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STUDIES ON ANALYSIS OF ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF *PRUNUS PERSICA* (L.) BATSCH

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ABSTRACT
The antibacterial activity of methanol, acetone and aqueous leaf extracts of *Prunus persica* was determined *in vitro* against medically important pathogens such as *Escherichia coli*, *Yersinia pestis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Listeria monocytogenes* and *Staphylococcus aureus* following agar well diffusion method using different concentrations (25%, 50%, 75% and 100%). Results showed low to significant antibacterial activity against the mentioned bacterial species. Methanol leaf extract was found to be more effective against selected pathogenic bacteria as compared to acetone and aqueous leaf extracts. Furthermore, the leaf extracts inhibited gram-positive bacteria more efficiently than gram-negative bacteria. In addition, the antioxidant capacity of the different extracts of this plant was evaluated by DPPH (1,1-diphenyl-2-picrylhydrazyl) and reducing power tests. The plant exhibited moderate DPPH radical scavenging activity and good reducing power potential of greater than 50% in all the solvents used. Therefore, the leaf extracts of this plant can be selected for further investigation to determine their therapeutic potential.

KEYWORDS: *Prunus persica*, leaf extracts, pathogenic bacteria, agar well diffusion, DPPH, reducing power.

INTRODUCTION

Plants, as a source of medicine, have been playing an important role in the health services around the globe since ancient times. About three quarters of the world's population depends on plants and plant-derived products for health care. A good number of our population especially those living in rural areas depends largely on herbal remedies for the treatment of different kinds of diseases. Plants used for traditional medicine contain a wide range of compounds that can be used to treat chronic as well as infectious diseases This indicates the great importance of medicinal plants in the health care system (Periyasamy *et al*., 2010). Health care system in ancient times included the use of roots, leaves, stems, flowers and berries of herbs for their therapeutic or medicinal value. These medicines usually took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations (Balick and Cox, 1996). Different plant parts such as leaves, stems, roots, barks, twigs, tubers, bulbs, exudates, flowers and fruits were all used in the treatment of different disorders. These plant materials are used to prepare extracts, infusions, teas, snuffs and in many other forms which are generally administered in different ways (Van Wyk and Gericke, 2000).

An antimicrobial is a substance that retards or inhibits the growth of microorganisms such as bacteria, fungi or protozoans. These antimicrobial substances are of natural origin, and it is considered that their influences on the environment are few and can be used as effective biological control agents (McEwen and Fedorka-Cray, 2002). The development of continuous bacterial resistance to presently available antibiotics has necessitated the need to search for new antibacterial agents.

In the recent decade, the role of antioxidants has been continuously recognised as a critical influence on the biochemistry of living beings. Any substance that causes delay or prevents oxidative damage to a target molecule is often called an antioxidant. Antioxidants, also called inhibitors of oxidation, are compounds which retard or prevent the oxidation and in general prolong the life of oxidizable matter (Kokate and Purohit, 2004). The oxidants or free radicals are species with very short half-life, high reactivity and damaging activity towards macromolecules like proteins, lipids and DNA. Antioxidants prevent the oxidation of certain biomolecules such as nucleic acids, proteins, carbohydrates and fatty acids by various mechanisms (Gaikwad *et al*., 2011).

Prunus persica (L.) Batsch (Peach) also named as *Amygdalus persica* is a deciduous tree belonging to family Rosaceae and is commonly cultivated in West Asia, Europe, Himalayas and India upto an altitude of 1000 ft. The leaves of *P. persica* are astringent, anthelmintic, insecticidal, sedative, diuretic, demulcent, expectorant, vermicidal and are used in leucoderma and in piles. Leaf paste is prepared to kill worms in wounds and fungal infections. Aditionally, the plant exhibits antimicrobial, antioxidant, anti-tumour and anti-Oketsu syndrome effects (Christabel *et al*., 2012). Therefore, in the present work an attempt has been made to analyse the antibacterial and antioxidant potential of methanol, acetone and aqueous extracts of *P. persica* leaves against selected pathogenic bacterial strains.

MATERIALS & METHODS

Collection of Plant Material

Leaves of *Prunus persica* were plucked and collected from Devthana-Nandri area of District Sirmaur, Himachal Pradesh, India. The collected plant material was brought to the laboratory for further analysis.

