Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(2): 756-760
Received: 22-01-2019
Accepted: 24-02-2019

Richa Bhardwaj
Plant Physiology and Biochemistry laboratory, Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India

RA Sharma
Plant Physiology and Biochemistry laboratory, Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India

Correspondence
Richa Bhardwaj
Plant Physiology and Biochemistry laboratory, Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India

Boerhavia diffusa-GC-MS Analysis of alkaloids and their inhibitory activity against pathogenic microorganisms

Richa Bhardwaj and RA Sharma

Abstract
Boerhavia diffusa is a species of flowering plant in the four o'clock family which is commonly known as tar vine, punarnava meaning that which rejuvenates or renews the body, *Boerhavia* belongs to family Nyctaginaceae the plant holds tremendous potential of medicinal value and has been traditionally used in various ailments like syphilis, leukoderma, blood disorders to name a few. The plant has gained prominence due to presence of some prominent secondary metabolites. The present study focuses on the GC-MS analysis of extracts of all the plant parts of *B. diffusa* which revealed the presence of certain bioactive compounds like stigmasterol, sitosterol, thiazoline, phytol, pthalic acid, methyl alpha ketopalmitate and so forth. A total of about 20 bioactive compounds were identified. Antimicrobial activity of crude alkaloids obtained from different plant parts i.e roots stems and leaves. alkaloids from roots showed highest activity against F. oxysporium (15.33+1.52) and E. coli(17.33±0.58), alkaloids from stems showed highest activity against *C. albicans* (20.33+2.08)and *E. coli(*40.56±2.08) alkaloids from leaves showed highest activity against *C. albicans (*14.33 +2.31) and *S. aureus(*22.00±3.6). Alkaloids from the leaf extract also show highest inhibition zone than alkaloids from root and stem extract.

Keywords: *Boerhavia diffusa*, bioactive compounds, GC-MS, antimicrobial activity

Introduction
Healing with medicinal plants is as old as mankind itself, It was an integral part of the development of modern civilization. Utilization of plants for medicinal purposes in India has been documented long back in ancient literature (Rabia, 2005) [8]. Plants are a goldmine of novel chemicals; much impressive number of modern drugs has been developed from them. The Last decade has witnessed an increase in the investigation on plants as a source of new biomolecules for human disease management (Grierson and Afolayan, 1999) [3]. Today plants are sources of many conventional drugs (Vickers *et al.,* 2001) [14]. The importance of medicinal plants in traditional healthcare practices has provided a clue to new areas of research and biodiversity conservation is now well recognized (Uniyal, 2006) [12]. Plants synthesize a vast range of organic compounds that are traditionally classified as primary and secondary metabolites (Croteau *et al.,* 2000) [2]. Most pharmaceutically important secondary metabolites are isolated from wild or cultivated plants because their chemical synthesis is not economically feasible. Chemodiversity in nature offers a valuable source as secondary metabolites, previously regarded as waste products are now recognized for their resistant activity against pests and diseases (Verpoorte, 1998) [13]. *Boerhavia diffusa* is a species of flowering plant in the four o'clock family which is commonly known as tar vine, punarnava meaning that which rejuvenates or renews the body, or red spiderling. A metabolite profiling and biological study undertaken in *Boerhavia* leaves and roots, phytochemical characterization revealed rotenoids and alkaloids terpenes, phenylpropanoids, indol compounds, norisoprenoids, among others which were identified. Organic acid analysis was also performed, allowing their characterization in this species for the first time, and oxalic, ketoglutaric, pyruvic, quinic and fumaric acids were identified. Additionally, several flavonoids and one phenolic acid were also confirmed. (Pereira *et al*, 2009) [7]. Alkaloids belong to the broad category of secondary metabolites. This class of molecule has historically been defined as naturally occurring substances that are not vital to the organism that produces them. alkaloids do have important ecochemical functions in the defense of the plant against pathogenic organisms and herbivores or, as in the case of pyrrolizidine alkaloids, as pro-toxins for insects, which further modifiy the alkaloids and then incorporate them into their own defense secretions (Kutchan, 1995) [5] Alkaloids in medicinal plants are reported for their antimicrobial and antimalarial activity (Hadi and Bremner, 2001, Ameyaw and Eshun, 2009) [4, 1].

