

Original Research Article

Analysis of Bioactive Compounds in Latex of *Croton bonplandianum* (Baill.) using GC-MS

ABSTRACT

Background: *Croton bonplandianum* Baill. belongs to the family of Euphorbiaceae and commonly known as 'Bantulsi'. Traditionally, latex of this plant is used for cuts and wounds.

Objective: The present investigation was designed to determine the bioactive constituents of latex of *C. bonplandianum* using GC-MS. In this study, mass spectra of the compounds were found in the ethanolic extract of latex were matched with the National Institute of Standards and Technology (NIST) library.

Place and Duration of Study: Post Graduate and Research Department of Biochemistry at Government Arts College (Autonomous), Kumbakonam and Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, Tirumalaisamudram, Thanjavur, Tamilnadu, India, during the months between October to December, 2015.

Methodology: 10 ml of fresh latex was mixed with 10 ml of ethanol and kept at shaker for 3 hours. The sample was filtered and concentrated through nitrogen flushing to 1 ml. From this 2 µl of prepared sample was injected into GC-MS instrument for phytochemical analysis.

Results: The GC-MS analysis of the ethanolic extract of latex of *C. bonplandianum* revealed that the presence of twenty five bioactive compounds including the anti-inflammatory, antioxidant and antimicrobial compounds. The major chemical constituents present in the latex are Myo inositol, 2-C-methyl- (32.4%), Mequinol (0.74%), Phenol, 4-methyl- (6.86%), 1,2,3-Benzenetriol (3.54%), Quinolins, 3-methyl- (0.44%), n-Hexadecanoic acid (5.36%) and Octadecanoic acid (1.95%).

Conclusion: Further study is needed to isolate, identify, characterize and elucidate the structure of bioactive compounds responsible for medicinal values of latex of *C. bonplandianum*.

Keywords: *Croton bonplandianum*, latex, GC-MS, ethanolic extract, bantulsi

1. INTRODUCTION

Medicinal plants have been used by human being since ages in traditional medicine due to their therapeutic potential. The search on medicinal plants have led to the discovery of novel drug candidates used against diverse diseases. According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs [1]. Phytochemical is a natural bioactive compound found in plants such as vegetables, fruits, medicinal plants, flowers, leaves and roots that act as a defense system against diseases [2]. Screening of biologically active medicinal compounds have been conducted on well-known species of plants used in traditional medicines [3]. In the recent years GC-MS have been applied to unambiguously identify the structures of different phytoconstituents in plant extracts and biological samples with great success [4,5]. GC-MS is one of the best techniques to identify the constituents of volatile matter, long and branched chain hydrocarbons, alcohols, acids, esters etc [6]. The phytochemical screening of GC-MS analysis proved that the plants are pharmaceutically important due to presence of different medicinally important phytochemical compounds and secondary plant metabolites. The GC-MS analysis of the plants showed that the presence of important bioactive compounds.

The plant latex is a natural polymer, which composed of 320 to 35,000 isoprene molecules and it is stored in specialized cells called laticifer [7,8]. Latex to be phytochemically diverse that contains complex mixtures of terpenoids, phenolics, proteins and alkaloids [9]. Although, all parts of the plant have been used for medicinal purposes [10,11], it is toxic to human and animals. Comprehensive literature survey on

* 2-C-methylmyoinositol (32.4), mequinol (0.74), 4-methylphenol (6.86), 1,2,3-benzenetriol (3.54), 3-methylquinoline (0.44), n-hexadecanoic acid [palmitic acid] (5.36) and octadecanoic acid [stearic acid] (1.95)

the plant latex revealed that it contains wide diversities of defense chemicals and proteins, which displayed different biological activities [12,13]. Moreover, some compounds with wound healing properties were reportedly found in latex [11]. *C. bonplandianum* Baill. belongs to the family of Euphorbiaceae. It is widely distributed throughout the plains of India. It is a small perennial herb, green and laticiferous growing upto 1-2 ft tall. *C. bonplandianum* is also well known for its medicinal value. Seeds of *C. bonplandianum* is used for the treatment of jaundice, acute constipation, abdominal dropsy and internal abscesses [14]. Its ethanolic extract has been found to possess hypotensive, spasmolytic and antimicrobial activities [15,16]. Latex of *C. bonplandianum* is used to treat cuts and wounds traditionally [17,18]. The latex of *C. bonplandianum* (1:5 v/v in 50% acetone) showed antifungal activity [19]. Many pharmacological studies are available using latex of *C. bonplandianum*. But there is no study about the screening of bioactive compounds of latex of *C. bonplandianum*. Therefore, the present study was aimed to investigate the phytoconstituents of ethanolic extract of latex of *C. bonplandianum* using GC-MS.