Processing of Plant Material

Leaves of *P. persica* were washed thoroughly under tap water and then with 2% Mercuric chloride. After that the leaves were cut into smaller pieces for quick drying. Cleaned leaves were shade dried for 15-20 days. The dried plant material was crushed into fine powder with the help of pestle mortar. Finally the fine powder was stored in an air tight container at room temperature.

Preparation of Plant Extracts

5 gm dried leaves of *P. persica* were taken in separate Erlenmeyer flasks to which 50 mL of required solvents *i.e.* methanol, acetone and aqueous were added. The flasks were covered with aluminium foil and allowed to stand for 3-5 days for extraction. These extracts were filtered through Whatman filter paper no. 1 and evaporated at 40°C using rotary evaporator. The extracts were collected and weighed. Finally, stock solution of concentration 50 mg/mL was prepared.

Procurement of Bacteria

Bacterial strains used for antibacterial studies were procured from Department of Biotechnology, Himachal Pradesh University, Summer Hill Shimla, India. Pathogens used in the study were *Escherichia coli, Pseudomonas aeruginosa, Yersinia pestis, Staphylococcus aureus, Bacillus cereus* and *Listeria monocytogenes.*

Revival of Pathogen

The pathogens were revived in nutrient broth and stored in nutrient agar slants at 4°C.

Screening the Antibacterial Activity of *P. persica*

Screening of plant extracts (methanol, acetone and aqueous) of *P. persica* was done using agar well diffusion method. Nutrient agar medium (Beef extract 1 g, Yeast extract 2 g, Sodium Chloride 1 g, Peptones 5 g, Agar 20 g, Distilled Water 1000 mL) was used throughout the investigation. The medium was autoclaved at 121.6°C for 30 minutes and poured into Petri plates. Bacteria were grown in nutrient broth for 24 hours. A 100 µL of bacterial suspension was spread on each nutrient agar plate. Agar wells of 8 mm diameter were prepared with the help of sterilized stainless steel cork borer in each Petri plate. The wells in each plate were loaded with 25%, 50%, 75% and 100% concentration of prepared leaf extracts of *P. persica.* The Petri plate kept as a control contained pure solvent only. The plates were incubated at 37± 2^0C for 24 hours in the incubation chamber. The zone of growth inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in perpendicular direction in all the three replicates and the average values were tabulated. Percentage inhibition of bacterial species was calculated after subtracting control from the values of inhibition diameter using control as standard (Hemashenpagam and Selvaraj, 2010).

$$\text{Percentage of growth inhibition (\%)} = \left(\frac{\text{Control}-\text{Test}}{\text{Control}}\right) \times 100$$

Control = average diameter of bacterial colony in control.
Test = average diameter of bacterial colony in treatment sets (Kannan *et al*., 2002).

Antioxidant Activity Test

DPPH Radical Scavenging Activity Assay

The free radical scavenging activity of plant extracts was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as described by Blois (1958). Briefly, to 1ml of different concentrations (20, 40, 60, 80 and 100 µg/mL) of plant or test extract, 1mL of DPPH (0.1 mM in methanol) was added. Corresponding blank sample was prepared and ascorbic acid was used as reference standard. Mixture of 1ml methanol and 1mL DPPH solution (without plant extract) was used as control. All the tests were carried out in triplicate and the decrease in absorbance was measured at 517 nm after 30 minutes in dark using UV-VIS spectrophotometer. The percentage of inhibition was calculated using the following formula:

$$\text{DPPH scavenging effect (\%)} = \left(\frac{\text{Acontrol}-\text{Asample}}{\text{Acontrol}}\right) \times 100$$

Where, $A_{control}$ is the absorbance of control; A_{sample} is the absorbance of sample

Reducing Power Assay

The reducing power was determined according to the method described by Oyaizu (1986). Different concentrations of plant extract (20, 40, 60, 80 and 100 µg/mL) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 minutes. A portion (2.5 mL) of Trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%) and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as the standard. Phosphate buffer (pH 6.6) was used as blank solution. Higher absorbance of the reaction mixture indicated greater reductive potential. Experiment was performed in triplicates at each concentration to evaluate percent reducing power. The % reducing power (antioxidant activity) was calculated by using the formula:

$$\text{\% Reducing power} = \left(\frac{\text{Acontrol}-\text{Asample}}{\text{Acontrol}}\right) \times 100$$

