Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 02, pp.26071-26075, February, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITY OF PHENOLIC ACIDS FROM SOME SELECTED MEDICINAL PLANTS OF SOUTH INDIA

[1]Mohan, S., [*,2]Subramani, K. and [3]Tangavelou, A.C.

[1]Department of Chemistry, Kanchi Mamunivar Centre for Post Graduate Studies, Lawspet, Pondicherry 605 008
[2]Department of Chemistry, Bharathidasan Government College for Women, Pondicherry 605 001
[3]Post Graduate & Research Department of Botany, Periyar Arts College, Cuddalore 607 001

ARTICLE INFO

Article History:
Received 18th November, 2015
Received in revised form
15th December, 2015
Accepted 25th January, 2016
Published online 14th February, 2016

Key words:

Medicinal plants,
Phenolic acids,
Antimicrobial activity,
Drug development.

ABSTRACT

In the search of plants as a source of natural antibiotics, four medicinal plants namely *Anisomeles malabarica* (L.) R. Br. ex Sims (Lamiaceae), *Erythrina variegata* L., (Leguminosae) and *Merremia gangetica* Cufod., (Convolvulaceae) and *Operculina turpethum* (L.) Silva Manso (Convolvulaceae) were selected for the present investigation. In the present study, four bioactive phenolic acids were isolated from methanolic extract of the selected plants viz., tannic acid (*A. malabarica*), ferulic acid (*E. variegata*), chlorogenic acid (*M. gangetica*), *p*-coumaric acid (*O. turpethum*) and their identities were confirmed with previous reports. The isolated molecules were subjected to determine the antimicrobial activity by Agar well diffusion method. The study reveals that all the phenolic acids showed significant antimicrobial activities against tested gram-negative, gram-positive and fungal organisms. Based on this, it is concluded that all the phenolic acids exhibited significant antibiotic activity and thus it proved as a potent antibiotic therapeutic agent to develop a plant based drug from these bioactive molecules against various pathogenic infections.

Citation: **Mohan, S., Subramani, K. and Tangavelou, A.C, 2016.** "Antimicrobial activity of phenolic acids from some selected medicinal plants of South India", *International Journal of Current Research,* 8, (02), 26071-26075.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine (Cragg and Newman, 2001; Lopez, 2011). These plant-based traditional medicine systems continue to play an essential role in health care and 80% of the world population relies on Traditional Medicine for their primary health care needs (Farnsworth and Soejarto, 1991; Pei, 2001; WHO, 2002; Robinson and Zhang, 2011). Modern medicine draws heavily from traditional medicine which exists in every continent of the globe and in every cultural area of the world. The global demand for Traditional Medicine is not only large, but is growing (Srivastava, 2006) and 85% of the traditional medicines involve the use of plant extracts. During the past decade, traditional medicine became more and more important for preventive and therapeutic purposes (Tistaert *et al*., 2011).

****Corresponding author: Subramani***
Department of Chemistry, Bharathidasan Governement College for Women, Pondicherry 605 001

Nearly 25% of modern medicines are derived from plants first used in traditional medicine (WHO, 2007&2011). Modern pharmacopoeias contain at least 25% of drugs derived from plants and many others, which are synthetic analogues built on prototype compounds isolated from plants (Farnsworth *et al*., 1985; Astin, 1998; De Silva, 2005; Douglas Kinghorn, 2011). It is interesting to note that the ethnomedicinal approach of plants is the most successful criteria used by the pharmaceutical industry in finding new therapeutic leads for various fields of biomedicine (Cox and Balick, 1994). Interest in the antimicrobial properties of active compounds is strengthened by the findings that they affect the behavior of pathogenic bacteria or fungi of agro-food or medical field. Indeed, their use as natural additives in food industry is increased in recent years (Nazzaro *et al*., 2009). The antimicrobial activity of phenolics and flavonoids are also well documented (Erdemoglu *et al*., 2007; Milovanović *et al*., 2007; Xia *et al*., 2011a). Due to their antibacterial, antifungal and antiviral activity, phenolic biomolecules were the subject of anti-infective research for many years (Hulin *et al*., 1998; Suppakul *et al*., 2003; Lai and Roy 2004; Cushnie and Lamb 2005; Fattouch *et al*., 2007; Szabo *et al*., 2010). These

activities suggested that phenolic compounds can be used as chemotherapeutic agents, food preserving agents and disinfectants (Dorman and Deans 2000). They can affect the growth and metabolism of bacteria, activating or inhibiting the microbial growth according to their constitution and concentration (Nazzaro *et al*., 2009). With this background, in this paper, the isolated bioactive molecules from the four important selected medicinal plants were subjected for antimicrobial potential.

