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A Comparitive Study on Phytochemical Analysis, Antibacterial Activity and Antioxidant Activity of *Barleria prionitis* leaves extract of Petroleum ether and Ethanol extract

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Abstract: In recent time, traditional medicines have been brought a huge potential in herbal medicine with lots of therapeutic potential to heal many infectious diseases without associated with the side effects unlike synthetic drugs. In indigenous system of medicine in India, the aerial parts of Barleria prionitis are used in the treatment of anemia, toothache, and bacterial disorders. In the present work, an attempt has been made to study the efficacy of non-polar compounds fractionated from *Barleria prionitis* has been evaluated for the anti bacterial activity and compared with the efficacy of polar fraction of the same plant. Leaves of *Barleria prionitis* were subjected to extraction with petroleum ether which could have extract the non-polar compounds followed by ethanol could extract the polar compounds of *Barleria prionitis*. The non polar compounds present in both the extracts were identified through GC-MS Analysis and their efficacy in biological activities was evaluated by antioxidant and antibacterial properties.
Key Words: *Barleria prionitis*; petroleum ether extract; ethanol extract; anti bacterial activity; antioxidant activity.

Introduction

Medicinal plants are used world wide in management of healthcare problems since time immemorial and approximately 60-80% of the world's population still depending on the traditional medicines [1-6]. Currently, the global demand of herbal medicines is increasing rapidly because of their higher safety margin and low cost [7].With the help of medicinal plants still many of the diseases are getting cured. medicinal plants are believed to be a potential source for the discovery source for the discovery of new drugs candidates.[8-11] numbers of active compounds classes like alkaloids, terpenes, flavanoids, glycosides, lignans, phenolics, saponins etc has been used in the modern system of medicines for their wide therapeutic activities [12-16] *Barleria prionitis* or porcupine flower is an ornamental plant belongs to Acanthaceae family. This plant has a long history as an important herb possessing healing and curative properties. It is known as vajradanti because it is used to make teeth strong and free from all diseases. *Barleria prionitis* Linn is widely distributed throughout Africa, India, Srilanka and tropical Asia [17]. *Barleria prionitis* is an annual shrub, 1-3 feet high. They possess 2-4 sharp long axillary spines which about 11m long. Antioxidants, antibacterial are very important because it can be used therapeutically. phyotochemical compounds from this plant have been found to possess wide range of pharmacological include antimicrobial, anthelemintic, antifertility, antioxidant, anti-diabetic, anti-inflammatory, anti-arthritic, cytoprotective, hepatoprotective, diuretic, antidirrhoeal, enzyme inhibitory without any toxic effects. The shade dried plant was subjected to petroleum ether and ethanol extraction, the both the extract were evaluated for their antioxidant activity and antibacterial activity.

Materials and methods

Plant material: collection and authentication

At December month the *Barleria prionitis* leaves were collected from Ammapet, Thanjavur district, TamilNadu, India. The leaves of plant used for study was authenticated by Dr S. Jayendran, Assistant Professor, Government Arts College Ooty. Herbarium document number-182[B]

Preparation of Extract

The freshly collected leaves of *Barleria prionitis* were thoroughly washed in tap water and washed with distilled water to remove all type of contamination. Then washed leaves were air dried in the shade and the dried material [1.5kg] was powdered. The powdered plant material was percolated first with petroleum ether for 72 hours 3 times to extract fatty substances and non polar substances and that was further extracted with ethanol for 72hous for 3 times. Both the extract were concentrated using by rotor vaccum evaporator.. Then both the crudes extract [petroleum ether-10gm],[ethanol-11gm] were used for further analysis.

Materials

Ethanol, 2,2-diphenyl- 1-picryl-hydrazyl, Pottasium Dihydrogen Phosphate, Pottasium Ferricyanide, Trichloro acetic acid, Ascorbic acid, BHT, NBT(Nitroblue Tetrazolium), Phosphate buffer, EDTA, Riboflavin. Petroleum ether.