2. MATERIAL AND METHODS

2.1 Collection of Plant Material

C. bonplandianum latex was collected from the waste lands in and around Mayiladuthurai, Nagapattinam District, Tamilnadu, India, where it was found naturally. The plant was identified by Rev. Dr. John Britto, Director, Rabinat Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchirappalli, Tamilnadu, India. The latex of *C. bonplandianum* was obtained as exudates by and plucking of fresh leaves from actively growing plants. The latex was collected into sterile, plastic containers by pressing and squeezing in between fingers, the apex of the leaves to release as much as possible latex into the containers. After collection, the latex was centrifuged with 5000 rpm for 15 minutes to remove any solid particles present in it. The latex was decanted off into containers and plugged with cotton and stored at 4°C until required for the time of use. Collections were made in the mornings during the days of each analysis [20].

2.2 Identification of Phytochemicals by GC-MS

2.2.1 Sample Preparation

10 ml of fresh latex was mixed with 10 ml of ethanol and kept at shaker for 3 hours [21]. The sample was filtered and concentrated through nitrogen flushing to 1 ml. From this 2 µl of prepared sample was injected into GC-MS instrument for phytochemical analysis.

2.2.2 Equipment

The GC-MS analysis was carried out using a Perkin Elmer Clarus 500 for the analysis of phytochemicals of *C. bonplandianum* [22]. The data were obtained on a Capillary Column Elite- 5MS (5% phenyl 95% dimethyl poly siloxane). Helium was used as the carrier gas with a flow rate of 1ml/min in the split mode (10:1). An aliquot of 2 µl of ethanol solution of the sample was injected into the column with the injector temperature at 250°C. GC oven temperature started at 110°C and holding for 2 min and it was raised to 200°C at the rate of 10°C/min without holding. Holding was allowed at 280°C for 9 min with program rate of 5°C/min. The injector and detector temperatures were set at 250°C and 280°C, respectively. GC interface and ion source temperature was maintained at 200°C. The mass spectrum of compounds in the sample was obtained by electron ionization at 70 eV and the detector was operated in scan mode from 40-450 amu (atomic mass units). A scan interval of 0.5 second and fragments from 40 to 450 Da were maintained. The total running time was 36 minutes.

2.2.3 Interpretation of Mass Spectrum (MS)

In the MS Programme, NIST Version 2.0 library database of National Institute of Standard and Technology (NIST) having more than 2,00,000 patterns was used for identifying the phyto components of *C. bonplandianum*. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

3. RESULTS AND DISCUSSION

Now a days the study of the phytochemicals from plants and their activity has increased. The combination of a best separation technique (GC) with the best identification technique (MS) made GC-MS an ideal technique for qualitative analysis for volatile and semi-volatile bioactive compounds [23]. In the present investigation twenty five bioactive chemical constituents were identified in the latex of *C. bonplandianum* with their retention time (RT), peak area percentage and molecular weight are given in Tables 1. The phytochemical prediction is based on NIST Version 2.0 library database of National Institute of Standard and Technology (NIST). The highest peak area percentage was obtained by Myo inositol, 2-C methyl (32.4%) with the retention time of 10.48 and the lowest peak area percentage was obtained by 3,5-Triazin-2-amine, N-ethyl-4-methoxy- (0.07%) with the retention time of 23.31. The most abundant components were found in the latex such as Myo inositol, 2-C methyl (42.40%), 13-Octadecenal (23.31%), 2-Methoxy-4-methyl benzoic acid (10.90%), D-Allose (10.63%) and Phenol, 4-methyl- (6.86%).

In our previous study, the latex extract of *C. bonplandianum* was subjected to GC-MS analysis [24]. In that 10 ml of latex was mixed with 90 ml of ethanol and then prepared extract as mentioned in the sample preparation. The extract showed that the presence of only one compound, Myo-inositol, 2-C-methyl (30.8%). But in the present study, the ethanolic extract of latex of *C. bonplandianum* showed that the presence of twenty five compounds including Myo-inositol 2-C-methyl (42.4%). This variation may be due to the dilution of the latex with ethanol during extract preparation because, in this study the latex was mixed with equal volume of ethanol for extract preparation, but in the previous study the latex was mixed with 9 fold volume of ethanol for extract preparation. So, this study confirmed that the dilution of the latex play a main role in the presence of phytochemicals and appearance of peak in the GC-MS chromatogram.