MATERIALS AND METHODS

Plant material

Four medicinal plants namely *Anisomeles malabarica* (L.) R. Br. ex Sims (Lamiaceae), *Erythrina variegata* L., (Leguminosae) and *Merremia gangetica* Cufod., (Convolvulaceae) and *Operculina turpethum* (L.) Silva Manso (Convolvulaceae) were selected for the present study to determine the antioxidant activity of the isolated bioactive molecules. Fresh aerial parts of all the plants were collected from Marakanam forest vicinity of Villupuram district, Tamil Nadu. The herbarium specimens were prepared for each plant, botanically identified and submitted at the Department of Botany, Kanchi Mamunivar Centre for Post Graduate Studies, Puducherry.

Extraction and Isolation

The air dried aerial parts of the plant (1 kg) were extracted thrice with boiling 95% EtOH (3×5L) and concentrated in vacuo to 500ml. The aqueous alcoholic concentrate was fractionated into benzene, ether, ethyl acetate, ethyl methyl ketone solubles. Benzene, fraction on paper chromatography gave (15% AcOH) no characteristic spots for polyphenolics and was not worked up further. The ether fraction was column chromatographed over sephadex LH-20. 20 fractions each of 10 ml were collected.

Antimicrobial activity

Test organisms

All the microbial strains of human pathogens were assigned from Institute of Microbial Technology (IMTECH), Chandigarh. These microorganisms include the Gram-negative bacteria, viz. *Escherichia coli* (MTCC 724), *Proteus vulgaris* (MTCC 426), *Salmonella typhi* (MTCC 733), *Vibrio parahaemolyticus* (MTCC 451); the Gram-positive bacteria, viz *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96) and *Streptococcus pneumoniae* (MTCC 655) and fungi *viz*., *Candida albicans* (MTCC 227) respectively.

Agar well-diffusion method

Agar well-diffusion method (Perez *et al*. 1990) was used to determine the antimicrobial activity. Mueller-Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) (Hi-Media, Mumbai) were used respectively for testing the antibacterial and antifungal activities. MHA and SDA plates were swabbed (sterile cotton swabs) with 8 h old - broth culture of respective bacteria and fungi. Four wells (10mm diameter) were made in each of these plates using sterile cork borer. About 0.3 ml of different concentrations of plant extracts were added using sterilized dropping pipettes into the wells and allowed to diffuse at room temperature for 2 h. Control experiments comprising inoculums without plant extract were set up. Respective solvents were used as control. The plates were incubated at 37°C for 18-24 h for bacterial pathogens and 28°C for fungal pathogens. Diameter of the inhibition zones was recorded. Triplicates were maintained and the experiment was repeated thrice and the average values were recorded.

RESULTS

Antimicrobial activity

In the present study, all the tested extracts showed concentration-dependent activity against the tested organisms.

Chlorogenic acid

In chlorogenic acid, the zone of inhibition recorded was ranged from 11 to 18mm against gram-positive bacteria, 12 to 16mm against gram-negative bacteria and 16mm against fungi at various concentrations. For gram-positive bacteria, maximum zone of inhibition was recorded as 18mm against *S. pneumoniae* at 100µg/ml concentration. Moderate zone of inhibition was recorded as 17mm against *B. subtilis* at 100µg/ml concentration, 13mm against *S. pneumoniae* at50µg/ml concentration and minimum zone of inhibition was recorded as 11mm at 25µg/ml concentration respectively. For gram-negative bacteria, maximum zone of inhibition was recorded as 16mm each against *E.coli* and *S. typhi* at 100µg/ml concentrations, moderate zone of inhibition was recorded as 14mm each against *E.coli* at 25µg/ml concentration and against *P. vulgaris* at1000µg/ml concentration and 13mm against *V. parahaemolyticus* at 50µg/ml concentration while minimum zone of inhibition was recorded as 12mm each against *E.coli* at 25µg/ml, *S. typhi* at 50µg/ml and *V. parahaemolyticus* at 25µg/ml concentrations respectively. For fungi, 16mm was recorded against *C. albicans* at 100µg/ml concentration respectively. Moreover, no inhibitory zone was recorded against *S. aureus*.