Superoxide Anion Scavenging Activity:

The superoxide anion scavenging activity is measured as described by Robak and Gryglewski(1988). The superoxide anion radicals are generated in 3.0 ml of Tris-HCJ buffer (16 mM.pH 8.0), containing 0.5 ml of NBT (o.3 mM), 0.5 ml NADH (0.036 mM) solution, 1.0 ml extract and 0.05 ml. Tris- HCI buffer (16 mM. pH 8.0). The reaction is started by adding 0.5 ml PMS solution(o.12 mM) to the mixture, incubated at 25 degree Celsius for 5 minutes and then the absorbance is measured at 560 mM) against a blank sample. Ascorbic acid is used as a positive control, Formula: Inhibition (%)=(Ao-A1/Ao) x 100

DPPH Free Radical Scavenging Activity

Dpph is used as a main substrate to evaluate antioxidant activity. DPPH assay is based on the measurement of ability of antioxidant towards dpph radical. The method is based on a change in purple coloured ethanol solution of DPPH in presence of hydrogen donating antioxidants, by formation of yellow coloured non radical form. The scavenging ability of petroleum ether, ethanol extract was compared with ascorbic acid. The DPPH free radical scavenging activity was calculated using the following formula:

The Percentage of inhibition can be calculated using the formula.

Inhibition (%) = 100- (Ao-A1/Ao) x 1

Where : **Ao** is the absorbance of control and **A1** is the absorbance of test.

Frap (Ferric Reducing Ability of Plasma)

In FRAP analysis various concentrations of extract were taken like 0.1, 0.2, 0.3, 0.4, 0.5ml .with that 2.5ml of phosphate buffer is added. Now 2.5 ml of potassium ferricyanide is added to each. Instead of plant extract if ascorbic acid were added then that is taken as standard. Then all the samples and standard were allowed to heat at 50degree Celsius for 20minutes. 2.5ml of trichloro acetic acid is added and centrifuged at 2000rpm for 10minutes. The upper layer of 2.5ml supernatant and 2.5 ml of distilled water, ferric chloride and absorbance at 700nm under UV visible spectrophotometer. without extract ascorbic acid is considered as standard.

Antibacterial Assay

Bacterial strains, *Escherchia coli, Psedumonas beteli, Psedumonas fluroscence, Salmonella paratyphi, Staphyllococcus aureus, Bacillus subitilus, Psedumonas putida.*

These were strains used for anti bacterial activity. The Bacteria were incubated on a nutrient agar-slant for 48 hours at 37 degree Celsius.

Screening for Antibacterial Activty

The standard antibiotics used were chloramphenicol and Tetracycline. The antibacterial activity was demonstrated using a modified method originally described which is widely used from antibacterial susceptibility testing. A loopful of bacteria was taken from the stock culture and dissolved in O.1ml of saline. All the tests were done by well diffusion method. Various crude solvent extracts are impregnated with discs and placed on Nutrient Agar plates. These plates are already inoculated with 20 ml of Nutrient broth medium with Gram positive and Gram negative bacteria. Respective solvent without plant extracts served as negative control. Standard antibiotics were used as reference or positive control. Plates were incubated at 37 degree Celsius for 24 hours. After the incubation period, the diameter of the inhibition zone around the leaf extracts were measured and also compared with the diameter of inhibition zone of commercial standard antibiotics.

Results and Discussion:

Phytochemical studies

It is revealed that from various tests of phytochemical analysis, Alkaloids, Terpenoids, Phenolic compounds, Tannins, Carbohydrates and Flavonoids are present in the plant *Barleria prionitis* and the response is tabulated in the Table 1.

Table 1: Phytochemical Analysis of *Barleria prionitis* Petroleum ether extract and ethanol extract

Detection	Test	Response	Result
Alkaloids	Mayer's test	White or creamy precipitated	+
Carbohydrates	Fehling's test	Red precipitate color	+
Phenolic compounds and Tannins	Ferric chloride test	Dark Green color	+
Flavonoids	Alkaline Reagent Test	Yellow color	+
Terpenoids	Dinitrophenyl hydrazine test	Yellow interface layer	+

Results for Antibacterial Activity

The anti bacterial activity was examined for both petroleum ether extract and ethanol extract. Totally 7 strains were used, *B.subtilus, S.aureus, P.betili, S.paratyphi, P.florosence, E.coli, P.putide*.