The biological activities of identified phytochemicals in *C. bonplandianum* were predicted based on Dr. Duke's phytochemical and Ethnobotanical Databases. Among the twenty five phytochemicals in latex of *C. bonplandianum*, a few compounds like, Myo inositol, 2-C methyl (42.4%), Quinoline, 3-methyl- (0.44%), 4-Amino-6-methyl-piperidin-2-one (5.24%), Cytidine, 2'-deoxy- (5.24%), 1,2,3-Benzenetriol (3.54%), 2-Methoxy-4-methyl benzoic acid (10.90%) and n-Hexadecanoic acid (5.36%) have antimicrobial activity. 1,2,3-Benzenetriol (pyrogallol) is an important, allelochemical which belong to phenolic compounds present in plants and it is used in controlling bacterial diseases [25].

The phytochemicals such as Mequinol (0.74%), Phenol, 4-methyl- (6.86%) and Quinoline, 3-methyl- (0.44%) have anti-inflammatory activity. Mequinol is a common active ingredient in topical drugs used for skin depigmentation and it is a derivative of hydroquinone (4-hydroxyanisole, hydroquinone monomethyl ether). The phenolic compounds are one of the largest and most ubiquitous group of plant metabolites. A number of studies have focused on the phytochemicals of biological properties such as antiapoptosis, anti-ageing, anticarcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of the endothelial functions, as well as inhibition of angiogenesis and cell proliferation activity [26]. Phenolic compounds have been extensively used in disinfections and as bacteriocides [27]. Quinoline compounds has excellent antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* [28].

Based on the Dr. Duke's phytochemical and ethnobotanical database, the Phenol, 2-methoxy-4-methyl- (9.55%), D-Allose (10.63%), 1H-Indole-3-carbaldehyde, 6-methoxy- (5.98%) and 2-Methoxy-4-methyl benzoic acid (10.90%) have antioxidant activity. n-Hexadecanoic acid (5.36%) and 2-Methoxy-1-naphthyl thiocyanate (2.58%) have antitumor activity and Quinoline, 3-methyl- (0.44%) has antimalarial activity.

The antioxidant properties of phenolic compounds are due their redox properties, ability to chelate metals and quenching of singlet oxygen [29]. Natural phenolic compounds have great potential as natural antimicrobials and food preservatives [30]. D-Allose is a novel antitumor monosaccharide, which causes cell growth inhibition on cancer cells by inducing the tumor suppressor gene [31]. n-Hexadecanoic acid has anti-tumor activity to human leukemic cells as well as murine cells [32]. The fatty acids exhibit

cytotoxicity against HeLa cells and retard tumor growth [33]. The n-Hexadecanoic acid and octadecanoic acid were showed significant cytotoxicity against oral cancer (KB), breast cancer (MCF-7) and lung cancer (NCI-H187) [34].

The peak area of 1.54% was obtained by benzyl alcohol and it occurs naturally in a number of plants and it has strong activity against gram positive bacteria and has some weak activity against gram negative bacteria, yeasts and moulds [35]. Benzyl alcohol also has some mild local anaesthetics and anti-spasmodic properties [36].

The peak area of 0.51% was obtained by dibutyl phthalate. There is an available reports on antimicrobial activities of dibutyl phthalate from flowers of *Lea indica* [37]. Roy *et al.* reported that the strong bioactive nature of dibutyl phthalate against bacteria as well as fungi [37]. Dibutyl phthalate isolated from *Ipomoea carnea* stem showed significant activity against microbes like *Klebsiella pneumonia*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. Dibutyl phthalate possesses pharmacological activity leading to selective deterioration of leukemic cells, with less harmful effect on the growth of normal hemopoietic progenitors [38].