Ferulic acid

In ferulic acid, the zone of inhibition recorded was ranged from 13 to 17mm against gram-positive bacteria, 11 to 17mm against gram-negative bacteria and 17mm against fungi at various concentrations. For gram-positive bacteria, maximum zone of inhibition was recorded as 17mm each against *B. subtilis* and *S. penumoniae*at 100µg/ml concentration. Moderate zone of inhibition was recorded as 15mm each against *B. subtilis* and *S. pneumoniae*at 50µg/ml concentration and against *S. aureus* at 100µg/ml concentration, 14mm against *B. subtilis*at25µg/ml concentration and minimum zone of inhibition was recorded as 13mm against *S. pneumoniae*at 25µg/ml concentration respectively. For gram-negative bacteria, maximum zone of inhibition was recorded as 17mm each against *S. typhi* at 100µg/ml concentration, moderate zone of inhibition was recorded as 15mm each against *S. typhi* at

50µg/ml concentration and *V. parahaemolyticus* at 100µg/ml concentrations while minimum zone of inhibition was recorded as 11mm each against *V. parahaemolyticus*li at 25 and 50µg/ml concentrations respectively. For fungi, 17mm was recorded against *C. albicans* at 100µg/ml concentration respectively. Moreover, no inhibitory zone was recorded against *E. coli, P. vulgaris*.

50µg/ml concentration and against *V. parahaemolyticus* at 100µg/ml concentrations. While minimum zone of inhibition was recorded as 10mm *V. parahaemolyticus* at 25µg/ml concentration respectively. There is no activity against *E.coli*. For fungi, 14mm was recorded against *C. albicans* at 100µg/mlconcentration respectively.

Table 1. Antimicrobial activity of isolated Phenolic Compounds

Microrganisms	Chlorogenic acid (µg/ml)			Ferulic acid (µg/ml)			p-coumaric acid (µg/ml)			Tannic acid (µg/ml)			Standard (10µg/ml)
	25	50	100	25	50	100	25	50	100	25	50	100	
B. subtilis	-	14	17	14	15	17	-	14	16	13	15	18	33 (A)
S. aureus	-	-	-	-	-	15	11	13	14	-	-	16	34 (A)
S. pneumoniae	11	13	18	13	15	17	-	-	-	14	15	16	35 (C)
E. coli	12	14	16	-	-	-	10	12	16	-	-	-	32 (A)
P. vulgaris	-	-	14	-	-	-	10	13	14	12	14	15	33 (Cl)
S. typhi	-	12	16	12	15	17	-	-	-	-	13	15	32 (Cf)
V. parahaemolyticus	12	12	13	11	11	15	12	15	15	10	12	14	33 (K)
C. albicans	-	-	16	-	-	17	12	13	15	-	-	14	31 (N)

p-coumaric acid

In *p*-coumaric acid, the zone of inhibition recorded was ranged from 11 to 16mm against gram-positive bacteria, 10 to 16mm against gram-negative bacteria and 12 to 15mm against fungi at various concentrations. For gram-positive bacteria, maximum zone of inhibition was recorded as 16mm against *B. subtilis* at 100µg/ml concentration. Moderate zone of inhibition such as 14mm each was recorded against *B. subtilis* at 50µg/ml concentration *S. aureus*at 100µg/ml concentration. There is no activity against *S. pneumoniae*. For gram-negative bacteria, maximum zone of inhibition was recorded as 16mm each against *E.coli*, moderate zone of inhibition was recorded as 15mm against *V. parahaemolyticus* at 50 and 100µg/ml concentrations. Moderate zone of inhibition such as 12mm against *P. vulgaris* at 50µg/ml concentration while minimum zone of inhibition was recorded as 12mm each against *E.coli* at 50µg/ml and *V. parahaemolyticus*at 25µg/ml concentrations and 10mm against E.coli and P. vulgaris at 25µg/ml respectively. For fungi, 15mm was recorded against *C. albicans* at 100µg/ml, 13 mm at 100µg/ml and 12mm at 100µg/ml concentrations respectively. Moreover, no inhibitory zone was recorded against *S. pneumoniae* and *S. typhi*.

