EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 8
584-589
Year: 2018

GC-MS ANALYSIS AND ANTIMICROBIAL POTENTIAL OF *GARCINIA XANTHOCHYMUS*, Hook. F. ex J. Anders., LEAF EXTRACT

Kandasamy Palanisamy Jaiganesh*[1], Nataraj Priya[2], Ramamoorthi Karthik[1] and Balakrishnan Sreedharren[1]

[1]Division of Pharmacognosy and Phytochemistry Research Laboratory, Nehru College of Pharmacy, Pampady, Thiruvilwamala, Thrissur, Kerala, India.
[2]Ahalia School of Pharmacy, Kozhippara, Palakkad, Kerala, India.

***Corresponding Author: Dr. Kandasamy Palanisamy Jaiganesh**
Division of Pharmacognosy and Phytochemistry Research Laboratory, Nehru College of Pharmacy, Pampady, Thiruvilwamala, Thrissur, Kerala, India.

Article Received on 20/06/2018 **Article Revised on 10/07/2018** **Article Accepted on 31/07/2018**

ABSTRACT
Garcinia xanthochymus, is a medium-sized, glabrous, deciduous tree, is one of the medicinally important plant belonging to the Family Clusiaceae. Traditionally fruits of the plant are used as anthelmintic, cardio tonic and improve appetite. Ripe fruit is tonic, invigorating and alexipharmic, good in heart trouble and biliousness. In the present investigation, preliminary phytochemical screening, GC-MS analysis and antimicrobial studies were carried out for ethanol extracts (leaf and stem) against gram positive bacteria (*Staphylococcus aureus*), gram negative bacteria (*Escherichia coli*) and fungi (*Aspergillus niger*) by detecting the zone of inhibition using agar well diffusion method. Preliminary phytochemical screening showed the presence of phenolic compounds and flavonoids. GC-MS analysis of ethanolic of leaf and stem extracts of *Garcinia xanthochymus* were exhibited the presence of 24 and 17 compounds respectively as reported 2,3-dihydro benzofuran, nepetalactol, neophytodiene and squalene. Ethanol of leaf and stem extracts (25, 50, 100 mg/ml) exhibited significant antimicrobial activity against bacteria and fungi using the respective standard antibiotics (10µg/ml).

KEYWORDS: *Garcinia xanthochymus, Phytochemical study, GC-MS analysis, Antimicrobial activity.*

INTRODUCTION
For thousands of years, plant products and their modified derivatives have been rich source for clinically useful drugs. Even today, about 80% of the world's population relies predominantly on plants and plant extracts for health care.[1] Medicinal plants have been used for centuries as remedy for human diseases because they contain the compounds of therapeutic values.[2]

Infectious diseases are the leading cause of death worldwide. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, as a pure compound or as a standardized plant extracts provide unlimited opportunities for new drug lead because of the unmatched availability of chemical diversity. An increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic and infectious agents has led to the screening of medicinal plants for their antimicrobial potential. In recent years, secondary metabolites (phytochemicals) previously with unknown pharmacological activities have been extensively investigated as a source of medicinal plants. Thus, it is anticipated that the phytochemicals with adequate anti-infective efficacy will be used for the treatment of various infections caused by pathogens.[3] Therefore, there is a need to develop the efficient, safe and inexpensive drugs from plant source are of great importance.

Garcinia species *G. indica* is predominantly seen in the northern parts of Western Ghats. Other species located were *G. morella* in Siruvani forest of Palaghat district of Kerala and *G. xanthochymus* and *G. hombroniana* in the forests of South Kanara in Karnataka, indicating the poor distribution of these species. Out of the 12 species reported to exist in South India [4] only five species, *G. cambogia*, *G. indica*, *G. hombroniana*, *G. xanthochymus* and *G. morella* are available. Antimicrobial xanthochymol,[5] NGF potentiating prenylated xanthones and free radical scavenging phenolic compounds,[6] antioxidant principles like stearic, oleic, linoleic, linolenic, palmitic, myristic and arachidic acid,[7] and chemopreventive bi flavonoid mrelloflavone [8] were reported from the different parts of *Garcinia xanthochymus*.