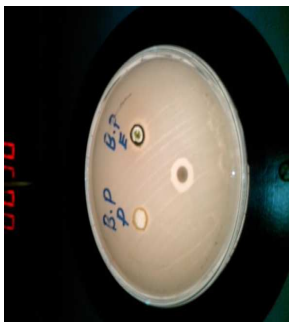


Fig 1: P.beteli

Fig 2: P.aureus

Fig 3: B.subtilus

Fig 5: P.putida

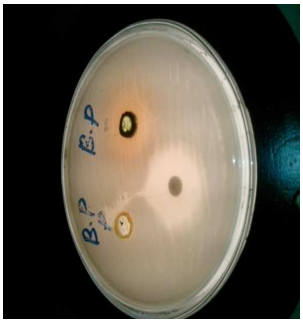


Fig 5: P.flurosence

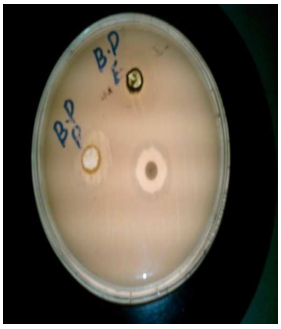


Fig 6: S.paratyphi


Fig 7. E.coli

Petroleum ether extract

The petroleum ether extract of *Barleria prionitis* showed good antibacterial activity. The petroleum ether extract of *Barleria prionitis* was most effective against *Pseudomonas putida* and *Bacillus subitilus* with a zone of inhibition of 28mm. Zone of inhibition for petroleum ether extracts of *Barleria prionitis* was compared with standard antibiotics.

Ethanol extract

The ethanol extract of *Barleria prionitis* showed good antibacterial activity. The ethanol extract of *Barleria prionitis* was most effective against *Pseudomonas putida* with a zone of inhibition of 25mm. Zone of inhibition for petroleum ether extracts of *Barleria prionitis* was compared with standard antibiotics.

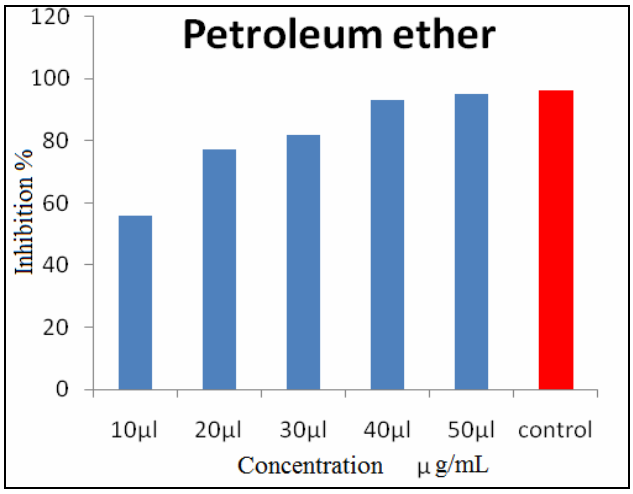
Table 2: diameter of zone of inhibition

Name of the species	Standard used	Diameter of zone of inhibition (mm)		
		Petroleum ether fraction	ethanol fraction	Standard
Escherichia coli	Chloromphenical	15	11	25
Pseudomonas fluorescence	Tetracycline	16	19	25
Pseudomonas putida	Chloromphenical	28	25	30
Pseudomonas beteli	Chloromphenical	-	-	20
Salmonella paratyphi	Tetracycline	18	-	19
Staphylococcus aureus	Tetracycline	-	15	17
Bacillus subitilus	Tetracycline	28	11	18

DPPH assay:
Petroleum ether

Table 3:Free radical scavenging activity *for Barleria prionitis of* petroleum ether extract:

Concentration of samples per µg/1ml	% of inhibition	Control % Ascorbic acid/BHT
10	26	96
20	76	
30	87	
40	90	
50	94	

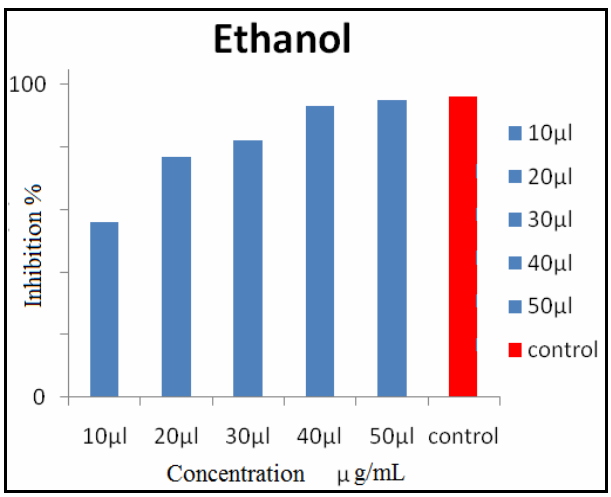


Graph shows that the DPPH free radical scavenging capacity of petroleum ether extract of *Barleria prionitis* is proportional to the increasing concentration of *Barleria prionitis* extract. It shows that *Barleria prionitis* is a better scavenger. it is compared to standard ascorbic acid.