Tannic acid

In tannic acid, the zone of inhibition recorded was ranged from 13 to 18mm against gram-positive bacteria, 12 to 15mm against gram-negative bacteria and 14 against fungi at various concentrations. For gram-positive bacteria, maximum zone of inhibition was recorded as 18mm against *B. subtilis* at 100µg/ml concentration. Moderate zone of inhibition such as 16mm each was recorded against *S. aureus* and *S. pneumoniae* at 100µg/ml concentrations, 15mm each against B. subtilis and S. pneumoniae at 50µg/ml concentrations, 14mm against S. pneumoniae at 25µg/ml concentrations respectively. Minimum zone of inhibition was recorded as 13mm againt B. subtilisat 25µg/ml concentration. For gram-negative bacteria, maximum zone of inhibition was recorded as 15mm each against*P. vulgaris* and *S. typhi* at 100µg/ml concentrations and moderate zone of inhibition such as 14mm each *against P. vulgaris* at

DISCUSSION

Microorganisms have been developing resistance to many antibiotics, due to the indiscriminate use of antimicrobial drugs inducing thus an increase of problems in clinical treatment of infectious diseases (Rios and Recio, 2005; Kilani-Jaziri, 2011). In addition, antibiotics are sometimes associated with adverse effects in the host which include hypersensitivity, depletion of gut and mucosal microorganisms, immunosuppressant and allergic reactions. Therefore, there is a need for alternative antimicrobial drugs for the treatment of infectious diseases. One approach is to screen local medicinal plants for possible antimicrobial properties. Medicinal herbs represent a rich source from which novel antibacterial and antifungal chemotherapeutic agents may be obtained (Rates, 2001; Kilani-Jaziri, 2011).

In the present study, all the tested phenolic acids showed concentration-dependent activity against the tested organisms. The present study revealed that the phenolic acids have the medicinal potential to develop a drug against various diseases such as food poison caused by B. subtilis, skin infections (such as pimples, impetigo, boils, folliculitis, carbuncles, scalded skin syndrome, abscesses), pneumoniae, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), chest pain, bacteremia, sepsis, nosocomial infections, hospital infections, gastroenteritis, urinary tract infections, neonatal meningitis, haemolytic uremic syndrome, peritonitis, mastitis, septicaemia caused by S. aureus, pneumococcal infections other than pneumonia, including acute sinusitis, meningitis, bacteremia, sepsis, osteomyelitis, septicarthritis, endocarditis, peritonitis caused by S. pneumoniae and gram-negative pneumonia caused by E. coli, urinary tract infections caused by P. vulgaris, headache, weakness, fatigue, sore throat infection, abdominal pain, typhoid fever, severe constipation or diarrhea, weight loss or even delirium caused by S. typhi, gastroenteritis caused by V. parahaemolyticus and candidiasis caused by C. albicans respectively. The isolated molecules have been previously reported as antimicrobial potential such as chlorogenic acid (Babic *et al*., 1994; Albayrak *et al*., 2010; Xia *et al*., 2011a& 2011b; Karunanidhi *et al*., 2013; Nirmal

kumar *et al*., 2014), coumarin (Maddox *et al*., 2010), tannic acid (Taguri *et al*., 2004; Bancirova, 2010). The mechaniasm for antimicrobial activities of phenolics products involves destabilization and permeabilization of cytoplasmatic membrane and enzyme inhibition by the oxidized products, possibly through reaction with sulfhydyl groups or through more nonspecific interactions with the proteins and create significant change in cell surface hydrophobicity, charge, induced PI uptake, and K+ leakage (Cowan, 1999; Puupponen-Pimia *et al*., 2005; Bittner, 2006; Muthuswamy and Rupasinghe, 2007; Maddox *et al*., 2010; Borges *et al*., 2013). Phenols can also inhibit the synthesis of nucleic acids of both Gram-negative and Gram-positive bacteria (Cushnie and Lamb, 2005).

Conclusion

Thus from the present study it is concluded that the isolated phenolic acids such as chlorogenic acid, ferulic acid, p-coumaric acid and tannic acid possess antibiotic potential which would be very useful to develop a potent antibiotic drug for several diseases.

REFERENCES

Albayrak S., Aksoy A., Sagdic O., Budak, U. 2010. Phenolic compounds and antioxidant and antimicrobial properties of Helichrysum species collected from eastern Anatolia, *Turkey. Turk. J. Biol.* 34(4):463-473.

