly, from the stock solution of (1 mg/ml ethanol), 200 µl of both of the crude extracts were made up to 3 ml with distilled water then mixed thoroughly with 0.5 ml of Folin–Ciocalteu reagent for 3 min, followed by the addition of 2 ml of 20% (w/v) sodium carbonate. The mixture was allowed to stand for a further 60 min in the dark and absorbance of the reaction mixtures was measured at 650 nm. Quantification was done on the basis of the standard curve of Gallic acid concentration range from 50 to 500 mg/ml ($r^2 = 0.998$). Total phenolic content calculated from the calibration curve was expressed as mg of gallic acid equivalent (GAE)/g of dry weight.

2.5. Determination of total flavonoid content

Total flavonoid content of both crude extracts was determined using the aluminium chloride colorimetric method as described earlier.[18] Briefly, from the stock solution of 1 mg/ml crude extracts, 50 µl of each extract was made up to 1 ml with methanol, mixed with 4 ml of distilled water and subsequently with 0.3 ml of 5% $NaNO_2$ solution. 0.3 ml of 10% $AlCl_3$ solution was added after 5 min of incubation and then allowed to stand for 6 min. This was followed by the addition of 2 ml of 1 M NaOH solution to the mixture and final volume of the mixture was brought to 10 ml by the addition of double distilled water. The mixture was allowed to stand for 15 min and absorbance was measured at 510 nm. Quantification was done on the basis of the standard curve of rutin concentration ranging from 50 to 500 mg/ml ($r^2 = 0.999$). Total flavonoid content calculated from a calibration curve was expressed as mg of rutin equivalent (RU)/g of dry weight.

2.6. DPPH assay

The antioxidant activity was determined by DPPH assay as described earlier with some modifications.[19] From the stock solution different concentrations of extract (100 µg–600 µg/ml) were prepared. 200 µl of each concentration was mixed with 3.8 ml DPPH solution and incubated in the dark at room temperature for 60 min. Absorbance of the mixture was then measured at 517 nm control and Vitamin E was used as a positive. Scavenging ability of the sample to DPPH radical was determined according to the following equation

$$\%\ \text{DPPH scavenging activity} = \frac{(\text{Control absorbance} - \text{Sample absorbance}) \times 100}{\text{Control absorbance}}$$

2.7. Ferric reducing power assay

Ferric reducing/antioxidant power (FRAP) was determined following the method as described earlier.[20] Briefly, 100 µl of each concentration of the extracts (100–600 µg/ml) was mixed with 2.5 ml of 200 mM phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide and incubated at 50 C for 20 min. After this, 2.5 ml of 10% trichloroacetic acid was added and the tubes were centrifuged at 10,000 rpm for 10 min. Five milliliters of the upper layer of the solution was mixed with 5.0 ml of distilled water and 1 ml of 0.1% ferric chloride and the absorbance of the reaction mixtures was measured at 700 nm. The final results were expressed as mg ascorbic acid equivalent/g of dry weight.

2.8. NBT assay

Superoxide anion scavenging activity was performed as described earlier.[21] From the stock solution (1 mg/ml) different concentrations of extract (100 µg g–500 µg g/ml) were prepared. The reaction was performed in 50 mM phosphate buffer (pH 7.8) containing extracts of various concentrations (100–600 lg/ml), 1.5 mM riboflavin, 50 mM NBT, 10 mM DL-methionine and 0.025% v/v Triton X-100. The reaction was initiated by illuminating the reaction mixture and absorbance of formazan was recorded at 560 nm and percentage scavenging activity was described.

2.9. Antibacterial assay

The antimicrobial activity was tested against both Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) obtained from the Department of Microbiology, Yogivemana University, Kadapa, India. The bacterial strains were grown on Mueller Hinton agars plates and suspended in MH broth. The minimum inhibitory concentration (MIC) values were determined using the broth microdilution method as described earlier.[22] Serial dilutions of the stock solutions of the crude extracts in broth medium were prepared in a microtiter plate and the microbial suspensions were added in the microwells at the concentration of 5×10^5 organisms/ml. The MIC values were determined as the lowest concentrations preventing visible growth. Streptomycin was used as a positive control. Each assay was repeated three times.