Ethanol extract:

Table 4: Free radical scavenging activity *for Barleria prionitis of* ethanol extract:

Concentration of samples per µg/1ml	% of inhibition	Control % Ascorbic acid/BHT
10	46	
20	77	
30	82	96
40	93	
50	95	

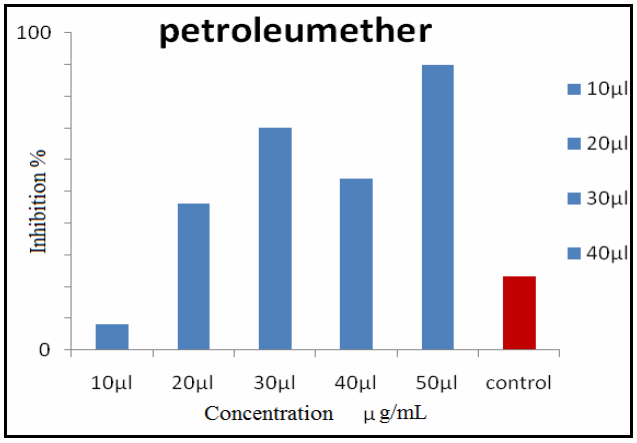


The graph shows that the DPPH free radical scavenging capacity of ethanol extract of *Barleria prionitis* is proportional to the increasing concentration of *Barleria prionitis* extract. It shows that *Barleria prionitis* is a better scavenger. It is compared to standard ascorbic acid.

Super Oxide:

Table 5: Superoxide scavenging activity for *Barleria prionitis* of petroleum ether extract:

Concentration of sample Per µg/ml	% Inhibition	Control% Ascorbic acid
10	8	23
20	46	
30	70	
40	54	
50	90	

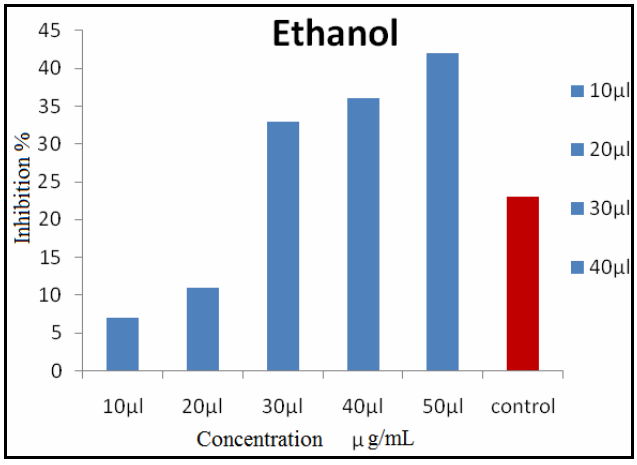


The graph shows that the super oxide anion free radical scavenging capacity of *Barleria prionitis* shows increasing and decreasing concentration of petroleum ether extract of Barleria prionitis. This shows that *Barleria prionitis* has less scavenging activity. The standard used is ascorbic acid

Ethanol

Table 6: Superoxide scavenging activity for *Barleria prionitis* of Ethanol extract:

Concentration of sample Per µg/ml	% Inhibition	Control% Ascorbic acid
10	7	23
20	11	
30	33	
40	36	
50	42	



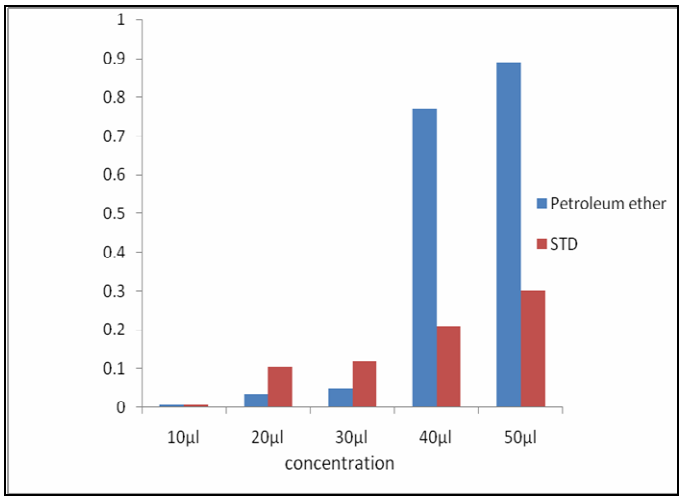
The graph shows that the super oxide anion free radical scavenging capacity of *Barleria prionitis* shows increasing and decreasing concentration of ethanol extract of *Barleria prionitis*. This shows that *Barleria prionitis* has less scavenging activity. The standard used is ascorbic acid.