Where, $A_{control}$ is the absorbance of control; A_{sample} is the absorbance of sample

RESULTS & DISCUSSION

Agar well diffusion method is widely used for screening the antimicrobial activity of plant extracts. The antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular since there will be a confluent lawn of growth (Magaldi *et al*., 2004; Valgas *et al*., 2007).The results of agar well diffusion method for determining antibacterial activity of various plant extracts are shown in Table 1.1. Methanol extract of *Prunus persica* inhibited all the test bacteria except *E. coli*. The diameter of zone of inhibition (ZOI) ranged from 10.00 to 23.20 mm (maximum ZOI = 23.20 ±0.88 mm against *L. monocytogenes*). Acetone extract showed maximum inhibition of 21.04 ±0.40 mm against *P. aeruginosa* and no inhibition against *E. coli* and *B. cereus*. Aqueous extract was found to be effective against *P. aeruginosa*

and *L. monocytogenes* only and exhibited no inhibition against rest of the bacteria. The finding agrees with previously published results of Gilani *et al.* (2000), Deb *et al*. (2010) and Baranwal *et al*. (2013) for *P. persica* leaf extracts that exhibited antimicrobial activity against a group of gram-positive and gram-negative bacteria.

TABLE 1.1 Zones of inhibition (ZOI) produced by leaf extracts of *Prunus persica* at different concentrations

Plant Extract	Concentration in %	Inhibition zone diameter in mm (± SD)					
		E. coli	*Y. pestis*	*P. aeruginosa*	*B. cereus*	*L. monocytogenes*	*S. aureus*
Methanol	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	0.00±0.00	11.00±0.10	10.00±0.10	10.00±0.00	12.08±0.09	12.00±2.05
	50	0.00±0.00	12.20±0.05	11.92±0.19	12.00±0.00	14.80±1.20	14.15±0.95
	75	0.00±0.00	15.60±0.46	13.80±2.20	13.44±0.25	18.70±1.76	19.00±0.00
	100	0.00±0.00	17.00±0.00	16.10±0.20	15.66±1.70	22.30±0.88	23.20±0.70
Acetone	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	0.00±0.00	9.90±2.00	12.00±0.00	0.00±0.00	10.30±1.92	9.00±0.05
	50	0.00±0.00	11.67±0.33	14.20±1.80	0.00±0.00	13.24±0.28	9.70±1.30
	75	0.00±0.00	13.00±1.99	17.90±1.56	0.00±0.00	15.50±2.20	12.00±1.45
	100	0.00±0.00	15.00±0.45	21.04±0.40	0.00±0.00	19.33±1.00	13.90±0.10
Aqueous	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	10.00±2.22	0.00±0.00
	50	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	12.90±1.65	0.00±0.00
	75	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	15.57±0.20	0.00±0.00
	100	0.00±0.00	0.00±0.00	9.08±1.10	0.00±0.00	18.00±0.80	0.00±0.00

TABLE1.2 Free radical (DPPH) scavenging activity (%) of the tested medicinal plants at different concentrations

Concentration (µg/mL)	Methanol extract	Acetone extract	Aqueous extract	Ascorbic acid
20	12.12±1.00	10.05±1.17	10.40±0.56	35.24±0.50
40	23.87±1.30	16.90±0.15	18.00±0.00	50.54±0.42
60	30.40±0.56	20.00±0.00	24.88±0.66	62.35±1.20
80	36.30±2.22	27.00±0.00	32.30±1.56	74.14±0.00
100	48.08±0.88	38.15±1.67	38.10±1.33	83.26±2.20