2.10. Statistical analysis

Data were expressed as Mean ± SD. Statistical analysis was performed by SPSS 11.5. One-way analysis of variance (ANOVA) was utilized to evaluate differences.

3. RESULTS

3.1. Total phenol content

The total phenolic content of the stem and leaves ethanolic extracts of *Salvadora persica* was determined by the method described above. The total phenolic content for the leaves extract was found to be 68 ± 2.2 (GAE)/g DW and for the stem extract was 42 ± 2.4 (GAE)/g DW (Table 3.1).

3.2. Total flavonoid content

The total flavonoid content of the stem and leaves ethanolic extracts of *Salvadora persica* is given in Table 3.1. The total flavonoid content for the leaves extract was found to be higher (38 ± 1.8 rutin equivalent/g DW) than the stem extract (21 ± 1.5 rutin equivalent/g DW).

Table 3.1 Total phenolic and flavonoid content of stem and flower extracts of *Salvadora persica.*

Extract	Total phenolic content[a]	Total flavanoid content[b]
leaves extract	68 ± 2.2	38 ± 1.8
Stem extract	42 ± 2.4	21 ± 1.5

Each value is a mean of three biological replicas.

[a]mg gallic acid equivalent (GAE)/g DW.

[b]mg rutin equivalent/g DW

3.3. Antioxidant activity

Plants rich in secondary metabolites including phenolics, flavonoids and carotenoids exhibit antioxidant activities which are due to their redox properties and chemical structures. The antioxidant property of the crude extracts was investigated and compared by various biochemical assays like, DPPH and NBT assay. The ethanolic extract of stem demonstrated comparatively stronger antioxidant activity as compared to the leaves extract. The DPPH scavenging activity was found to be 54%, 76% and 91% at 600 µg/ml for leaves extract, stem extract and Vitamin C respectively (Fig. 3.1).

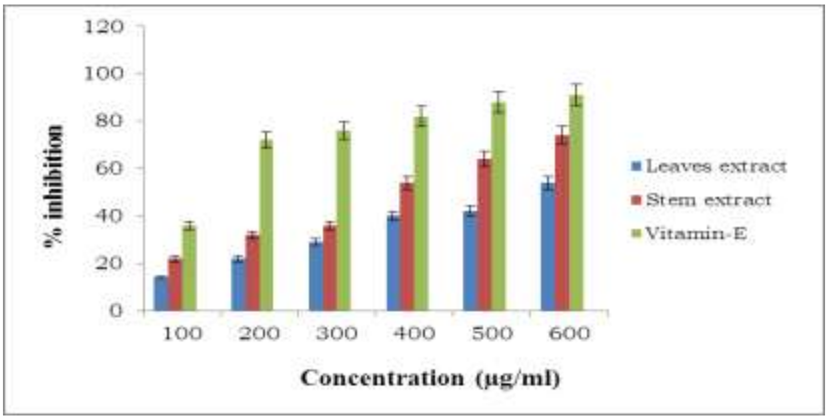

Fig 3.1 Free radical scavenging activity of ethanolic extracts of leaves and stem of *Salvadora persica.* Vitamin E was included as a positive control. Activity was measured by the scavenging of DPPH radicals and each value is expressed as the mean ± standard deviation.

Superoxide scavenging activity determined by NBT assay was found to be 54%, 69% and 91.7% at 600 µg/ml for leaves extract, stem extract and ascorbic acid respectively (Fig. 3.2). Presence of antioxidant substances or reductants in the plant extracts leads to the reduction of $Fe^{3}+$ ferricyanide complex to the ferrous form (Fe^{2+}). We also evaluated the reducing power of the crude extracts and significant changes were observed with the increase in the concentration of the extract (100–500 µg /ml). For leaves extract absorbance values ranged from 0.26 to 0.6 and for stem extract the values were between 0.3 and 0.65 (Fig 3.3). Ascorbic acid was used as a positive control.