Plant extracts and essential oils have been used as alternatives to antibiotic due to their antimicrobial activities and the favorable effect on the animal intestinal system (Ljubiša *et al*., 2009) [6]. Plant extracts are able to restrict the growth of bacteria due to the presence of active principles in it.

Materials and methods

Plant material

The *B. diffusa* was collected from the University of Rajasthan, Jaipur, Campus. A specimen was submitted to the Herbarium, Department of Botany, University of Rajasthan, Jaipur and the voucher specimen no. RUBL211299 was given. The plant material was shade dried and different plant parts were collected separately, powdered and used as the experimental plant material for further experimentation.

Chemicals

All the chemicals used were of analytical grade and purchased from Hi Media from Hi-media Laboratory Pvt. Ltd. Mumbai.

Tests for Alkaloids

Iodine Test: A few drops of dilute iodine solution were added into 3 ml test solution. Blue colour appeared; and disappeared on boiling and reappeared on cooling (Khandewal, 2008).

Alklaoids

Extraction Procedure (Family: Nyctaginaceae)

Each of the dried and powdered test sample (10gm) (plant and callus) was soaked in 200 mL methanol for several days at room temperature. The mixture was filtered and methanol was removed by rotary evaporator to give the crude methanolic extract. Procedure of extraction and evaporation was repeated three times. The dry methanolic extract was dissolved again in 20 mL methanol in separating funnel and mixed with 200 mL of 0.5 N sulphuric acid. A few drops of ammonia were added to the solution till the whole solution became basic (pH 9-12). 50 ml of chloroform was added in separating funnel and kept at room temperature for 24 hours. Discard the upper layer and taken the lower layer which was further evaporated to give the crude alkaloid mixture (Singh *et al*., 2000) [10], which was analysed for chromatographic and GC-MS analysis.

GC-MS analysis of alkaloids

GC-MS conditions

GCMS-QP 2010 Plus was used for identification and quantification of phytoconstituents, using MS libraries previously compiled from purchased standards. For the acquisition of an electron ionization mass spectrum, an ion source temperature of 250 °C was used. The GC was equipped with a SE-30 capillary column a split injection piece (270 °C) and direct GC-MS coupling (280 °C). Helium (1.2 mL/min) was used as the carrier gas with a split ratio of 1:10. The oven temperature program for analyzing the extracts utilized an initial oven temperature of 100 °C, maintained for 2 min, followed by a steady climb to 200 °C at a rate of 7 °C/min allowed to increase to 190 °C at a rate of 30 °C/min. This oven temperature was again maintained at 190 °C for 5 min and then allowed to increase to 300 °C at a rate of 7 °C/min. This oven temperature was maintained for 2 min and finally ramped to 300 °C at a rate of 10 °C/min and maintained for a further 22 min. Injection temperature was 270 °C and volume 250 °C and 1 µL, respectively. The total GC running time was about 43.28 min. The MS operating conditions were as follows, Interference temperature of 260 °C, Ion source temperature of 250 °C, mass scan (m/z)-40-450, solvent cut time 7 min, scan speed 2000 amu/s total MS running time-50.28 min and Threshold -1000.

Identification

GC-MS is a valuable aid for identifying unknown peak as well as for confirming the identification of identified phytoconstituents. In some cases when no identical spectra were found, the structural type of the corresponding component was suggested only on the basis of its mass spectral fragmentation and retention data. Identification of components was based on directs comparison of the retention times and mass spectral data with those for standard compounds and computer matching with the library (Wiley library, NIST data bank, database NIST 98) as well as by comparison of the retention time.

Sources of test organisms

a. Fungi

The fungal strains *Aspergillus niger* (NCIM 0616), *Fusarium oxysporum* (NCIM 1228), Trichoderma reesei (NCIM 0992), *Penicillium funiculosum* (NCIM 1075), *Candida albicans* (NCIM- 3501), *Trichoderma viride* are procured from the National Institute for Complementary Medicine.

b. Bacteria

The bacterial strains *Escherichia coli* (MTCC 1652), *Staphylococcus aureus* (MTCC 0087) (Gram +ve), *Pseudomonas aeruginosa* (MTCC 4646) (Gram +ve), Bacillus subtilis (MTCC 0121), *Klebsiella pneumoniae* (MTCC-0109) (Gram –ve) and *Streptomyces albudencus* (MTCC 1764), *Enterococcus faecalis* (ATCC- 29212) (gram +ve) were procured from the microbial type culture collection (Institute of Microbial Technology, Chandigarh, India).