Values are given as mean ± SD

The results of present study are in accordance to earlier reports where organic extracts exhibited more activity than aqueous extract. Although traditional healers used primarily water but plant extracts from organic solvents have been found to give more consistent activity when compared with aqueous extract (Parekh *et al*., 2006). The higher activity of methanol and acetone extracts can be attributed to the presence of higher amount of polyphenols as compared to aqueous extracts. This may be due to the better solubility of their active components in organic solvents (Boer, 2005). Moreover, gram-negative bacteria are found to be more resistant than gram positive bacteria to different plant extracts. These observations are likely to be the result of the differences in cell wall structure between gram-positive and gram-negative bacteria, with gram-negative bacteria outer membrane acting as a barrier to many environmental substances (Rabe and Staden, 1995; Pages *et al*., 2008). In the present study, extracts of *P. persica* in three different solvents (methanol, acetone and aqueous) were tested for their free radical scavenging ability by using DPPH assay and it was observed that the plant extracts showed moderate potency for scavenging free radicals as shown in Table 1.2. The extracts were tested on a concentration range (20-100 µg/mL) and it was found that the activity altogether increased with increase in concentration of plant extracts (Fig. 1.1). In all cases, methanol extracts proved to be better antioxidants than the corresponding acetone and aqueous extracts. A pattern of increasing antioxidant activity with increasing polarity has been reported (Goze *et al*., 2000).

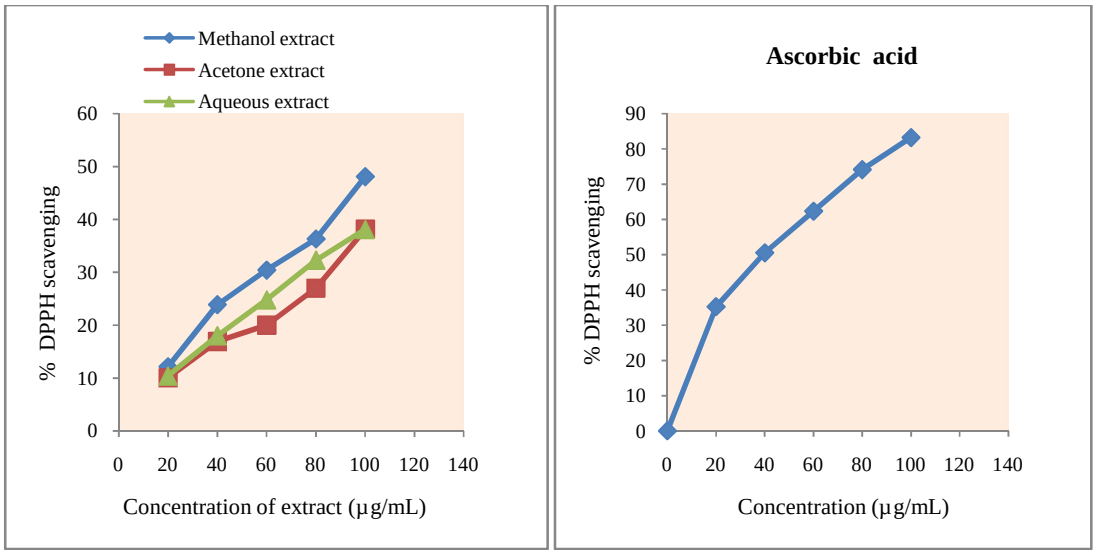
Deb *et al*. (2010) employed DPPH free radical scavenging assay on aqueous extract of *P. persica* and reported 13.28-64.24% inhibition. But in present study, *P. persica* exhibited 38.10% and 45.08% inhibition of free radicals by aqueous and methanol extracts respectively. Reducing power experiment is a good reflector of antioxidant activity of the plants. The reducing capacity of compounds serves as an important indicator of their potential antioxidant activity. The plant having high reducing power generally reported to carry high antioxidant potential too (Meir *et al*., 1995).

We investigated the reducing capacity of *P. persica* by measuring Fe^{3+}-Fe^{2+} conversion as given in Table 1.3 and Fig. 1.2. In this experiment, Ferric ions reduced to ferrous ions with the colour of the reaction mixture changes from yellow to bluish green. Reducing power potential of extracts increased with the dose, however, plant extracts exhibited low reducing power than that of standard ascorbic acid. The methanol extract showed more reductive ability than the acetone and aqueous extracts, which was capable for neutralizing the free radicals. All the extracts of *P. persica* showed greater than 50.00% reducing power when tested on a concentration range of 20-100 µg/mL. Gulfishan and Akhtar (Gulfishan and Akhtar, 2014) tested the methanol extract of *P. persica* for reducing power assay and observed 76.60% antioxidant activity. While in present study, 73.05% (maximum) antioxidant activity was reported for the methanolic extract of *P. persica*.