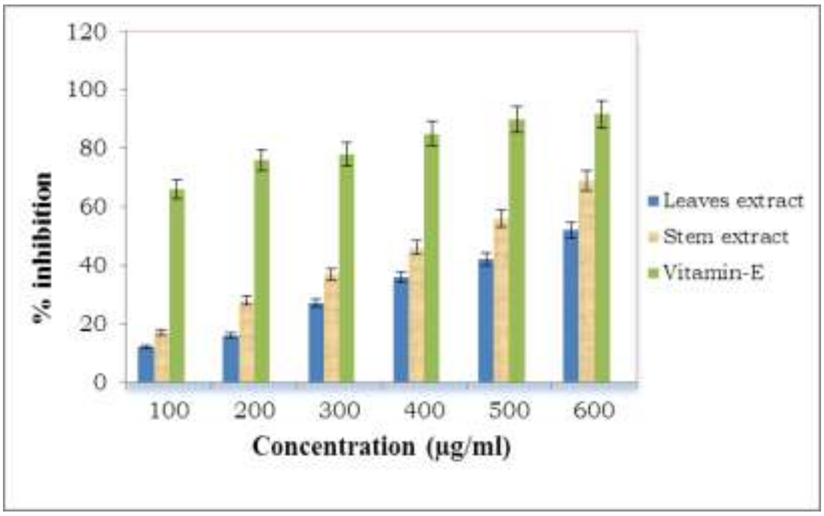

Fig 3.2 Superoxide scavenging activity of ethanolic extracts of flower and stem of *Butea monosperma.* Vitamin E was included as a positive control. Activity was measured using NBT assay and each value is expressed as the mean ± standard deviation.

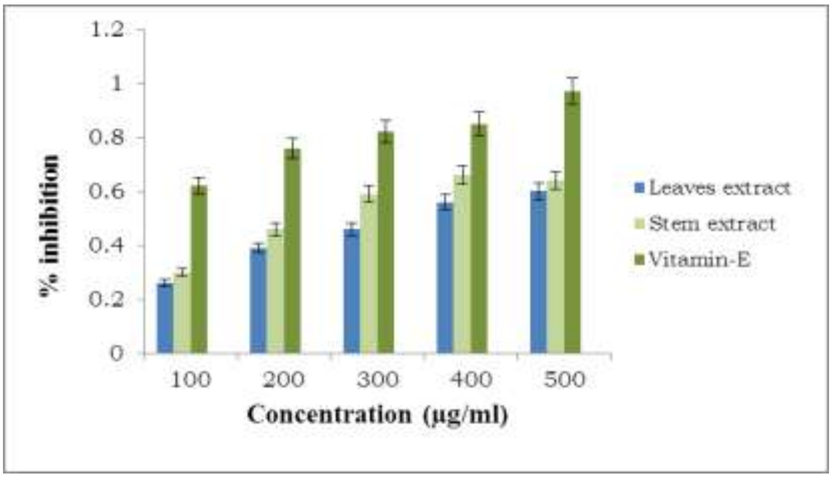

Fig 3.3 Determination of ferrous reducing capacity of ethnolic extracts of leaves and stem of *Salvadora persica*. Vitamin E was taken as a positive control. Each value is expressed as the mean ± standard deviation.

3.4. Antibacterial activity

The in vitro antibacterial properties of ethanolic extracts of leaves extract, stem extract of *Salvadora persica* are presented in Table 4.1. The tested extracts of *Salvadora persica* leaves and stem possessed antibacterial activity against both Gram positive and Gram negative bacteria. The antibacterial activity of stem extract was found to be comparatively higher than that of leaves extract. The MIC value of the stem extract ranged from 0.22 ± 0.04 to 0.72 ± 0.04 mg/ml and that of stem from 0.30 ± 0.01 to 0.91 ± 0.04 mg/ml. The stem extract exhibited highest antibacterial activity against *Pseudomonas aeruginosa* (0.22 mg/ml) and lowest activity against *Staphylococcus aureus* (0.72 ± 0.06). A similar trend was exhibited by the leaves extract although the MIC values were higher than those of the leaves extract.