Astin, J.A. 1998. Why patients use alternative medicine: results of a national study. *J. American Med. Associ* .279: 1548-1553.

Babic I., Nguyenthe C., Amiot M., Aubert S. 1994. Antimicrobial Activity of Shredded Carrot Extracts on Foodborne Bacteria and Yeast. *J. Appl. Bacteriol.* 76(2):135- 141.

Bancirova M. 2010. Comparison of the antioxidant capacity and the antimicrobial activity of black and green tea. *Food Research International* 43(5):1379-1382.

Bittner, S. 2006. When quinones meet amino acids: chemical, physical and biological consequences. Amino Acids 30:205– 224.

Borges, A., Ferreira, C., Saavedra, M.J. and Simo˜es, M. 2013. Antibacterial Activity and Mode of Action of Ferulic and Gallic Acids Against Pathogenic Bacteria. *Microbial Drug Resistance* Doi: 10.1089/mdr.2012.0244.

Cowan, M.M. 1999. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* 12:564–582.

Cox, P.A. and Ballick, M.J. 1994.The ethnobotanical approach to drug discovery. *Sci. American* 270(6): 82-87.

Cragg, G.M. and Newman, D.J. 2001.Medicinals for the millennia. Ann. New York Acad. Sci.953: 3-25.

Cushnie, T.P.T., and A.J. Lamb. 2005. Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents* 26:343–356.

De Silva, T. 2005. Industrial Utilization of Medicinal Plants in Developing Countries. Chemical Institutes Branches, Industrial Sectors and Environment Division, United Nation Industrial Development Organization, pp. 1–11, http://www.fao.org/docrep/W7261e07.html.

Dorman H., Deans S. 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J. Appl. Microbiol.* 88(2):308-316.

Douglas Kinghorn, A., Pan, L., Fletcher, J.N. and Chai, H. 2011. The Relevance of Higher Plants in Lead Compound Discovery Programs. *J. Nat. Prod.* dx.doi.org/10.1021/np200391c.

Erdemoglu, N., Ozkan, S., Tosun, F. 2007. Alkaloid profile and antimicrobial activity of Lupinus angustifolius L. alkaloid extract. *Phytochemistry Reviews* 6(1):197-201.

Farnsworth, N. R., Akerele, O., Bingel, A. S., Soejarta, D. D. and Eno, Z. 1985. Medicinal plants in therapy. *Bulletin of World Health Organization*63:226–230.

Farnsworth, N.R. and Soejarto, D.D. 1991. Global importance of medicinal plants. In: The conservation of medicinal plants (ed. O. Akerele, V. Heywood and H. Synge), pp. 25-51. Cambridge University Press, Cambridge, UK.

Fattouch, S., Caboni, P., Coroneo, V., Tuberoso, C., Angioni, A., Dessi, S., Marzouki, N., Cabras, P. 2007. Antimicrobial Activity of Tunisian Quince (Cydonia oblonga Miller) Pulp and Peel Polyphenolic Extracts. *J. Agric. Food Chem.* 55(3):963-969.

Hulin, V., Mathot, A., Mafart, P., Dufosse, L. 1998. Antimicrobial properties of essential oils and flavour compounds. *Sci. Aliments* 18(6):563-582.

Karunanidhi, A., Thomas, R., Belkum, A.V. and Neela, V. 2013. In Vitro Antibacterial and Antibioflm Activities of Chlorogenic Acid against Clinical Isolates of Stenotrophomonas maltophilia including the Trimethoprim/Sulfamethoxazole Resistant Strain. BioMed Research International 2013, Article ID 392058, doi.org/10.1155/2013/392058.

Kilani-Jaziri, S., Bhouri, W., Skandrani, I., Limem, I., Chekir-Ghedira, L. and K. Ghedira. 2011. Phytochemical, antimicrobial, antioxidant and antigenotoxic potentials of Cyperus rotundus extracts. *South African Journal of Botany* 77: 767-776.

Lai, P., Roy, J. 2004. Antimicrobial and chemopreventive properties of herbs and spices. *Curr. Med. Chem.* 11(11):1451-1460.

Lopez, V. 2011. Are Traditional Medicinal plants and ethnobotany still valuable approaches inPharmaceutical Research?.Boletín Latinoamericanoy del Caribe de Plantas Medicinales y Aromáticas 10(1): 3-10.