In addition to this, the results of the GC-MS profile of latex of *C. bonplandianum* can be used as pharmacognostical tool for the identification of bioactive compounds. The presence of various bioactive compounds confirms that the use latex of *C. bonplandianum* for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

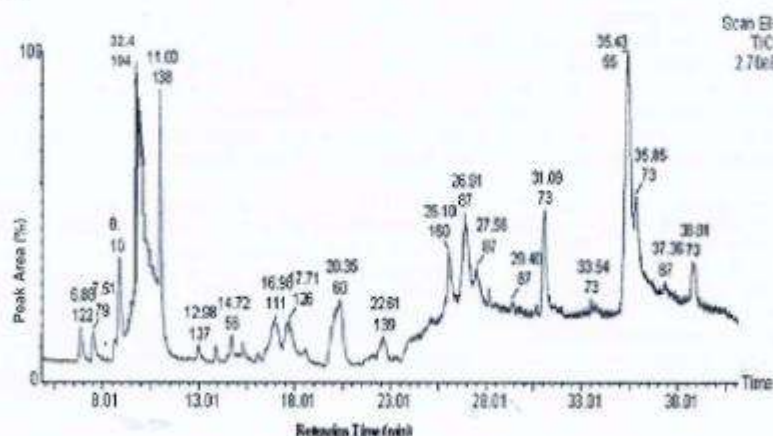


Fig. 1. GC-MS chromatogram of ethanolic extract of latex of *C. bonplandianum*

Table 1. Screening of the phytochemicals of ethanolic latex extract of *C. bonplandianum* by GC-MS

Sl. No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %	Nature of compound	Activity
1.	6.88	1,3,5-Cycloheptatriene, 7-methoxy-	$C_8H_{10}O$	122	1.52	Ether compound	No activity reported
2.	7.51	Benzyl Alcohol	C_7H_8O	108	1.54	Alcoholic compound	Antibacterial
3.	8.62	Mequinol	$C_7H_6O_2$	124	0.74	Alcoholic compound	Anti-inflammatory
4.	8.87	Phenol, 4-methyl-	C_7H_8O	108	6.86	Alcoholic compound	Anti-inflammatory
5.	10.48	Myo-inositol, 2-C-methyl	$C_7H_{17}O_6$	194	32.4	Inositol compound	Antimicrobial
6.	11.00	Phenol, 2-methoxy-4-methyl-	$C_9H_{10}O_2$	138	9.55	Alcoholic compound	Antioxidant Cytotoxicity
7.	12.98	Phenol, 4-ethyl-2-methoxy-	$C_9H_{12}O_2$	152	0.77	Alcoholic compound	No activity reported
8.	13.90	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	0.54	Alcoholic compound	No activity reported
9.	14.72	1,3-Cyclopentanediol, 4,4-dimethyl-	$C_7H_{12}O_2$	126	1.01	Ketone compound	Herbicidal activity
10.	15.33	Quinoline, 3-methyl-	$C_{10}H_9N$	143	0.44	Alkaloid compound	Antimalarial Antibacterial Antifungal Anti-inflammatory
11.	16.98	4-Amino-6-methyl-piperidin-2-one	$C_8H_{16}N_2O$	128	5.24	Alkaloid compound	Antimicrobial
12.	16.98	Cytidine, 2'-deoxy-	$C_9H_{13}N_5O_4$	227	5.24	Nucleoside	Antimicrobial
13.	17.71	1,2,3-Benzenetriol	$C_6H_6O_3$	126	3.54	Alcoholic compound	Antimicrobial
14.	18.55	1H-Indol-5-ol	C_8H_7NO	133	0.35	Alcoholic compound	No activity reported
15.	20.34	D-Aliose	$C_6H_{12}O_4$	180	10.63	Sugar compound	Antiproliferative,

6.	22.61	3-Acetyl-2,5-dimethyl thiophene	$C_9H_{10}OS$	154	2.45	thiophene derivative Sulfur compound	Antioxidant Endocytic
7.	23.31	1,3,5-Triazin-2-amine, N-ethyl-4-methoxy-	$C_9H_{10}N_4O$	154	0.07	Amine Ether compound	No activity reported
8.	26.10	1-H-Indole-3-carbaldehyde, 6-methoxy-	$C_{10}H_9NO_2$	175	5.98	Indole aldehyde Ether compound	Antioxidant
9.	26.91	2-Methoxy-4-methyl benzoic acid	$C_{10}H_{10}O_3$	166	10.90	Benzoic acid compound Amino acid	Antimicrobial Antioxidant
10.	27.56	1-H-Indole-3-carboxaldehyde, 5-methoxy-	$C_{10}H_9NO_2$	175	4.20	Indole aldehyde Ether compound	Antioxidant
11.	28.16	Dibutyl phthalate	$C_{18}H_{26}O_4$	278	0.51	phthalate derivative (Plasticizer compound)	No activity reported
12.	31.09	n-Hexadecanoic acid (palmitic acid)	$C_{16}H_{32}O_2$	256	5.35	Fatty acid	Antitumor Antibacterial Antifungal
13.	35.43	13-Octadecenal (Stearaldehyde)	$C_{18}H_{34}O$	266	23.31	Fatty Aldehyde compound	No activity reported
14.	35.84	Octadecanoic acid (stearic acid)	$C_{18}H_{36}O_2$	284	1.95	Fatty acid	Antibacterial
15.	38.91	2-Methoxy-1-naphthyl thiocyanate	$C_{15}H_{11}NOS$	215	2.58	naphthalene derivative Sulfur compound	Antitumor Antifungal