4. DISCUSSION

In present study, we determined the total phenolic and flavonoid content of ethanolic extracts of flower extract, stem extract of *Salvadora persica* and both of the extracts showed high phenolic and flavonoid content. Antioxidant and antibacterial activity of these crude extracts may be attributed to the high phenolic and flavonoid content. Phenolic compounds are important plant constituents because of their free radical scavenging ability facilitated by their hydroxyl groups and the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity.[23] Phenolic compounds are also involved in conferring plants with oxidative stress tolerance. Flavonoids are highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various other free radicals implicated in several diseases.[24] Flavonoids, on the other hand, suppress reactive oxygen formation, chelate trace elements involved in free-radical production, scavenge reactive species, and up-regulate and protect antioxidant defenses.[25] Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials rich in phenolics and flavonoids, are increasingly being used in the food industry for their antioxidative properties and health benefits. In present study, the ethanolic extracts of leaves showed comparatively higher antioxidant activity than the ethanolic extract of stem of *Salvadora persica*, which is in accordance with the total phenolic and flavonoid content of the two extracts. The tested extracts of *Salvadora persica* leaves and stem possessed relatively higher antibacterial activity against Gram positive than Gram negative bacteria. The antibacterial activity of stem extract was found to be comparatively higher than that of leaves extract. The reason for higher sensitivity of the Gram-positive bacteria than Gram negative bacteria could be attributed to their differences in cell membrane constituents. Gram-positive bacteria contain an outer peptidoglycan layer, which is an ineffective permeability barrier.

Table 3.2 Antimicrobial activity of *Salvadora persica* extracts (MIC value expressed in mg/ml)

Microorganism	Leaves extract	Stem extract	Streptomycin
Pseudomonas aeruginosa	0.30±0.01	0.22±0.04	0.055±0.002
Bacillus subtilis	0.34±0.02	0.25±0.04	0.025±0.001
Escherichia coli	0.79±0.01	0.66±0.06	0.055±0.001
Staphylococcus aureus	0.91±0.04	0.72±0.04	0.0020±0.03

Similar results were obtained from a research carried on strawberry tree leaves.[26] These results suggest that *Salvadora persica* may be a potential source of broad spectrum of antibacterial agents. The antibacterial activity of the extracts could be attributed to the high content of flavonoids which have been reported to be involved in the inhibition of nucleic acid biosynthesis and metabolic processes.[27]

5. CONCLUSION

The results suggest that *Salvadora persica* is a potential source of antibacterial and antioxidant molecules. The stem and leves of the plant can be used as natural antioxidants and preservatives in food and non-food systems. However, further phytochemical analysis is required for the isolation of bioactive molecules from the plant that may show a broad spectrum of pharmacological activities.

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REFERENCES

1. Karthikeyan A, Shanti, V, Nagasathaya A. Preliminary phytochemical and antibacterial screening of crude extract of the leaf of Adhatoda Vasica, Int. J. Green Pharm, 1991; 3: 78-80.
2. Halliwell, B. Oxidative stress and cancer: have we moved forward? Biochem. J, 2007; 401: 1–11.
3. Halliwell, B. Oxidative stress and neurodegeneration; where are we now? J. Neurochem, 2006; 97: 1634–1658.