Maddox, C.E., L.M. Laur, and L. Tian. 2010. Antibacterial activity of phenolic compounds against the phytopathogen Xylella fastidiosa. *Curr. Microbiol.* 60:53–58.

Milovanović, V., Radulović, N., Todorović, Z., Stanković, M., Stojanović, G. 2007. Antioxidant, Antimicrobial and Genotoxicity Screening of Hydro-alcoholic Extracts of Five Serbian Equisetum Species. Plant Foods for Human Nutrition (Formerly Qualitas Plantarum) 62(3):113-119.

Muthuswamy, S., and H.P.V. Rupasinghe. 2007. Fruit phenolics as natural antimicrobial agents: selective antimicrobial activity of catechin, chlorogenic acid and phloridzin. *J. Food Agr. Environ.* 5:81–85.

Nazzaro F., Caliendo G., Arnesi G., Veronesi A., Sarzi P., Fratianni F. 2009. Comparative Content of Some Bioactive Compounds in Two Varieties of Capsicum Annuum L. Sweet Pepper and Evaluation of Their Antimicrobial and Mutagenic Activities. *J. Food Biochem.* 33(6):852-868.

Nirmal kumar, G, Yogesh, S., Saravanakumar, P., Dhayananth, N. and Rameshbabu, N. G. 2014. Chlorogenic Acid From

Coffee Waste As A Preservativeagainst Bread Spoilage. *Int. J. Engg. Res. & Sci. & Tech.* 3 (2): 374-378.

Pei, S. 2001. Ethnobotanical approaches of traditional medicine studies: some experiences from Asia. *Pharmaceu. Botany*. 39:74-79.

Perez, C., Pauli, M. and Bazerque, P.1990.An antibiotic assay by agar-well diffusion method. *Acta Biol. Med. Exp.*15: 113-115.

Puupponen-Pimia¨, R., L. Nohynek, H.L. Alakomi and K.M. Oksman-Caldentey. 2005. Bioactive berry compounds—novel tools against human pathogens. *Appl. Microbiol. Biotechnol.* 67:8–18.

Rates, S.M.K. 2001. Plants as source of drugs. Toxicon.39: 603-613.

Rios, J.L. and Recio, M.C. 2005. Medicinal plants and antimicrobial activity. *J. Ethnopharmacol.*100: 80-84.

Robinson, T. 1963. The organic constituents of higher plants, bargles, U.S.A. p. 107.

Srivastava, R.C. 2006. Plant identity crisis in Indian systems of Medicines. *Curr. Sci.* 91(9): 1125.

Suppakul, P., Miltz, J., Sonneveld, K., Bigger, S. 2003. Antimicrobial properties of basil and its possible application in food packaging. *J. Agric. Food Chem.* 51(11):3197-3207.

Szabo M., Radu D., Gavrilas S., Chambre D., Iditoiu C. 2010. Antioxidant and Antimicrobial Properties of Selected Spice Extracts. *Int. J. Food Prop.* 13(3):535-545.

Taguri T., Tanaka T., Kouno I. 2004. Antimicrobial activity of 10 different plant polyphenols against bacteria causing food-borne disease. *Biol. Pharm. Bull.* 27(12):1965-1969.

Tistaert, C., Dejaegher, B. and Heyden, Y.V. 2011. Chromatographic separation techniques and data handling methods for herbal fingerprints: A review. *Analytica Chimica Acta*. 690: 148-161.

WHO, 2002. Traditional Medicine Strategy2002-2005. World Health Organization, Geneva.

WHO, 2007. WHO Country Cooperation Strategy 2006-2011. Supplement on Traditional Medicine. World Health Organization, New Delhi.

WHO, 2011. WHO Country Cooperation Strategy 2006-2011. Supplement on Traditional Medicine. World Hel;ath Organizartion, New Delhi.

Xia D., Wu X., Shi J., Yang Q., Zhang Y. 2011a. Phenolic compounds from the edible seeds extract of Chinese Mei (Prunus mume Sieb. et Zucc) and their antimicrobial activity. LWT - *Food Science and Technology* 44(1):347-349.

Xia D., Wu X., Shi J., Yang Q., Zhang Y. 2011b. Phenolic compounds from the edible seeds extract of Chinese Mei (Prunus mume Sieb. et Zucc) and their antimicrobial activity. *LWT-Food Sci. Technol*. 44(1):347-349.

****