Culture of test microbes

For the cultivation of bacteria, Nutrient Broth Medium (NB) was prepared using 8% Nutrient Broth (Difco) in distilled water and agar-agar and sterilized at 15 lbs psi for 25-30 min. Agar test plates were prepared by pouring ~15 ml of NBM into the petri dishes (10 mm) under aseptic conditions. A peptone saline solution was prepared (by mixing 3.56 g KH2PO4 + 7.23 g NaH2PO4 + 4.30 g, NaCl + 1 g peptone in 1000 ml of distilled water, followed by autoclaving) and the bacterial cultures were maintained on this medium by regular sub-culturing and incubation at 37 °C for 24 hrs. However, for the cultivation of fungi, Potato Dextrose Agar (PDA) medium was prepared by mixing 100 ml potato infusion + 20 g agar + 20 g glucose, followed by autoclaving) and the test fungi were incubated at 27 °C for 48 hrs and the cultures were maintained on same medium by regular subculturings.

Fungicidal and Bactericidal Assay

For both, fungicidal and bactericidal assays agar well diffusion method was adopted (Bauer *et al*., 1996), because of reproducibility and precision. The different test organism were proceeded separately using a sterile swab over previously sterilized culture medium plates and the zone of inhibition were measured around wells in solidified medium (5 mm in diameter), which were containing 2mg/ml and 4mg/ml of the test extracts, control solvent or steptomycin (1mg/ml) or ketokenazol (1mg/ml) as reference separately. These plates were initially placed at low temperature for 1 hr, so as to allow the maximum diffusion of the compounds from the wells into the plate and later, incubated at 37 °C for 24 hrs in case of bacteria and 48 hrs at 27 °C for fungi, after which

the zones of inhibition could be easily observed. Three replicates of each test extract were examined and the mean values were then referred.

Results and Discussion

Antifungal activity recorded against *Fusarium* was maximum by root and minimum by stem and leaf (root; IZ=15.33±1.52mm > stem; IZ= 13.33±0.57mm > leaf; IZ=12.16 ±1.04 mm). Against *P. funiculosum*, leaf showed maximum whereas stem showed minimum inhibition (leaf; IZ=18.66 ± 0.57 mm > root; IZ= 14.33 ± 0.57 mm > bark; IZ=13.00 ± 0.00 mm > stem; IZ=12.00 ± 2.00). Maximum activity against *C. albicans* was recorded by the leaf extract while minimum by root (leaf; IZ=27.66 ± 1.52 mm > stem; IZ= 19.66 ± 1.52 mm > bark; IZ=14.33 ± 2.51 mm > root; IZ=9.33 ± 1.52 mm). Leaf extracts showed maximum activity against *T. virdae* whereas bark extract showed minimum inhibition (leaf; IZ=26.66 ± 1.05 mm > stem; IZ= 20.00 ± 2.64 mm > root; IZ=12.33 ± 2.51 mm > bark; IZ=12.00 ± 2.54 mm).

Table 1: Retention time, molecular weight and % area by setting the total peak area to 100% of Alkaloids identified by GC-MS in Leaves of *Boerhavia diffusa*

Peak#	R. Time	Area%	Name	Mol. Formula	Mol. wt
1	18.397	81.22	Bezyloxy-1	$C_{21}H_{34}O_4Si$	378
2	18.815	0.94	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-6-hy	$C_{11}H_{16}O_3$	196
3	19.22	0.36	1-Decene, 8-methyl-	$C_{11}H_{22}$	154
4	19.31	5.23	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*	$C_{20}H_{40}O$	296
5	19.398	0.61	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}$	280
6	19.947	3.06	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
7	21.213	0.24	Hexadecanoic acid	$C_{16}H_{32}O_2$	256
8	21.534	1.04	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	442
9	23.188	0.6	Phytol	$C_{20}H_{40}O$	296
10	23.779	0.12	Cyclododecene, (Z)-	$C_{10}H_{18}$	138
11	23.84	0.58	Ethyl 9-hexadecenoate	$C_{18}H_{34}O_2$	282
12	25.761	0.39	trans-2-Decen-1-ol, trifluoroacetate	$C_{12}H_{19}F_3O_2$	252
13	26.025	0.81	Cyclohexane, eicosyl-	$C_{26}H_{52}$	364
14	36.684	0.33	Cholesta-4, 6-dien-3-ol, benzoate, (3.beta.)-	$C_{34}H_{50}O_2$	488
15	36.836	2.64	Cholest-5-en-3-ol (3.beta.)-, propanoate	$C_{30}H_{50}O_2$	442
16	38.126	0.63	Stigmasterol	$C_{29}H_{48}O$	412
17	38.592	0.33	Cholest-5-en-3-ol (3.beta.)-, tetradecanoate	$C_{42}H_{72}O_2$	596