RT - Retention Time, MW - Molecular Weight, *Dr. Duke's phytochemical and Ethnobotanical Databases

4. CONCLUSION

In the present study, the GC-MS analysis of ethanolic latex extract of *C. bonplandianum* revealed that the presence of twenty five compounds. Among these compounds according to the Dr. Duke's phytochemical and ethnobotanical database, eight compounds have antimicrobial activity, three compounds have antiinflammatory activity, five compounds have antioxidant activity, two compounds have antitumour activity and one compound has antimalarial activity. Thus, the GC-MS analysis is the first step towards understanding the nature of active principles in the latex of *C. bonplandianum* and this study will also be helpful for further research related to pharmacological properties.

REFERENCES

1. Pierangeli G, Vital G, Rivera W. Antimicrobial activity and cytotoxicity of *Chromolaena odorata* (L.) Klag and Robinson and *Uncaria perrottetii* (A. Rich) Merr extracts. J Medicinal Plants Res. 2009;3(7):511-518.
2. Krishnaiah D, Devi T, Bono A, Sarbaty R. Studies on phytochemical constituents of six Malaysian medicinal plants. J Med Plant Res. 2009;3(2):67-72.
3. Rabe T, Van Staden J. Antibacterial activity of South African plants used for medicinal purposes. J Ethnopharmacol. 1997;56(1):81-87.
4. Prasain JK, Wang CC, Barnes S. Mass spectroscopic methods for the determination of flavonoids in biological samples. Free Radic Biol Med. 2004;37:1324-1350.
5. De Rijke E, Out P, Neissen WMA, Ariese F, Spoiler C, Brinkman UA. Analytical separation and detection methods for flavonoids. J Chromatogr. 2006;1112:31-63.
6. Anjali Ruikar, Rasika Torane, Amruta Tambe, Vedavati Puranik, Nirmala Deshpande. GC-MS study of a steam volatile matter from *Mimusops elengi*. International Journal of Chem Tech Research. 2009;1:158-161.
7. Kang H, Kang MY, Han KH. Identification of natural rubber and characterization of rubber biosynthetic activity in Fig tree. Plant Physiol. 2000;123:1133-1142.
8. Upadhyay RK. Plant latex: A natural source of pharmaceuticals and pesticides. Int J Green Pharm. 2011;5:169-80.
9. Agrawal AA, Konno K. Latex: A model for understanding mechanisms, ecology and evolution of plant defense against herbivory. Ecology Evolution System. 2009;40: 311-315.
10. Duke JA. Medicinal plant science. London: Macmillan press limited, 1985.
11. Gilbitz GM, Mittelbach M, Trabi M. Exploitation of the tropical oil seed plant *Jatropha curcas* L. Bioresour Technol. 1999;67:73-82.
12. Konno K. Plant latex and other exudates as plant defense systems: Roles of various defense chemicals and proteins contained therein. Phytochemistry. 2011;72(13):1510-1530.
13. Kitajima S, Kamel K, Taketani S, Yamaguchi M, Kawai F, Komatsu A, Inukai Y. Two chitinase-like proteins abundantly accumulated in latex of mulberry show insecticidal activity. BMC Biochem. 2010;11:6-11.
14. Reddy KR. Folk medicines from Chittoor District, Andhra Pradesh, used in the treatment of jaundice. Pharm Biol. 1995;26:137-140.
15. Mishra D, Chaturvedi RV, Tripathi SC. The fungitoxic effect of the essential oil of the herb *Nardostachys jatamansi* DC. Tropical Agric. 1995;72(1):48-52.
16. Verma SP, Kannaliya RS, Upadhyay RR. Antifungal activities of some Euphorbiaceae plants. Ind J Plant Path. 1998;16(1-2):62-63.
17. Das AJ, Dutta BK, Sharma GD. Medicinal plants use by different tribes of Cachar District, Assam. Indian J Tradit Know. 2008;7(3):446-454.
18. Chandel KPS, Shukla G, Sharma N. Biodiversity in medicinal and aromatic plants in India, Conservation and utilization, New Delhi, National Bureau of Plant Genetic Resources. 1996: 361.
19. Asthana A, Mall HV, Dixit K, Gupta S. Fungitoxic properties of latex of plants with special reference to that of *Croton bonplandianum* Baill. Pharm Biol. 1989;27(1):25-28.
20. Oyi R, Onaolapo JA, Haruna AK, Morah CO. Antimicrobial screening and stability studies of the crude extract of *Jatropha curcas*(L) latex. Nig J Pharmac Sci. 2007;6(2):14-20.