MATERIALS AND METHODS
Collection of plant material
The taxonomically identified *Garcinia xanthochymus* plant (leaf and stem) was collected from Bangalore, Karnataka, India. A herbarium for morphological studies

was prepared, identified and authenticated by a taxonomist Dr. A.C. Tangavelou, M.Sc., M.Phil., Ph.D., The School of Liberal Arts and Sciences, Trans Disciplinary University, Yelahanka, Bengaluru 500064, Karnataka, India.

Preparation of crude drug powder

The collected leaves and stems of *Garcinia xanthochymus* were cleaned and washed with running water and dried at room temperature for 2 to 3 weeks. Then the dried plant materials were powdered by using pulverizer and stored in air tight container.

Preparation of extracts

The powdered plant materials were extracted with 70% ethanol by continuous hot percolation method using soxhlet apparatus.[9] Then, ethanol extracts (leaf, stem) were collected and distilled off on a water bath at atmospheric pressure and the last trace of the solvent was removed *in vaccuo*. The resulted extracts were used for preliminary phytochemical screening, GC-MS analysis and antimicrobial studies.

Preliminary phytochemical screening

Ethanol leaf and stem extracts were subjected to preliminary phytochemical screening by adopting standard methods.[9,10]

GC- MS Analysis of Plant Extracts[11]

The GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer system, comprising a Aoc-20i Auto sampler and Gas chromatograph interfaced to a mass spectrometer instrument employing the conditions: Column Elite. 5Ms fused silica capillary column (30 × 0.25 mm, 1D × 1mm df, composed of 95% Dimethyl Poly diloxane, 5% Diphenyl Polysixone), operating in electron impact mode at 70ev; helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 2 ml was employed (split ratio of 10: 1), injector temperature 2000˚C; ion-source temperature 2000˚C. The oven temperature was programmed from 1100˚C (isothermal for 2 min), with an increase of 100˚C/min, to 2000˚C, then 50˚C/min, to 2800˚C, ending with a 9 min isothermal at 2800˚C. Mass spectra were taken at 70ev; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36 min.

Identification of Compounds

GC-MS chromatogram was subjected to interpretation by using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of test materials were characterized and identified.

Antimicrobial screening

Test Microorganisms

The following bacterial and fungal strains were used for the screening of antimicrobial activity. All the microbial strains of human pathogens used were procured from IMTECH (Institute of Microbial Technology), Chandigarh and the procured microbes are the Gram-negative bacteria, such as *Escherichia coli* (MTCC 724), Gram-positive bacteria *Staphylococcus aureus (*MTCC 96), and fungi *Aspergillus niger* (MTCC 1344). The cultures were emulsified in 5ml of Nutrient Broth (NA) and incubated for 24 hrs. Fresh cultures were employed for assessing antimicrobial potential of the prepared extracts.

Antimicrobial Activity[12]

Agar well-diffusion method[13] was followed to determine the antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 8 hours old - broth culture of respective bacteria and fungi. Two wells (10mm diameter) were made in each of these plates using sterile cork borer. About 0.3 ml of different concentrations (25, 50, 100mg/ml) of plant extracts were added using sterilized dropping pipettes into the wells and allowed to diffuse at room temperature for 2 hours. The plates were incubated at 37˚C for 18-24 hours for bacterial pathogens and 28˚C for fungal pathogens. Respective solvent control for bark extracts was also maintained and the diameter of zone of inhibition was recorded in mm and compared with standard values. Triplicates were maintained and the experiment was repeated thrice and the average values were recorded for antimicrobial activity.

RESULTS AND DISCUSSION

Phytochemical screening

Qualitative tests of ethanol extracts leaf and stem extracts *Garcinia xanthochymus* showed the presence of phenolic compounds, flavonoids, tannins, lignins, carbohydrates and amino acids and were depicted in Table 1.