Table 2: Retention time, molecular weight and % area by setting the total peak area to 100% of Alkaloids identified by GC-MS in stems of *Boerhavia diffusa*

Peak#	R. Time	Area%	Name	Mol. Formula	Mol. Wt
1	14.351	6.96	.beta.-D-Glucopyranose, 1,6-anhydro-	$C_6H_{10}O_5$	162
2	16.244	2.64	Phen-1,5-diol, 2-[3-oxododecanoyl]-	$C_{18}H_{26}O_4$	306
3	17.261	3.73	Methyl 4-o-methyl d-arabinopyranoside	$C_7H_{14}O_5$	178
4	17.514	1.64	Oxirane, tetradecyl-	$C_{16}H_{32}O$	240
5	19.218	1.29	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}$	280
6	19.306	13.96	2,6,10-Trimethyl,14-ethylene-14-pentadecne	$C_{20}H_{38}$	278
7	19.673	8.98	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
8	20.596	2.71	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298
9	21.262	0.35	Phthalic acid, 4-bromophenyl octyl ester	$C_{22}H_{25}BrO_4$	432
10	21.529	3.3	Ethyl pentadecanoate	$C_{17}H_{34}O_2$	270
11	22.649	0.47	Oxirane, octyl-	$C_{10}H_{20}O$	156
12	22.92	0.48	6-Tridecane	$C_{13}H_{24}$	180
13	22.991	2.3	7-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	268
14	23.182	4.38	Phytol	$C_{20}H_{40}O$	296
15	23.303	0.87	Methyl 17,18-dideuteriooctadecanoate	$C_{19}H_{36}D_2O_2$	300
16	23.774	0.86	9-Hexadecyn-1-ol	$C_{16}H_{30}O$	238
17	23.836	2.79	Ethyl 9-hexadecenoate	$C_{18}H_{34}O_2$	282
18	24.151	0.9	Tetradecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	256
19	24.223	0.92	Dodecanamide	$C_{12}H_{25}NO$	199
20	25.286	3.1	Octanoic acid, 2-dimethylaminoethyl ester	$C_{12}H_{25}NO_2$	215
21	25.479	1.78	Docosanoic acid	$C_{22}H_{44}O_2$	340
22	25.758	1.87	5-Dodecen-1-al	$C_{12}H_{22}O$	182
23	26.02	2.25	Cyclohexane, eicosyl-	$C_{26}H_{52}$	364
24	26.15	0.66	Cyclohexane, (3-methylpentyl)-	$C_{12}H_{24}$	168
25	26.436	2.46	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281
26	27.45	0.46	Fumaric acid, 2-dimethylaminoethyl nonyl ester	$C_{17}H_{31}NO_4$	313
27	27.53	1.81	2-(Dimethylamino)ethyl 2-methylacrylate	$C_8H_{15}NO_2$	157
28	27.715	1.91	8-Hexadecenal, 14-methyl-, (Z)-	$C_{17}H_{32}O$	252
29	28.837	1.47	1,2-Benzenedicarboxylic acid	$C_{24}H_{38}O_4$	390
30	36.317	0.41	Cis-2-phenyl-1, 3-dioxolane-4-methyl octadec	$C_{28}H_{40}O_2$	440
31	36.377	0.87	Cholesta-6,22,24-triene, 4,4-dimethyl-	$C_{29}H_{46}$	394
32	36.557	1.2	Methyl 10,12-pentacosadiynoate	$C_{26}H_{44}O_2$	388