FRAP:

Table 7: FRAP (ferric reducing ability of plasma)scavenging activity of *Barleria prionitis* of petroleum ether extract:

Concentration of sample Per µg/ml	FRAP scavenging activity of petroleum ether Fe^{+}/µg	FRAP scavenging activity of control ascorbic acid Fe^{+}/µg
10	0.007	0.008
20	0.036	0.105
30	0.048	0.119
40	0.77	0.21
50	0.89	0.302

Petroleum ether

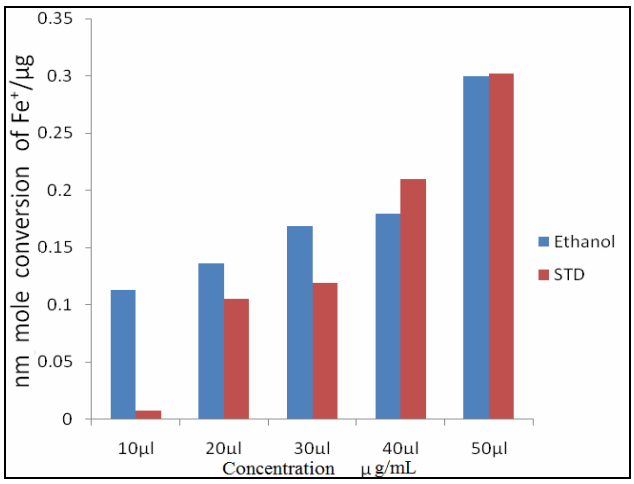


From the graph the FRAP values of petroleum ether extract of *Barleria prionitis* inhibited more ferric reducing activity .and it was observed that it increased with increasing concentration.

Table 8: FRAP scavenging activity of *Barleria prionitis* of Ethanol extract:

Concentration of sample Per µg/ml	FRAP scavenging activity Ethanol Fe^{+}/µg	FRAP scavenging activity of control ascorbic acid Fe^{+}/µg
10	0.11	0.008
20	0.136	0.105
30	0.169	0.119
40	0.18	0.21
50	0.3	0.302

Ethanol



From the graph the FRAP values of ethanol extract of *Barleria prionitis* inhibited more ferric reducing activity .and it was observed that it increased with increasing concentration.

Discussion:

The antioxidant activity of ethanolic extract was evaluated by various assays such as α,α-Diphenyl-β-picryl-hydrazyl radical scavenging(DPPH) assay, superoxide anion radical scavenging (SO) and Ferric reducing ability of plasma(FRAP). For DPPH activity it shows that the DPPH free radical scavenging capacity of petroleum ether extract of *Barleria prionitis* is proportionally increasing with the increase in the concentration of *Barleria prionitis* plant extract. Super oxide activity shows that the super oxide anion free radical scavenging capacity of *Barleria prionitis* showing non linear values with repect to concentration .revealed that petroleum ether extract of *Barleria prionitis* maybe a poor super oxide scavenger. FRAP assay shows that petroleum ether extract of *Barleria prionitis* inhibited more ferric reducing activity compared to standard. The antibacterial activity of was examined for both petroleum ether and ethanol leaf extract. Bacterial strains of *Escherchia coli ,Psedumonas beteli, Psedumonas fluroscence, Salmonella paratyphi, Staphyllococcus aureus ,Bacillus subitilus , psedumonas putide* were used. After the incubation period, diameter of inhibition zone of commercial standard antibiotics. Both the extracts showed good result. The petroleum ether extract of *Barleria prionitis* was most effective against *Pseudomonas putide* and *Bacillus subitilus.* Whereas the ethanol extract of *Barleria prionitis* was most effective against *Pseudomonas putide.* Isolation has to be carried out further through column chromotography in petroleum ether fraction and compounds has to be isolated.