Table 1: Preliminary phytochemical screening of *Garcinia xanthochymus.*

S. NO	Phytoconstituents	Ethanol leaf extract	Ethanol stem extract
1	Carbohydrates	+	+
2	Glycosides	-	-
3	Fixed oils and fats	+	+
4	Saponins	-	-
5	Phenolic compounds	+	+
6	Tannins	+	+
7	Proteins &amino acids	+	+
8	Flavonoids	+	+
9	Gums and mucilages	-	-
10	Lignins	+	+
11	Phytosterol	-	-
12	Alkaloids	-	-

(+) = Presence of phytoconstituents; (-) = Absence of phytoconstituents

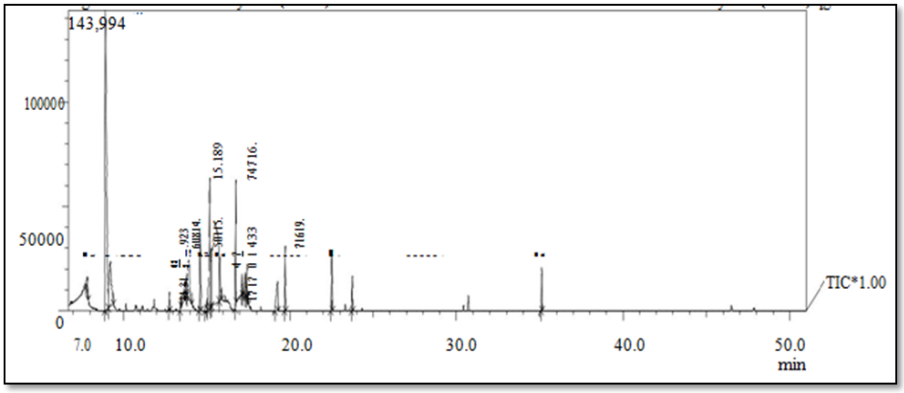

Figure 1: GC-MS chromatogram of *Garcinia xanthochymus leaf* extract.

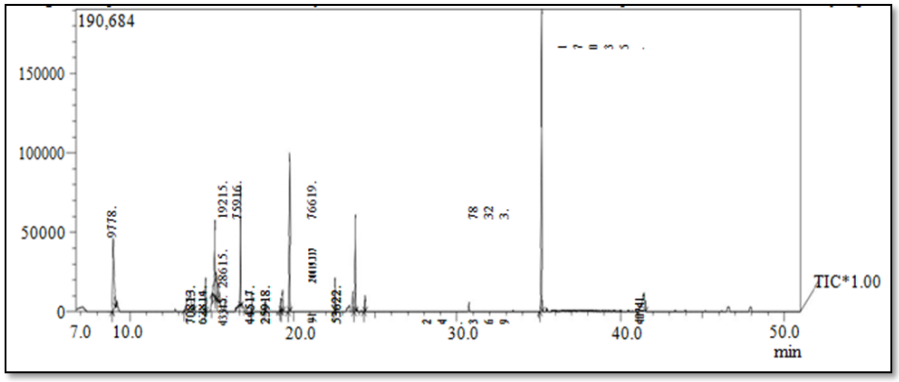

Figure 2: GC-MS chromatogram of *Garcinia xanthochymus* stem extract

Table 2: Components identified in ethanol leaf extract of *Garcinia xanthochymus.*

Peak #	R. Time	Area	Area%	Height	Height %	Name	Base m/z
1	7.839	34101	1.01	7626	1.21	(methoxy amino) methane	61.00
2	8.926	879094	26.09	143644	22.83	2,3-dihydro-benzofuran	120.10
3	9.224	198419	5.89	21582	3.43	5-hydroxymethyl furfural	97.00
4	12.766	10492	0.31	8599	1.37	benzene, 1-methyl-4-(1-nitrocyclohexyl)-	173.10
5	13.438	8803	0.26	5041	0.80	tridecanoic acid	60.00
6	13.717	23639	0.70	5095	0.81	glutaric anhydride, β, β.-dimethyl-	56.05
7	13.808	35723	1.06	9049	1.44	2-(N-ethyl) imino-3-pentaone	70.05
8	13.923	143456	4.26	18181	2.89	β.-D-glucopyranose, 1,6-anhydro-	60.00
9	14.608	110981	3.29	39047	6.20	9-octadecen-1-ol, (Z)-	85.05
10	14.917	22060	0.65	2032	0.32	β - methylvaleric acid	60.00
11	15.189	264117	7.84	62149	9.88	(-)- nepetalactol	93.05