33	36.832	7.93	.beta.-Sitosterol	$C_{29}H_{50}O$	414
34	37.04	0.35	2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecy	$C_{29}H_{50}O_2$	430
35	37.163	0.86	Urs-12-ene	$C_{30}H_{50}$	410
36	38.12	5.24	Stigmasta-5,22-dien-3-ol	$C_{29}H_{48}O$	412
37	38.648	0.87	Squalene	$C_{30}H_{50}$	410

Table 3: Retention time, molecular weight and % area by setting the total peak area to 100% of Alkaloids identified by GC-MS in roots of *Boerhavia diffusa*

Peak #	R. Time	Area%	Name	Mol. formula	Mol. Wt
1	8.194	1.97	Pentofluropropionic acid, heptyl ester	$C_{10}H_{15}F_5O_2$	262
2	11.933	1.23	1-Pentadecanol	$C_{15}H_{32}O$	228
3	14.169	0.89	2-Tert-butyl-4-(1,1,3,3-tetramethylbutyl)phen	$C_{18}H_{30}O$	262
4	16.083	0.41	Butane, 1-bromo-3-methyl-	$C_5H_{11}Br$	90
5	17.43	1.77	4-Octanol	$C_8H_{13}O$	130
6	19.233	15.10	2,6,10-Trimethyl,14-ethylene-14-pentadecne	$C_{20}H_{38}$	278
7	19.592	0.38	4-Nonen-1-ol, (E)-	$C_9H_{18}O$	142
8	21.559	10.37	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284
9	23.087	1.28	6-Octen-1-ol, 3,7-dimethyl-	$C_{10}H_{20}O$	156
10	23.797	1.14	Hexylidencyclohexane	$C_{12}H_{22}$	166
11	23.854	5.76	9-Octadecenoic acid, ethyl ester	$C_{20}H_{38}O_2$	310
12	25.39	1.9	Dichloroacetic acid, nonyl ester	$C_{11}H_{20}Cl_2O_2$	254
13	25.501	1.43	Eicosanoic acid, 2-ethyl-2-methyl-, methyl ester	$C_{24}H_{48}O_2$	368
14	26.053	0.97	(S)-(+)-5-Methyl-1-heptanol	$C_8H_{18}O$	130
15	27.708	3.28	Cholesta-8, 24-dien-3-ol, 4-methyl-, (3.beta., 4.alpha.)-	$C_{28}H_{46}O$	398
16	27.913	1.19	1-Hexanesulfonic acid, methyl ester	$C_7H_{16}O_3S$	180
17	28.856	1.11	1-Pentanone, 3-[4-(diphenylmethyl)phenyl]-4,4	$C_{32}H_{32}O$	432
18	36.382	1.1	Stigmasteryl tosylate	$C_{36}H_{54}O_3S$	566
19	36.55	1.4	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436
20	38.121	11.28	Stigmasta-5,22-dien-3-ol	$C_{29}H_{48}O$	412
21	38.598	26.09	.beta.-Sitosterol	$C_{29}H_{50}O$	414
22	39.668	1.39	.beta.-Sitosterol acetate	$C_{31}H_{52}O_2$	456

Table 4: Bactericidal and fungicidal efficacy of alkaloids crude extracts of *Boerhavia diffusa*

Microorganisms		Root	stem	Leaves
Fungi		**Alkaloid**	**Alkaloid**	**Alkaloid**
F. oxysporium	IZ	15.33+1.52	13.33+0.57	12.16 +1.04
NCIM 1228	AI	0.64	0.56	0.47
P.fumiculosum	IZ	11.33+1.52	8.00+1	10.33+3.51
NCIM 1075	AI	0.54	0.38	0.49
C.albicans	IZ	14.66+0.57	20.33+2.08	14.33 +2.31
NCIM 3501	AI	0.86	1.19	0.78
T.viridie	IZ	12.33+1.52	16.66+1.15	12.6+3.60
NCIM	AI	0.46	0.62	0.68
Bacteria				
S. aureus	IZ	13.66±3.05	12.33±2.30	22.00±3.6
MTCC 0087	AI	0.46	0.41	0.73
E. coli	IZ	17.33±0.58	40.56±2.08	20.33±1.52
MTCC 1652	AI	0.96	1.76	1.13
E. faecalis	IZ	11.67±2.81	11.66±2.08	14.66±0.57
ATCC 29212	AI	0.58	0.58	0.73
B. subtilis	IZ	16.00±0.0	11.33±2.08	14.33±4.04
MTCC 0121	AI	0.80	0.57	0.72
K. pneumonia	IZ	15.00±3.0	0.00	13.67±3.05
MTCC 0109	AI	0.94	0.00	0.85

