INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com ISSN 2230 – 8407
Research Article

# *BARLERIA CRISTATA* LINN.: PHYTOCHEMICAL SCREENING AND HPTLC ANALYSIS

Rajasekaran Narmadha and Kanakasabapathi Devaki*
Department of Biochemistry, Karpagam University, Coimbatore-641 021 India

Article Received on: 15/04/12 Revised on: 21/05/12 Approved for publication: 11/06/12

*Dr.K.Devaki, Assistant Professor in Biochemistry, Karpagam University, Coimbatore-641 021 Tamilnadu, India
Email: devakirajasekar@gmail.com

**ABSTRACT**
Phytochemical examination (qualitative and quantitative) and HPTLC analysis of phytochemicals of the crude extract *Barleria cristata* Linn. leaves were investigated. Preliminary phytochemical screening of various extracts of the leaves revealed the presence of compounds such as amino acids, carbohydrates, flavonoids, proteins, phenolic groups, saponins, steroids, tannins and terpenoids. HPTLC finger printing analysis support the presence of alkaloids and phenolic compounds (Quercetin) in this plant extract. The present study provides information with respect to phytochemicals of *Barleria cristata* L.
**Keywords**: HPTLC, secondary metabolites, *Barleria cristata* Linn., sequential extraction

## INTRODUCTION

Plants are rice source of large amount of drugs comprising to different groups such as antispasmodics, emetics, anti-cancer, antimicrobials etc. They have been an important source of medicine for thousands of years. Even today, the World Health Organization estimates that up to 80 percent of people still rely mainly on traditional remedies such as herbs for their medicines. Its civilization is very ancient and the country as a whole has long been known for its rich resources of medical plants[1-3]. The use of traditional medicines holds a great promise as a easily available source as effective medicinal agents to cure a wide range of ailments among the people particularly in tropical developing countries like India. Use of several plants or plant derived formulations to cure helmintic infections and treatment of wounds were also recorded [4, 5].

Herbal medicines are known to be oldest health care products that have been used by mankind all over the world in the form of folklore medicines or traditional medicines or ethnic medicines. The therapeutic use of herbal medicines is gaining considerable momentum in the world during the past decade. The World Health Organization (WHO) estimates that herbal medicine is still the mainstay of about 75-80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side-effects[6].

*Barleria cristata* Linn (family acanthaceae) is a shrub found widely in subtropical Himalaya, Sikkim, Khasi Hills, central, and southern India at a height of 1,350 m. The chemical constituents of the plant have been identified as flavonoid type phenolic compounds, especially apigenin, quercetin, quercetin-3-O-β-D-glucoside, naringenin, luteolin, and apigenin glucuronide. *Barleria cristata* L. have been used traditionally for the treatment of variety of diseases including anemia, toothache, and cough. Leaves were used to reduce swellings in inflammation[7].

The main objective of the present study was to investigate the phytochemical screening and HPTLC finger printing analysis of the leaves of *Barleria cristata.*

## MATERIALS AND METHODS

### Collection of Plant Material:

The leaves of *Barleria cristata* used for the investigation were obtained from Coimbatore district, Tamilnadu, India. The plant was authenticated by Botanical Survey of India, TNAU Campus, Coimbatore. The voucher number is **BSI/SRC/5/23/2011-12/Tech.-n62**.

### Preparation of Plant Extract:

The leaves of *Barleria cristata* L. were air dried in the absence of sunlight and powdered well using a mixer and stored in an air tight container. The powdered plant material (50 g) was taken and subjected to successive solvent extraction (250ml) with increasing order of polarity like petroleum ether, chloroform, ethyl acetate, ethanol and water. The plant extracts were concentrated and stored in an airtight vial for further studies.

### Phytochemical screening

### Qualitative estimation of phytoconstituents

Phytochemical screening was carried out to assess the qualitative chemical composition of crude extracts using commonly employed precipitation and coloration to identify the major natural chemical groups such as steroids, reducing sugars, alkaloids, phenolic compounds, saponins, tannins, flavonoids, amino acids, fixed oils and fats and cardio glycosides. General reactions in these analysis revealed the presence or absence of these compounds in the crude extracts tested [8,9].