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21. Varsha Jadhav, Vaibhav Kalase, Poonam Patil. GC-MS analysis of bioactive compounds in methanolic extract of *Holigama grahamii* (Wight) Kurz. Int J Herb Med. 2014;2(4):35-39.
22. Janakiraman N, Johnson M, Sahaya Sathish S. GC-MS analysis of bioactive constituents of *Peristrophe bicalyculata* (Retz.) Nees. (Acanthaceae). Asian Pac J Trop Biomed. 2012;46-49.
23. Grover N, Patni V. Phytochemical characterization using various solvent extracts and GC-MS analysis of methanolic extract of *Woodfordia fruticosa*(L) Kurz. leaves. Int J Pharm Pharm Sci. 2013;5(4):291-295.
24. Vennila V, Udayakumar R. GC-MS Analysis of leaf, fruits and latex of *Croton bonplandianum* Baill. Int J Biochem Res Rev. 2015;5(3):187-197.
25. Ismail Kocacalisikana, Ismet Talanb, Irfan Terzio. Antimicrobial activity of catechol and pyrogallol as allelochemicals. Naturforsch. 2006;3(61c):639-642.
26. Han X, Shen T, Lou H. Dietary polyphenols and their biological significance. Int J Mol Sci. 2007;8:950-988.
27. Okwu DE. Evaluation of the chemical composition of indigenous species and flavouring agents. Global J Pure Appl Sci. 2001;8:455-459.
28. Pintilie Lucia. Quinolones: Synthesis and Antibacterial Activity. Antimicrobial Agents. 2012;255-272.
29. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med. 1996;20:933-956.
30. Hayriye Cetin-Karaca. Evaluation of natural antimicrobial phenolic compounds against foodborne pathogens. University of Kentucky Master's Theses. Paper 652. http://uknowledge.uky.edu/gradschool_theses/652.
31. Yamaguchi E, Kamitori K, Sanada K, Horii M, Dong Y, Sui L, Tokuda M. Rare sugar D-allose enhances anti-tumor effect of 5-fluorouracil on the human hepatocellular carcinoma cell line HuH-7. J Biosci Bioeng. 2008;106(3):248-52.
32. Harada H, Yamashita U, Kurihara H, Fukushi E, Kawabata J. Antitumor activity of palmitic acid found as a selective cytotoxic substance in a marine red alga. Anticancer Res. 2002;22:2587-2590.
33. Abdel Aziz A, Rady HM, Amer MA, Kiwan HS. Effect of some honey bee extracts on the proliferation, proteolytic and gelatinolytic activities of the hepatocellular carcinoma Hepg2 cell line. Aust J Basic Appl Sci. 2009;3:2754-2769.
34. Keawsa-Ard S, Liawruangrath B, Liawruangrath S, Teerawutgulrag A, Pyne SG. Chemical constituents and antioxidant and biological activities of the essential oil from leaves of *Solanum spirale*. Nat Prod Commun. 2012;7:955-958.
35. Bezyl alcohol. Committee for veterinary medical products. The European Agency for the Evaluation of Medicinal Products Veterinary Medicines Evaluation Unit. 1997.
36. Srinivasan GV, Sharanappa P, Leela NK, Sadashivam CT, Vijayan KK. Chemical composition and antimicrobial activity of the essential oil *Leuca indica* Merr. Flowers. Nat Prod Radiance. 2009;8(5):488-493.
37. Elija Khatiwora, Manik Kulkarni, Deshpande, Kashalkar. Antibacterial activity of Dibutyl Phthalate: A secondary metabolite isolated from *Ipomoea carnea* stem. Journal of Pharmacy Research. 2012;5(1):150-152.
38. Wu Chu-Tse, Pei Xue-Tao, Cao Jun-Rong, Xue Hui-Hua. A new pharmacological activity of dibutyl phthalate (DBP) on selective elimination of tumor cells from bone marrow. Leuk Res. 1993;17(7):557-560.