12	15.270	140709	4.18	27963	4.44	heptane, 1-fluoro-	69.00
13	15.501	868271	25.77	39053	6.21	β-D-glucopyranose, 1,6-anhydro-	60.00
14	15.792	74892	2.22	20746	3.30	neopentyl glycol	56.00
15	16.747	114673	3.40	59867	9.51	neophytadiene	68.05
16	17.121	18288	0.54	9944	1.58	hexanenitrile	55.00
17	17.308	42282	1.25	11018	1.75	pentane, 2,3,4-trimethyl-	71.00
18	17.340	25011	0.74	11173	1.78	4H-1,2,4-triazole, 3,4,5-trimethyl-	110.05
19	17.433	32393	0.96	17022	2.70	(-)- β -Citronellene	82.10
20	19.256	95295	2.83	14072	2.24	pentachlorobromobenzene	73.00
21	19.716	76838	2.28	31223	4.96	Hexadecanoic acid, ethyl ester	88.05
22	22.507	63074	1.87	27269	4.33	(+-)-citronellol	71.05
23	23.739	39736	1.18	16909	2.69	6(E),9(Z),13(E)-pendectriene	55.05
24	35.116	47102	1.40	20997	3.34	(E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene	69.05
		3369449	100.00	629301	100.00		

Table 3: Components identified in ethanol stem extract of *Garcinia xanthochymus.*

Peak #	R. Time	Area	Area%	Height	Height%	Name	Base m/z
1	8.977	247868	11.07	45535	6.93	coumaran	120.10
2	13.708	165424	7.39	10158	1.55	valeric acid	60.00
3	14.628	42787	1.91	20684	3.15	2-tetradecynal, 4-hydroxy-	85.10
4	15.192	168660	7.53	48333	7.36	(+)-nerolidol	69.05
5	15.286	65821	2.94	16374	2.49	1-hexanol, 4-methyl-, (S)-	70.05
6	15.433	73145	3.27	7274	1.11	butyric acid	60.00
7	16.759	134043	5.99	75685	11.52	neophytadiene	68.05
8	17.445	21094	0.94	12664	1.93	2-decen-1-ol	57.05
9	18.259	15929	0.71	7070	1.08	nonanoic acid, methyl ester	74.05
10	19.200	32369	1.45	4778	0.73	isobruceine B	73.05
11	19.337	60418	2.70	13492	2.05	Naphtha (1,8-BC) pyran, 2,3-dihydro-3,6,9-trimethyl-7-nitro-, (.+-.)-	60.00
12	19.766	305044	13.62	100151	15.24	hexadecanoic acid, ethyl ester	88.05
13	22.536	52152	2.33	21194	3.23	(+-)-citronellol	71.05
14	23.783	183978	8.21	60938	9.27	3-tetradecyn-1-ol	55.05
15	24.369	24447	1.09	10232	1.56	heptadecanoic acid, ethyl ester	88.05
16	35.170	510548	22.80	190684	29.02	squalene	69.05
17	41.407	135910	6.07	11837	1.80	2Z,6E-farnesol	69.10
		2239637	100.00	657083	100.00		

GC-MS chromatogram of ethanol leaf extract of *Garcinia xanthochymus* (Fig. 1; Table 2) shows 24 peaks, represent the phytoconstituents were (methoxyamino)-methane (26.09%), β-D-glucopyranose-1,6-anhydro (25.77%), (-)-neptalactol (7.84%), 5-hydroxymethyl furfural (5.89%), heptanes-1-fluro (4.18%), and neophytodiene (3.40%).

GC-MS chromatogram of ethanol stem extract of *Garcinia xanthochymus* (Fig. 2; Table 3) shows 17 peaks, represent the phytoconstituents were squalene (22.80%), hexadecanoic acid (13.62%), coumaran (11.07%), 3-tetradecyn-1-ol (8.21%), (+)- nerlidol (7.53%), valeric acid (7.39%) and neophytodiene (5.99%).

The results of antimicrobial potential of ethanolic leaf and stem extracts of *Garcinia xanthochymus* against various pathogens were depicted (Table 4).

Table 4: Antimicrobial activity of *Garcinia xanthochymus* extracts.