### Quantitative estimation of phytoconstituents

### Estimation of Protein:

The amount of protein present in the ethanolic extract of *Barleria cristata* was determined by the standard method given by Lowry *et al.,*[10]

### Estimation of Total Phenolics:

Total phenolic content of ethanolic extract of *Barleria cristata* was measured based on Folin-Ciocalteu assay[11]. Briefly, 0.5 ml of ethanolic extract was mixed with distilled water (2.5 ml) and 0.5 ml of Folin-ciocalteu reagent was added. After 3 minutes 2 ml of 20% sodium carbonate was added and mixed thoroughly. The tubes were incubated in a boiling water bath for exactly one minute. It was then cooled and the absorbance was measured at 650nm using spectrophotometer against the reagent blank. Total phenolic

content was expressed as mg gallic acid equivalents (GAE)/g of extract.

**Estimation of Total Flavonoids:**

The flavonoid content was examined by adopting the methodology of Ordon *et al*., 2006[12]. Briefly, a volume of 0.5 ml of 2% $AlCl_3$ in ethanol solution was added to 0.5 ml of sample solution. After one hour incubation at room temperature, yellow colour was developed. This was measured at 420 nm with UV-Visible spectrophotometer. A standard graph was prepared using the quercetin and the total flavonoid content was expressed as quercetin equivalent (mg/g) of extract.

**HPTLC analysis**

2µl of the above test solution and 2µl of standard solution were loaded as 5mm band length in the 3 x 10 Silica gel $60F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with its respective mobile phases (alkaloid, flavonoids and phenols) and the plate was developed up to 90mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at white light, UV 254nm and UV366nm. The developed plate was sprayed with respective spray reagent and dried at 100º C in hot air oven. The plate was photo-documented at daylight and UV 366nm mode using Photo-documentation (CAMAG REPROSTAR 3) chamber. After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at 500nm. The peak table, peak display and peak densitogram were noted.

**RESULT AND DISCUSSION**

The phytoconstituents are the major important compounds which are responsible for the medicinal properties of the herbs. Hence medicinal plants could be a potential source for nutraceuticals. The phytochemical substances namely, phenols and flavonoids are the major important substances responsible for the medicinal values of the plants including antioxidant, anticancer, antimicrobial activities, etc[13]. In the present study the phytochemical screening results revealed the presence of phytoconstituents such as alkaloids carbohydrates, glycosides, proteins and amino acids, phenols, flavonoids, phytosterols, steroids, tannins and triterpenes (Table 1). Among extracts, most of the phytochemicals were found to be present in ethanol and aqueous extract of *Barleria cristata* L.

Table. 2 shows the percentage yield of different extracts of *Barleria cristata* L. In that, the maximum yield was found in both ethanolic and water extract. So the quantitative analyses of secondary metabolites were carried out by using these two extracts. In quantitative assay the ethanolic extract showed the high concentration of alkaloids (1.43±0.02), phenols (0.725±0.01), flavonoids (0.47±0.05), and proteins (42.5±5.13) when compared with aqueous extract (Table 3). Therefore, ethanolic extract of *Barleria cristata* was used for the subsequent studies.

HPTLC profile of ethanolic extract of *Barleria cristata* L. was recorded in Table 3, 4 and 5 for alkaloids, flavonoids and phenols respectively. Yellow-brown coloured zones were detected in day light and UV after derivetaization for alkaloid, yellow coloured fluorescent zone at UV 366nm for flavonoid and blue coloured zones for phenols in the chromatogram. The extracts were run along with the standard alkaloid, flavonoid and phenolic compounds. The leaf extract that shows the presence of these compounds in the chromatogram and densitogram are depicted in Figure 1-6.