TABLE 1.3 Antioxidant activity percentage (%) of tested medicinal plants by reducing power method at different concentrations

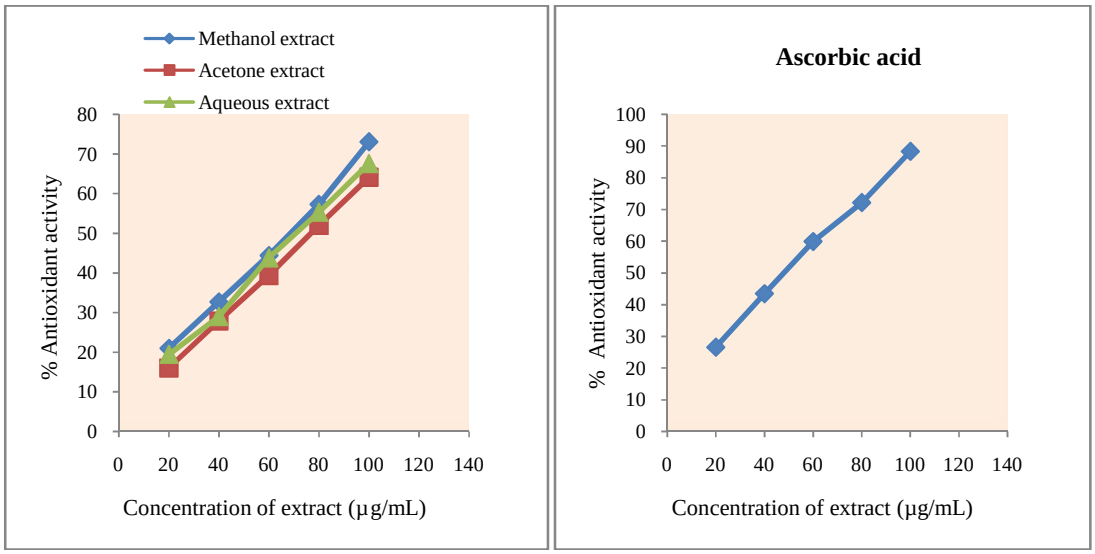
Concentration (µg/mL)	Methanol extract	Acetone extract	Aqueous extract	Ascorbic acid
20	21.00±1.00	16.05±1.17	19.45±0.50	26.55±2.25
40	32.70±0.30	27.90±0.15	29.00±0.00	43.44±0.45
60	44.40±0.52	39.40±0.80	43.80±0.66	59.90±1.20
80	57.30±2.59	52.00±0.00	55.30±0.56	72.15±0.54
100	73.05±0.45	64.15±1.60	67.60±1.30	88.30±1.50

Values are given as mean ± SD



(A) (B)

FIGURE 1.1 Percent scavenging (DPPH) activity of plant extracts at concentration range of 10-100 µg/mL (A) *P. persica* and (B) Standard curve of Ascorbic acid



(A) (B)

FIGURE 1.2 Antioxidant activity percentage (reducing power) of different plant extract at concentration range of 10-100 µg/mL (A) *P. persica*; (B) Standard curve of Ascorbic acid

CONCLUSION

It was concluded from the above experimental observations that the plant *Prunus persica* showed significant antibacterial and antioxidant activity at different concentrations. Methanol leaf extract was found to be more effective followed by acetone and aqueous leaf extracts. Further the leaf extract of *P. persica* showed more inhibitory effects in gram-positive bacteria than in gram-negative bacteria. This study also suggests that the plant extracts possess potent antioxidant activity, which might be helpful in preventing or slowing the progress of various oxidative stress-related diseases. Further investigation on the isolation and identification of

antioxidant component (s) in this plant may lead to chemical entities with potential for clinical use.

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COMPETING INTERESTS
Authors hereby declared that no competing interests exist.

REFERENCES

Balick, M.J. and Cox, P.A. (1996) Plants, people, and culture: the science of ethnobotany. New York: Scientific American Library.

Baranwal, A., Arora, S., Kumar, R.G., Prasad, B.K., Praharsha, J., Javed, A., Hidayatullah, M. and Sanobar, N. (2013) Evaluation of the combinational antimicrobial effect of *Prunus persica* and *Annona squamosa* seeds methanol extract on standard microbial strains. Global Journal of Bio-Science and Biotechnology, 2, 571-575.