S. NO	Micro organisms	Ethanol leaf Extract (mg/ml)			Ethanol stem extract (mg/ml)			Standard 10 µg/ml
		25	50	100	25	50	100	
1	*Escherichia coli*	12	17	24	11	15	24	27 (A)
2	*Staphylococcus aureus*	13	16	15	12	16	25	31 (A)
3	*Aspergillus niger*	13	16	23	11	14	24	29 (P)

A – Ampicillin; P - Pencillin

The objective of antimicrobial activity was to analyze past, present and future of medicinal plants to suggest as fundamental the research on plant extract mechanism of action, interactions with antibiotics or with other medicinal plants.[14]

The test organisms used in this study are associated with various forms of human infections. *Escherichia* coli causes septicemias and can infect the gall bladder, meningitis, surgical wounds, skin lesions and the lungs especially in debilitate and immune deficient patients. The demonstration of activity against both Gram-positive and Gram-negative bacteria is an indication that the plant can be a source of bioactive substances that could be broad spectrum of activity. Thus, the researchers to investigate the synergistic capacity of plants or other natural products, independent of antimicrobial activity they have. Therefore the results of the present study seems to be promising and may enhance the natural products and its uses, showing the potentiality of *Garcinia xanthochymus* in the treatment of various infectious diseases caused by bacteria. Further studies on the chemical characteristics of extract and active components should be carried out for the plant and its antimicrobial property.[15]

The possible activities of substances found in plant extracts on ribosome structure and bacterial enzymes inhibition appear to be related with synergism profile between plant extracts and the inhibitions of protein synthesis. Secondary metabolites in plant products are responsible for several biological activities in man and animals.[16] The active components usually interfere with growth and metabolism of microorganisms in a negative manner.[17] Antimicrobial properties of several plant extracts have been attributed due to the secondary metabolites.[18,19,20] The reason for the difference sensitivity between the gram-positive and gram-negative bacteria could be ascribed to the morphological differences between these microorganisms, gram-negative pathogens having an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes, while porins constitute a selective barrier to hydrophilic solutes with an exclusion limit of about 600 Da. The gram positive bacteria should be more susceptible having only an outer peptido glycone layer which is not an effective permeability barrier.[21] Phenolic content of plant extracts possess antimicrobial activity[22] and highly oxidized phenols are more inhibitory because of phenolic toxicity to microorganisms.[23] In addition, leaf and stem extracts exhibit antimicrobial potential against all pathogens which may be due to the presence of flavonoids and phenolic compounds.[24,25] It is not surprising that there are differences in the antimicrobial effects of plant groups, due to phytochemical properties and differences among species.

CONCLUSION

The present investigation revealed the that presence of phenolic compounds, flavonoids, tannins, lignins, amino acids and carbohydrates in *Garcinia xanthochynus*. GC-MS analysis exhibited the presence of 24 and 17 constituents in 70% ethanol leaf and stem extracts. The phytochemical profile of the plant extract attributes to the antimicrobial potential against the pathogens *Escherichia coli*, *Staphylococcus aureus* and fungi *Aspergillus niger*. Further explorations on plant derived antimicrobials are needed, to determine the identity of newer antimicrobial compounds from this plant and also to determine their full spectrum efficacy.

In conclusion, *Garcinia xanthochymus* leaf and stem ethanol extracts possess a broad-spectrum antimicrobial activity against pathogens which are responsible for various infectious diseases. The millenarian use of these plants in folk medicine suggested that they represent an economic and safe alternative to treat infectious diseases. Finally, it is recommended that awareness of local communities.

ACKNOWLEDGEMENT

The authors are highly thankful to The Chairman and Managing Trustee, **Adv. P. Krishna Das,** LLB, MBA, BEM, **Dr. P. Krishna Kumar,** CEO& Secretary, Nehru College of Pharmacy, Pampady, Thiruvilwamala, Thrissur, Kerala, for providing all the facilities to carry out this study.