**Table. 1. Qualitative analysis of selected phytochemicals in *Barleria cristata***

| Constituents | Solvents | | | | |
|---|---|---|---|---|---|
| | Petroleum ether | Chloroform | Ethyl acetate | Ethanol | Water |
| Alkaloids | + | + | + | + | + |
| Carbohydrates | + | + | - | + | + |
| Glycosides | - | - | - | + | + |
| Proteins and amino acids | + | + | + | + | + |
| Phenols | - | - | - | + | + |
| Flavonoids | - | - | - | + | + |
| Phytosterols | - | - | + | + | + |
| Steroids | - | - | - | + | - |
| Tannins | + | - | - | + | + |
| Triterpenes | - | + | - | + | + |

**Table. 2. Percentage yield of different extracts of *Barleria cristata***

| Extracts | % of yield (g) |
|---|---|
| Petroleum ether | 3 |
| Chloroform | 3.8 |
| Ethyl acetate | 4.5 |
| Ethanol | 8 |
| Aqueous | 5 |

**Table.3. Quantitative estimation of phytoconstituents in ethanolic and aqueous extracts of *Barleria cristata***

| Phytoconstituents | Ethanolic extract | Aqueous extracts |
|---|---|---|
| Protein (mg/g) | 42.5±5.13 | 33.69±4.66 |
| Phenol (mg/g) | 0.78±0.01 | 0.60±0.03 |
| Flavonoid (mg/g) | 0.47±0.05 | 0.34±0.02 |
| Alkaloids (% weight) | 1.43±0.02 | 0.44±0.01 |

**Table.4. Peak table with Rf values, height and area of alkaloids and unknown compounds**

| Track | Peak | Rf | Height | Area | Assigned substance |
|---|---|---|---|---|---|
| COL | 1 | 0.41 | 132.8 | 3583.7 | Colchicine standard |
| Sample C | 1 | 0.03 | 63.4 | 1250.7 | Alkaloid 1 |
| Sample C | 2 | 0.10 | 301.2 | 9273.8 | Alkaloid 2 |
| Sample C | 3 | 0.14 | 58.0 | 777.8 | Alkaloid 3 |
| Sample C | 4 | 0.17 | 99.0 | 1877.6 | Alkaloid 4 |
| Sample C | 5 | 0.22 | 116.7 | 2991.6 | Alkaloid 5 |
| Sample C | 6 | 0.27 | 142.1 | 2825.3 | Alkaloid 6 |
| Sample C | 7 | 0.41 | 16.2 | 379.1 | Unknown |
| Sample C | 8 | 0.44 | 42.7 | 496.1 | Unknown |
| Sample C | 9 | 0.47 | 29.5 | 691.9 | Unknown |
| Sample C | 10 | 0.50 | 41.5 | 366..2 | Unknown |
| Sample C | 11 | 0.53 | 21.6 | 327.3 | Unknown |
| Sample C | 12 | 0.61 | 165.5 | 4311.0 | Alkaloid 7 |
| Sample C | 13 | 0.74 | 22.7 | 443.5 | Unknown |
| Sample C | 14 | 0.91 | 390.4 | 20417.0 | Unknown |