Blois, M.S. (1958) Antioxidant determination by the use of stable free radicals. Nature. 26, 1199-1200.

Boer, D. (2005) Antifungal and antibacterial activity of some herbal remedies from Tanzania. Ethanopharmacol. 96, 461-469

Christabel, P.H., Nishaa, S., Vishnupriya, M., Sasikumar, J.M. and Gopalakrishnan, V.K. (2012) *In vitro* antioxidant studies and the scavenging potential of pulp and peel of *Prunus persica* L. fruit on different solvent systems. World J Pharm Res. 1, 1371-1386.

Deb, L., Gupta, R., Dutta, A., Yadav, A., Bhowmik, D. and Kumar, K. P. (2010) Evaluation of antioxidant activity of aqueous fraction of *Prunus persica* L. leaf aqueous extract. Der Pharmacia Sinica. 1, 157-164.

Gaikwad, S. A., Kamble, G. S., Devare, S., Deshpande, N. R. and Salvekar, J. P. (2011) *In vitro* evaluation of free redical scavenging potential of *Cassia auriculata*. Journal of Chem. Pharm. Res. 3, 766-772.

Gilani, A. H., Aziz, N., Ali, S. M. and Saeed, M. (2000) Pharmacological basis for the use of Peach leaves in constipation. Journal of Ethnopharmacology. 73, 87-93.

Goze, I., Alim, A., Tepe, A.S., Sokmen, M., Sevgi, K. and Tepe, B. (2009) Screening of the antioxidant activity of essential oil and various extracts of *Origanum rotundifolium* Boiss. from Turkey. Journal of Medicinal Plant Research. 3, 246-254.

Gulfishan and Akhtar, N. (2014) Phytochemical analysis and correlation of antioxidant activities of methanolic fruit extracts of family Rosaceae. Asian Journal of Chemistry. 26, 179-182.

Hemashenpagam, N. and Selvaraj, T. (2010) Antibacterial potential of different extracts of *Solanum xanthocarpum* Chard and Wendt. Plant Arch. 1, 387-390.

Kannan, P., Ramadevi, S.R. and Hopper, W. (2009) Antibacterial activity of *Terminalia chebula* fruit extract. Afr. J. Microbiol. 3, 180-184.

Kokate, C.K. and Purohit, A.P. (2004) Text book of pharmacognosy. Pune: Nirali Prakashan. 29, 542.

Magaldi, S., Mata-Essayag, S. and Capriles, H.C. (2004) Well-diffusion for antifungal susceptibility testing. Int. J. Infect. Dis. 8, 39-45.

McEwen, S.A. & Fedorka-Cray, P.J. (2002) Antimicrobial use and resistance in animals. Clin. Infect. Dis. 34, 93-106.

Meir, S., Kanner, J., Akiri, B. and Hadas, S.P. (1995) Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. J. Agric. Food Chem. 43**,** 1813-1817.

Oyaizu, M. (1986) Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition. 44, 307-315.

Pages, J.M., James, C.E. and Winterhalter, M. (2008) The porin and the permeating antibiotic: A selective diffusion barrier in gram-negative bacteria. Nat Rev Microbiol. 6, 893-903.

Parekh, J., Karathia, N. and Chanda, S. (2006) Evaluation of antibacterial activity and phytochemical analysis of *Bauhinia variegata* L. bark. Afr J Biomed Res. 29, 203-210.

Periyasamy, A., Rajkumar, R. and Mahalingam, K. (2010) Phytochemical screening and antimicrobial activity from five Indian medicinal plants against human pathogens. Middle-East Journal of Scientific Research. 5, 477-482.

Rabe, T. and Staden, V.J. (1995) Antibacterial activity of South African plants used for medicinal purposes. J. Ethanopharmacol**.** 56, 810-887.

Valgas, C., De Souza, S. M. and Smania, E.F.A. (2007) Screening methods to determine antibacterial activity of natural products. Braz. J. Microbiol. 38, 369-380.

Van Wyk, B.E. and Gericke, N. (2000) People's Plants: A guide to useful plants of Southern Africa. Pretoria. Briza Publications.