Antibacterial activity of alkaloids when tested against *S. aureus* leaf extracts showed higher activity as compared to stem extract which showed lower activity (leaf; IZ22.10 ± 1.00 mm > root; IZ= 13.66 ± 3.05 mm > stem; IZ=12.33 ± 2.30 mm). Stem showed maximum activity against *E. coli* and root showed minimum activity (stem; IZ40.66 ± 2.08 mm > leaf; IZ= 20.00 ± 1.00 mm > root; IZ=17.00 ± 1.00 mm. Root, stem and leaf showed moderate activity against *Enterococcus*. (Root, stem and leaf; IZ=11.66 ± 2.08 mm). Against *B. subtilis* roots exhibited maximum activity while stem exhibited minimum activity (root; IZ=17.66 ± 2.51 mm > leaf; IZ=14.66 ± 3.51 mm > stem; IZ=11.33 ± 2.08 mm). Against *K. pneumonia*, stem extracts showed no activity while a moderate activity was observed in root and leaf extract.

References

1. Ameyaw Y, Eshun DG. The alkaloid contents of the ethno-plant organs of three antimalarial medicinal plant species in the eastern region of Ghana. Int. J Chem. Sci. 2009; 7(1):48-58.
2. Croteau R, Kutchan TM, Lewis NG. Natural products (Secondary metabolites). In B. Buchanan, W. Gruissem, and R. Joneas, eds., Biochemistry and Molecular Biology

of Plants. American Society of Plant Biologists, Rockville, MD, 2000, 1250-1268.
3. Grierson DS, Afolayan AJ. Antibacterial activity of some indigenous plants used for the treatment of wound in the Eastern Cape; South Africa. Journal of Ethnopharmacology. 1999; 66:103-106.
4. Hadi S, Bremner JB. Initial Studies on Alkaloids from Lombok Medicinal Plants. Molecules. 2001; 6:117-129.
5. Kutchan TM. Alkaloid Biosynthesis-The Basis for Metabolic Engineering of Medicinal Plants. The Plant Cell. 1995; 7:1059-1070.
6. Ljubiša SC, Ivana CS, Bojana BM, Aleksandra MC, Marijana SB, Dragana PV. Antimicrobial activity of plant extracts from Serbia. Journal of the Institute for Food Technology in Novi Sad. 2009; 36(1-2):1-6.
7. Pereira DM, Faria J, Gaspar L, Valentão P, Andrade PB. Boerhaavia diffusa: Metabolite profiling of a medicinal plant from Nyctaginaceae Food and Chemical Toxicology. 2009; 47(8):2142-2149.
8. Rabia A. Urinary disease and ethnobotany among pastrol nomads in the Middle East. Journal of Ethnobiology and Ethnomedicine. 2005; 1:4-18.
9. Singh A. Herbal medicine–dream unresolved. Pharmacognosy Reviews. 2007; 2:375-376.
10. Singh DV, Maithy A, Verma RK, Gupta MM, Kumar S. Simultaniously Determination Of Catharanthus Alkaloids Using Reversed Phase High Performance Liquid Chromatography. J Liq. Chrom. & Rel. Technol. 2000; 23(4):601-607.
11. Singh RH, Udupa KN. Studies on the Indian indigenous drug punarnava (Boerhaavia diffusa Linn.). Part IV: Preliminary controlled clinical trial in nephrotic syndrome. Journal of Research in Indian Medicine. 1972; 7:28-33.
12. Uniyal SK, Singh KN, Jamwal P, Lal B. Traditional use of medicinal plants among the tribal communities of Chhata Bhangal. Western Himalayan. J Ethnobiol. Ethnomed. 2006; 2:1-14.
13. Verpoorte R. Exploration of nature's chemodiversity: the role of secondary metabolites as leads in drug development. Drug Discovery Today. 1998; 3(5):232-238.
14. Vickers A, Zollman C, Lee R. Herbal Medicine. 2001; 175(2):125-128.