**Figure. 1: Chromatogram result for alkaloids**

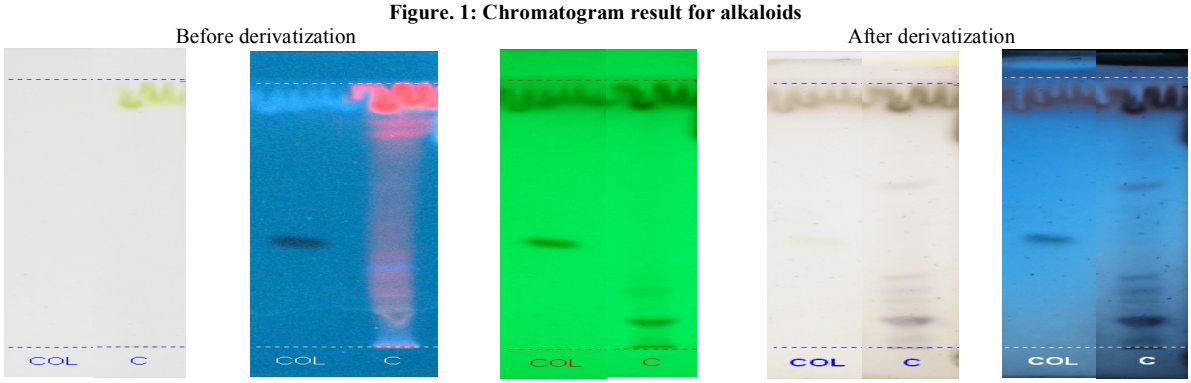
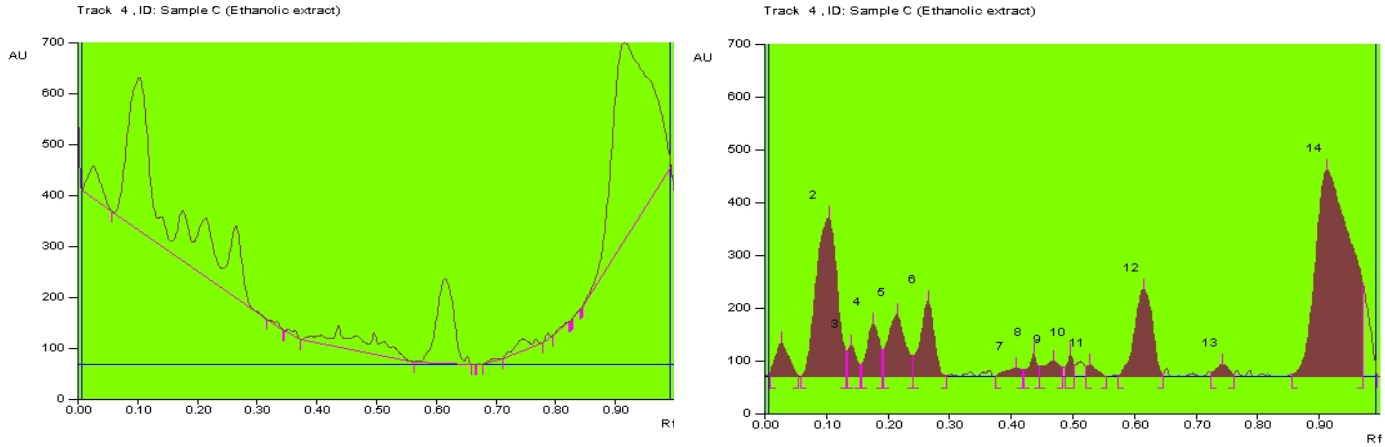
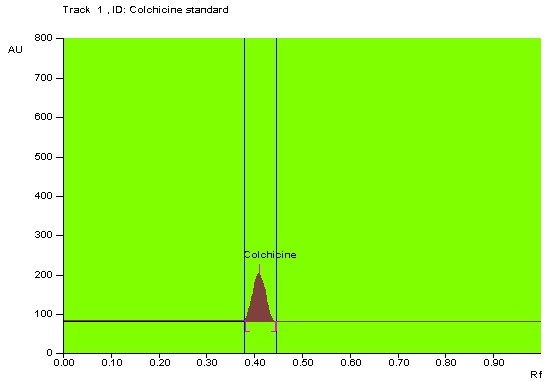



Fig. 1: Chromatograms of extract in HPTLC analysis -Before derivatization: under day light, Under UV 254 nm, Under UV 366 nm, After derivatization: under day light and UV 366 nm

**Figure. 2: Densitogram and Baseline for Alkaloid of ethanolic extract of *Barleria cristata***



**Track 4 – Sample C Ethanolic extract Baseline display (Scanned at 500nm)**
**Track 4 – Sample C Ethanolic extract Peak densitogram display (Scanned at 500nm)**




**Track COL – Colchicine standard Peak densitogram display (Scanned at 500nm)**

Fig. 2: HPTLC chromatogram of ethanolic extract at 500nm, showing different peaks (bands) of phytoconstituents in *Barleria cristata*. Concentration of sample 100mg/ml. Mobile phase Ethyl acetate: Methanol: Water (10 : 1.35 : 1).

The Rf value of *Barleria cristata* L. leaves extract was found to be 0.03, 0.10, 0.14, 0.17, 0.22, 0.27, 0.41, 0.44, 0.47, 0.53, 0.53, 0.61, 0.74, 0.91 for the peaks 1, 2, 3, 4, 5, 6 and 12. The graph illustrate that alkaloid numbered as 2 was found to be maximum in its concentration(Table 4, Figure 1 and 2).