References:

1. Dey, S.K., D. Banerjee, P.K. Nandi, S. Chattopadhyay and K. B. Karmakar,. Antimicrobial properties of Indian spices. Natural Prod., 2009, 5, 104-110
2. Ansari, J.A. and N.N. Inamdar,. The promise of traditional medicines. Int. J. Pharmacol., 2010, 6, 808-812.
3. Shafaei, A., E. Farsi, B.M.K. Ahamed, M.J.A. Siddiqui, I.H. Attitalla, I. Zhari and M.Z. Asmawi. Evaluation of toxicological and standardization parameters and phytochemical investigation of *Ficus deltoidea* leaves. Am. J. Biochem. Mol. Biol., 2011, 1, 237-243.
4. Menghani, E., A. Pareek, R.S. Negi and C.K. Ojha, Search for antimicrobial potentials from certain indian medicinal plants. Res. J. Med. Plant.,2011, 5, 295-301
5. Ramachandran, S., M. Vamsikrishna, K.V. Gowthami, B. Heera and M.D. Dhanaraju, Assessment of cytotoxic activity of agave cantula using brine shrimp (*Artemia salina*) lethality bioassay. Asian J. Sci. Res.2011, 4: 90-94
6. Kumar, T. and K.S. Chandrashekar, *Bauhinia purpurea* Linn.: A review of its ethnobotany, phyto chemical and pharmacological profile. Res. J. Med. Plant,2011, 5: 420-431
7. Musyimi, D.M., J.A. Ogur and P.M. Muema, . Phytochemical compounds and antimicrobial activity of extracts of aspilia plant (*Aspilia mossambicensis*) (Oliv) wild. Int. J. Bot.,2008, 4: 56-61

8. Mohajer, M., P. Sarkhail, N. Hajarolasvadi, M.J. Zamani and R. Khorasan *et al*., Antiinflammatory and analgesic effects of *Phlomis lanceolata* boiss. and hohen. extracts and examination of their components. Int. J. Pharmacol.,2006 2: 50-54.
9. Dey, S.K., D. Banerjee, S. Chattapadhyay and K.B. Karmakar, Antimicrobial activities of some medicinal plants of West Bengal. Int. J. Pharma Bio. Sci.2010, 1: 1-10.
10. Roy, S. and D. Banerjee, . Isolation of antimicrobial compound by endophytic bacteria from *Vinca rosea*. Int. J. Curr. Res.,2010 5: 47-51.
11. Kayode, A.A.A. and O.T. Kayode, . Some medicinal values of *Telfairia occidentalis*: A review. Am. J. Biochem. Mol. Biol.2011, 1: 30-38
12. Saadabi, A.M.A., A.G. AL-Sehemi and K.A. AL-Zailaie, . *In vitro* antimicrobial activity of some Saudi Arabian plants used in folkloric medicine. Int. J. Bot.,2006 2: 201-204.
13. Mukherjee, P.K., D. Mukherjee, A.K. Maji, S. Rai and M. Heinrich, The sacred lotus (*Nelumbo nucifera*)-phytochemical and therapeutic profile. J. Pharm. Pharmacol2009 61: 407-422.
14. Sohail, M.N., F. Rasul, A. Karim, U. Kanwal and I.H. Attitalla,. Plant as a source of natural antiviral agents. Asian J. Anim. Vet. Adv., 2011, 6: 1125-1152.
15. Agrawal, B., S. Das and A. Pandey, *Boerhaavia diffusa* Linn: A review on its phytochemical and pharmacological profile. Asian J. Applied Sci.,2011, 4: 663-684
16. Gantait, A., A. Maji, T. Barman, P. Banerji, P. Venkatesh and P.K. Mukherjee, Estimation of capsaicin through scanning densitometry and evaluation of different varieties of capsicum in India. Nat. Prod. Res.,2011, 26: 216-222.
17. Daniel, M., Medicinal Plants: Chemistry and Properties. 1st Edn., Science Publishers, USA.,2006 Pages: 78.
18. 22. Manek, R.A., N.R. Sheth, J.D. Vaghasiya, S.V. Malaviya, N.P. Jivani and J.R. Chavda, Study on herb-herb interaction potential of *Glycyrrhiza glabra* with *Solanum xanthocarpum* and *Adhatoda vasica* on mast cell stabilizing activity.2011 Int. J. Pharmacol.

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