4. Ferguson, L. R. Chronic inflammation and mutagenesis. Mutat. Res. Fund. Mol. M, 2010; 690: 3–11.
5. Peschel, W., Sanchez-Rabaneda, F., Dieckmann, W., Plescher, A., Gartzia, I., et al. An industrial approach in the search of natural antioxidants from vegetable and fruits wastes. Food Chem, 2006; 97: 137–150.
6. Sher, H., Al Yamani, M. N., L. Wijaya. Ethnobotanical and antibacterial potential of Salvadora persica: a well-known medicinal plant in Arab and union system of medicine," Journal of Medicinal Plants Research, 2011; 5(7): 1224–1229.
7. Goyal, M., Sasmal, D., B. P. Nagori. Salvadora persica (meswak): chewing stick for complete oral care, International Journal of Pharmacology, 2011; 7(4): 440–445.
8. Sofrata, A. H., Claesson, R. L. K., Lingström, P. K., Gustafsson, A. K. Strong antibacterial effect of miswak against oral microorganisms associated with periodontitis and caries, Journal of Periodontology, 2008; 79(8): 1474–1479.
9. Al-Bayat, F.A., Sulaiman, K. D. In vitro antimicrobial activity of Salvadora persica L. extracts against some isolated oral pathogens in Iraq, Turkish Journal of Biology, 2008; 32(1): 57–62.
10. Naseem,S., Hashmi, K., Fasih, F., Sharafat, S., Khanani, R. In vitro evaluation of antimicrobial effect of miswak against common oral pathogens, Pakistan Journal of Medical Sciences, 2014; 30(2): 398–403.
11. Al-Sieni, A. I. The antibacterial activity of traditionally used Salvadora persica L (Miswak) and Commiphora gileadensis (Palsam) in Saudi Arabia, African Journal of Traditional, Alternative and Complementary Medicines, 2014; 11(1): 23–27.
12. Sofrata, A., Brito, F., Al-Otaibi, M., Gustafsson, A. Short term clinical effect of active and inactive Salvadora persica miswak on dental plaque and gingivitis, Journal of Ethnopharmacology, 2011; 137(3): 1130–1134.
13. Bhat, P. K., Kumar, A., Sarkar, S. Assessment of immediate antimicrobial effect of miswak extract and toothbrush on cariogenic bacteria—a clinical study, Journal of Advanced Oral Research, 2012; 3(1): 13–18.
14. Fallah, M., Fallah, F., Kamalinejad, M., Malekan, M.A., Akhlaghi, Z., Esmaeili, M. The antimicrobial effect of aquatic extract of Salvadora persica on Mycobacterium bovis in vitro, International Journal of Myco bacteriology, 2015; 4(1): 167–168.

15. Alili, N., Türp, J. C., Kulik, E. M., Waltimo, T. Volatile compounds of Salvadora persica inhibit the growth of oral Candida species, Archives of Oral Biology, 2014; 59(5): 441–447.
16. Siddiqui S, Verma A., Rather A. A., Jabben F, Meghvansi M. K. Preliminary phytochemicals analaysis of some important medicinal and aeromatic plants, Advances in Biological Research, 2009; 3: 188-195.
17. Kaur, C., Kapoor, H. C. Anti-oxidant activity and total phenolic content of some Asian vegetables. Int. J. Food Sci. Technol, 2002; 37: 153–161.
18. Chang, C., Yang, M., Wen, H., Chern, J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal, 2002; 10: 178–182.
19. Villano, D., Fernandez-Pachon, M.S., Moya, M. L., Troncoso, A. M., Garcıa-Parrilla, M. C. Radical scavenging ability of polyphenolic compounds towards DPPH free radical. Talanta, 2007; 71: 230–235.
20. Zhao, H., Fan, W., Dong, J., Lu, J., Chen, J., et al. Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties. Food Chem, 2008; 107: 296–304.
21. Vyas, D., Kumar, S. Purification and partial characterization of a low temperature responsive Mn-SOD from tea (Camellia sinensis (L.) O. Kuntze). Biochem. Biophys. Res. Commun, 2005; 329: 831–838.
22. Ericcson, H.M., Sherris, J.C. Antibiotic sensitivity testing: report of an international collaborative study. Acta Pathol. Microbiol. Scand, 1971; 217: 1-9.
23. Yi, O., Jovel, E. M., Towers, N. G. H., Wahbe, T. R., Cho, D. Antioxidant and antimicrobial activities of native Rosa sp. From British Columbia, Canada. Int. J. Food Sci. Nutr, 2007; 58: 178–189.
24. Bravo, L. Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. Nutr. Rev, 1998; 56: 317–333.
25. Agati, G., Azzarello, E., Pollastri, S., Tattini, M. Flavonoids as antioxidants in plants: location and functional significance. Plant Sci, 2012; 196: 67–76.
26. Orak, H.H., Yagar, H., Isbilir, S. S., Demirci, A. S., Gu¨mu¨s, T., Ekinci, N. Evaluation of antioxidant and antimicrobial potential of strawberry tree (Arbutus Unedo L.) leaf. Food Sci. Biotechnol, 2011; 20: 1249–1256.
27. Cushnie, T. P. T., Lamb, A. J. Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents, 2005; 26: 343–356.