Table. 5 showed the Rf value of plant extract and unknown compounds of flavonoid (Figure 3 and 4). The flavonoid compound showed the Rf value of 0.07, 0.13, 0.29, 0.71 for the peaks of 2, 3, 4 and 8. The peak height of the respective flavonoid compounds was also given in the Table 5, Figure 3 and 4.

**Table.5. Peak table with Rf values, height and area of flavonoids and unknown compounds**

| Track | Peak | Rf | Height | Area | Assigned substance |
|---|---|---|---|---|---|
| RUT | 1 | 0.26 | 322.2 | 8991.4 | Rutin standard |
| Sample S2 | 2 | 0.07 | 60.3 | 1235.5 | Flavonoid 1 |
| Sample S2 | 3 | 0.13 | 71.6 | 1596.7 | Flavonoid 2 |
| Sample S2 | 4 | 0.29 | 105.8 | 3775.6 | Flavonoid 3 |
| Sample S2 | 5 | 0.43 | 13.4 | 354.2 | Unknown |
| Sample S2 | 6 | 0.55 | 13.1 | 333.4 | Unknown |
| Sample S2 | 7 | 0.64 | 26.2 | 948.4 | Unknown |
| Sample S2 | 8 | 0.71 | 147.4 | 5896.7 | Flavonoid 4 |
| Sample S2 | 9 | 0.89 | 16.2 | 752.5 | Unknown |

**Figure.3. Chromatogram result for flavonoids**

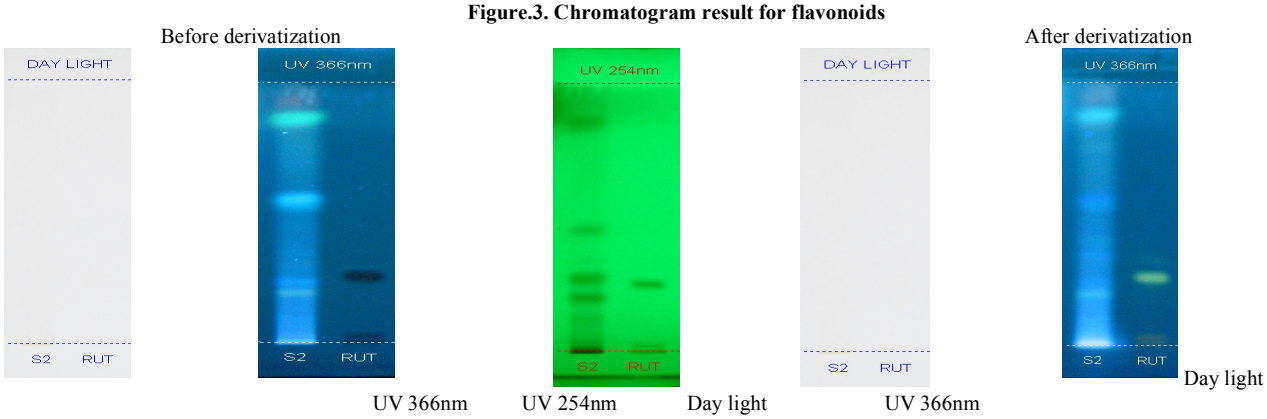
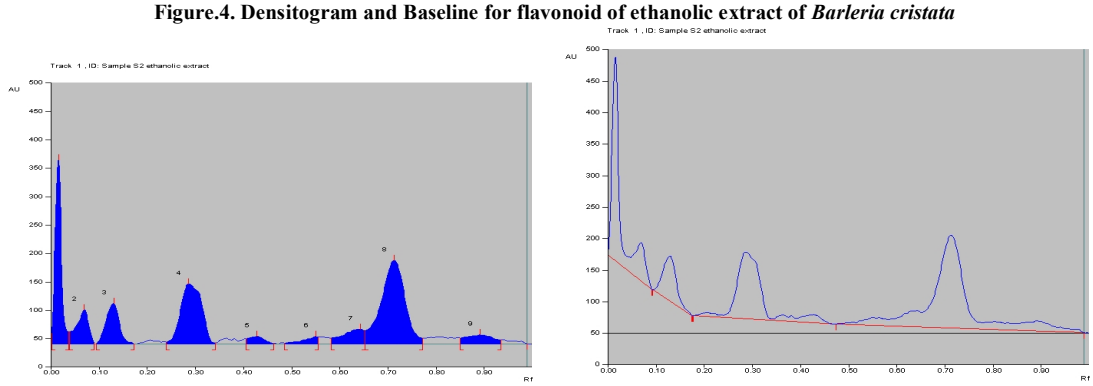