REFERENCES

1. Schmidt Werkaa J, Bochmeb AK, William N. Biological activities of essential oils from Monterverde, Costarica. Nat Prod Commun, 2007; 2(2): 1212 – 19.
2. Aliyu AB, Musa AM, Abdullahi MS, Oyewale AO. Phytochemical and antibacterial properties of *Ludwigia suffruticosa* (Willd.) Oliv. ex O. Ktze (Onagraceae). Int Jor Pharm App Sci., 2008; 2(4): 1-5.
3. Parekh J, Chanda SV. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Tur J Biol, 2007; 31 (1): 53-58.
4. Parthasarathy U, Nirmal Babu K, Senthil Kumar R, Ashis GR, Mohan S, Parthasarathy, VA. Diversity of Indian *Garcinia* – a medicinally important spice crop in India. Acta Hort, 2013; ISHS 979(50): 467-76.
5. Tandon RN, Srivastava OP, Baslas RK, Kumar P. Preliminary investigation on the antimicrobial activity of a phytochemical, xanthochymol from the fruits of *Garcinia xanthochymus* Hook. f. Curr Sci., 1980; 49(12): 472-73.
6. Meng Fu, Hui-Jin Feng, Yu Chen, De-Bin Wang, Guang-Zhong Yang, Antioxidant activity of *Garcinia xanthochymus* leaf, root and fruit extracts *in vitro*, Chi J Nat Med**.,** 2012; 10(2): 129-34.
7. Manohar SH, Naik PM, Patil LM, Karikatti SI, Murthy HN. Chemical composition of *Garcinia*

xanthochymus seeds, seed oil, and evaluation of its antimicrobial and antioxidant activity. J Herbs Spices Med Plants, 2014; 20(2): 148-55.
8. Silva TGA, Agustoni D, Fernades DC, Cafundo De Morais MC, Soares CP. Preliminary investigation on the chemopreventive profile of *Garcinia xanthochymus*. Int Res J Pharm, 2015; 6(3): 173-78.
9. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, 1st ed., Chapman and Hall Ltd., London, 1973; 49-188.
10. Trease GE, Evans WC. Pharmacognosy, 14th ed., London, W.B.Saunders Co. Ltd., 1996; 543.
11. Jaiganesh KP, Arunachalam G. GC-MS analysis of leaf extracts of *Pterospermum Canescens*, Roxb., (Sterculiaceae). Int J Chem Sci., 2012; 10(2): 1028-38.
12. Jaiganesh KP, Arunachalam G. Preliminary phytochemical screening and antimicrobial potential of *Pterospermum canescens* roxb, (Sterculiaceae). Int J Pharm Pharm Sci., 2011; 3(3): 139-41.
13. Perez C, Paul M, Bazerque P. Antibiotic assay by agar-well diffusion method. Acta Biol Med Exp., 1990; 15: 113-15.
14. Purushotham KG, Arun P, Johnsy Jayarani J, Vasnthakumari R, Sankar L, Bijjam AVI, Prakash Reddy. Synergistic *in vitro* antibacterial activity of *Tectona grandis* leaves with tetracycline. Int J Pharm Tech Res., 2010; 2(1): 519-23.
15. Betoni JEC, Mantouani RP, Barbosa LN, Sasti LCD, Fernender A. Synergism between plants extract and antimicrobial drugs used on *Stphylococcus aureus* diseases. Mem Inst Oswaldo Cruz, Rio De Janerio, 2006; 101(4): 387-90.
16. Sofowora A. Medicinal Plant and Traditional Medicine in Africa, 2nd ed., Chiechester, U.S.A. John Wiley and Sons, 1986; 567.
17. Aboaba OO, Efuwape BM. Antibacterial properties of some Nigerian species. Biol Res Commun, 2001; 13: 183-8.
18. Sharma DK. Pharmacological properties of flavonoids including flavonolignans - integration of Petrocrops with drug development from plants. J Sci Ind Res., 2006; 65(6): 477-84.
19. Kosalec I, Pepeljnjak S, Bakmaz M, Vladmir-Knezevic S. Flavonoid analysis and antimicrobial activity of commercially available *Propolis* products. Acta Pharm, 2005; 55(4): 423-30.
20. Tim Cushnie TP, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicro Ag, 2005; 26(5): 343-56.
21. Nikaido H, Vaara M. Molecular basis of bacterial outer membrane permeability. Microbiol Rev., 1985; 49(1): 1-32.
22. Acar G, Dogan NM, Duru ME, Kivrak I. Phenolic profiles, antimicrobial and antioxidant activity of the various extracts of *Crocus* species in Anatolia. Afr J Microbiol Res., 2010; 4(11): 1154-61.
23. Ciocan ID, Bara II. Plant products as antimicrobial agents. Genetică Si Biologie Moleculară, 2007; 8(1): 151-6.
24. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev., 1999; 12(4): 564-82.
25. Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origins: Current methods and future trends. Afr J Biotechnol, 2008; 7(12): 1797-06.