Fig. 3: Chromatograms of sample extract (100mg/ml) in HPTLC analysis -Before derivatization: under day light, Under UV 254 nm, Under UV 366 nm, After derivatization: under day light and UV 366 nm

**Figure.4. Densitogram and Baseline for flavonoid of ethanolic extract of *Barleria cristata***



**Track 1 – Sample S2 Ethanolic extract Baseline display (Scanned at 366nm)**
**Track 1 – Sample S2 Ethanolic extract Peak densitogram display (Scanned at 366nm)**
**Track RUT– Peak densitogram display**

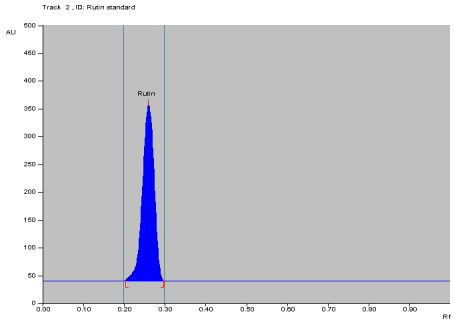


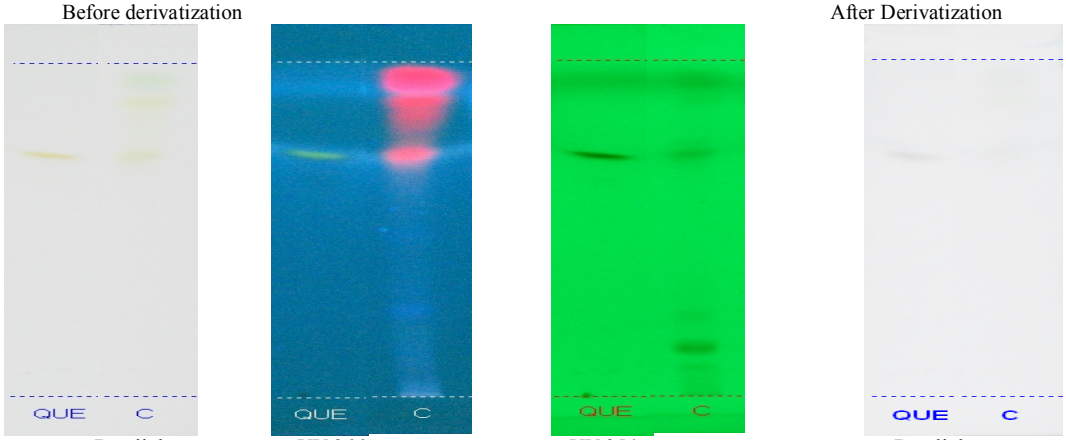

Fig. 4: HPTLC chromatogram of ethanolic extract at 366nm, showing different peaks (bands) of phytoconstituents in *Barleria cristata*. Concentration of sample 100mg/ml. Ethyl acetate: Butanone: Formic acid: Water (5 : 3 : 1 : 1)

**Table.6. Peak table with Rf values, height and area of phenols and unknown compounds**

| Track | Peak | Rf | Height | Area | Assigned substance |
|---|---|---|---|---|---|
| QUE | 1 | 0.73 | 392.4 | 7334.5 | Quercetin standard |
| Sample C | 1 | 0.05 | 28.0 | 435.0 | Unknown |
| Sample C | 2 | 0.08 | 40.0 | 716.4 | Unknown |
| Sample C | 3 | 0.14 | 182.9 | 5426.4 | Unknown |
| Sample C | 4 | 0.23 | 42.0 | 1185.3 | Unknown |
| Sample C | 5 | 0.73 | 120.6 | 5141.7 | Phenolic 1 (Quercetin) |
| Sample C | 6 | 0.94 | 79.4 | 3708.2 | Phenolic 2 |

**Figure.5. Chromatogram result for phenolic compounds**

Before derivatization

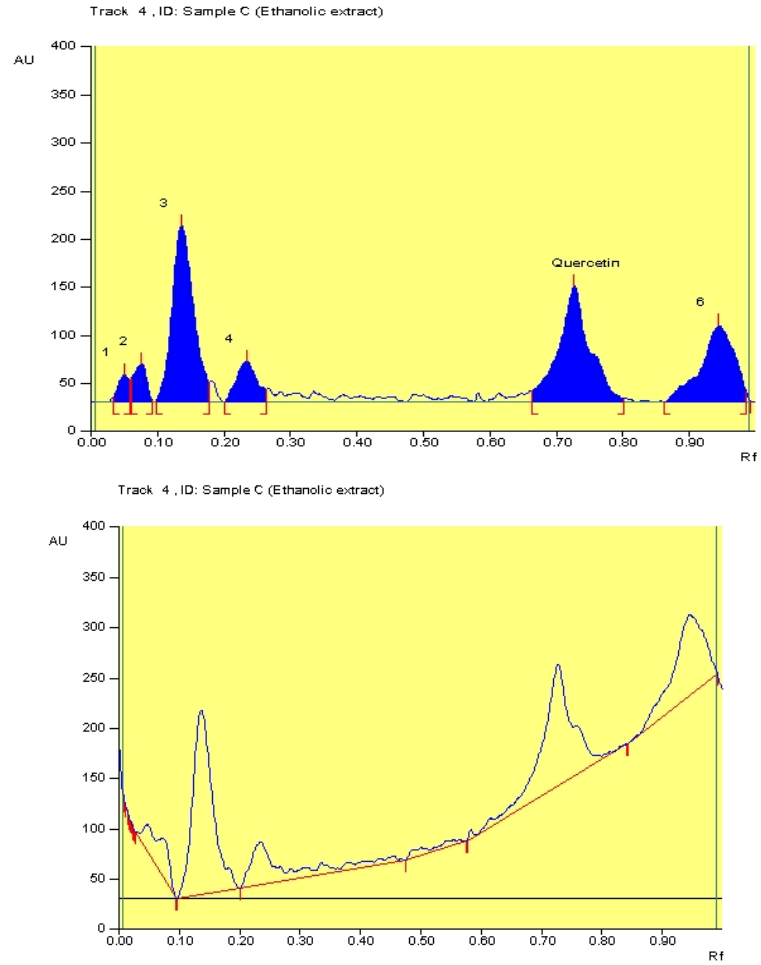



After Derivatization

Day light UV 366nm UV 254nm Day light

Fig. 5: Chromatograms of sample extract (100mg/ml) in HPTLC analysis -Before derivatization: under day light, Under UV 254 nm, Under UV 366 nm, After derivatization: under day light and UV 366 nm

**Figure. 6: Densitogram and Baseline for phenol of ethanolic extract of *Barleria cristata***




**Track C – Sample C Ethanolic extract Baseline display (Scanned at 254nm)**
**Track C – Sample C Ethanolic extract Peak densitogram display (Scanned at 254nm)**

Fig. 6: HPTLC chromatogram of ethanolic extract of ***Barleria cristata* L.** at 254nm, showing different peaks (bands) of phytoconstituents in which 8th peak was found to be quercetin with Rf value of 0.73. Concentration of sample 100mg/ml. Toluene: Acetone: Formic acid (4.5 : 4.5 : 1).

Table.6 showed the Rf value of plant extract and unknown compounds of phenol (Figure 3 and 4). The phenolic compound showed the Rf value of 0.73, 0.94 for the peaks of 5, 6. The peak height of the respective phenolic compounds was also given in the Table 6, Figure 5 and 6. The Rf value of one of the compound was similar to the quercetin standard and it is identified as quercetin. Phenolics compounds are one of the major groups of compounds acting as primary antioxidants or free radical terminators, it was reasonable to determine their total amount in the selected plant extract[17].

Finger print analysis by HPTLC has become an effective and powerful tool for linking the chemical constituent profile of the plants with botanical identity and for the estimation of chemical and biochemical markers[14-16]. It also offers the better resolution and estimation of active constituents with reasonable accuracy in a shorter time and it has been found to be rapid, sensitive, precise, and accurate and it has been applied for simultaneous quantitation of phytoconstituents[18]. The phenolic compounds such as flavonoids, phenolic acids and tannins are considered to be major contributors to the antioxidant capacity of plants. These antioxidants also possess diverse biological activities such as anticarcinogenic, anti-atherosclerotic and anti-inflammatory activities. These activities may be related to their antioxidant activity[19].

## CONCLUSION

The present study with *Barleria cristata* L. could be an answer to the people seeking for better therapeutic agents from natural sources which is believed to be more efficient with little or no side effects when compared to the commonly used synthetic chemotherapeutic agents. The commonly used medicinal plants, vegetables have not only possess essential nutrients but also contain secondary metabolites such as alkaloids, flavonoids, glycosides, steroids and phenols. The essences of these metabolites are beneficial for maintenance of human health and chronic degenerative diseases.

## ACKNOWLEDGEMENT

We, the authors are thankful to our Chancellor, Advisor, Vice Chancellor and Registrar of Karpagam University for providing facilities and encouragement.

## REFERENCES

1. Arunkumar S, Muthuselvam M. Analysis of phytochemical constituents and antimicrobial activities of *Aloe vera* L. against clinical pathogens. World Journal of Agricultural Sciences 2009; 5: 572-576.

2. Nostro A, Germano MP, D'Angelo A, Marino A, Cannatelli MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett. Appl. Microbiol 2000; 30: 379-385.
3. Tanaka H, Sato M, Fujiwara S. Antibacterial activity of isoflavonoids isolated from *Erythrina variegata* against methicillin resistant *Staphylococcus aureus*. Lett. Appl. Microbiol 2002; 35:228-489.
4. Raina R, Prawez S, Verma PK, Pankaj NK. Medicinal plants and their role in wound healing. Vet Scan 2008; 3:1-7.
5. Panda D, Dash SK, Dash GK. Qualitative phytochemical analysis and investigation of anthelmintic and wound healing potentials of various extracts of chromolaena odorata linn. collected from the locality of mohuda village, berhampur (south orissa). International Journal of Pharmaceutical Sciences Review and Research 2010; 1: 122-126.
6. Kumar S, Shukla GA, Rao SA. The Present Status of Medicinal Plants – Aspects and Prospects. International Journal of Research in Pharmaceutical and Biomedical Sciences 2011; 2 Supp 1: 2229-3701
7. Gambhire MN, Wankhede SS, Juvekar AR. Antiinflammatory activity of aqueous extract of *Barleria cristata* leaves; pharmacognosy 2009; 1: 220-224
8. Trease GE, Evans WC. Textbook of Pharmacognosy 1997; 14th edition. USA: W B Saunders, PP: 24-28.
9. Harborne JB. In: Phytochemical methods. London: Chapman and Hall Ltd, 1987: 356.
10. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. J Biol Chem 1951; 193: 265-275
11. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolbdic phosphor tugnstic acid reagents. Am J Enol Vitic 1965; 16:144-158
12. Ordon LE, Gomez JD, Vattuone MA, Isla MI. Antioxidant activities of *Sechium edule* (Jacq.) Swart extracts. Food Chem 2006; 97: 452-458.
13. Rajeswari P, Krishnakumari S. *Boerhaavia erecta*- A potential source for phytochemicals and antioxidants. Jounral of pharmaceutical sciences and research 2010; 2: 2010, 728-733.
14. Dash S, Das C, Sahoo DC. Phytochemical and anthelmintic screening of crude bark extract of *Adenanthera pavonina* Linn. IJCP 2010; 2 Supp 10: 1-4.
15. Patil AG, Koli SP, Patil DA, Chandra N. Pharmacognostical standardization and HPTLC finger print of *Crataeva tapia* Linn. SSP. Odora (Jacob.) Almeida leaves [J]. Int J Pharm Biosci 2010; 1 Supp 2: 1-14.
16. Ramya V, Dheena Dhayalan V, Umamaheswari S. *In vitro* studies on antibacterial activity and separation of active compounds of selected flower extracts by HPTLC [J]. J Chem Pharm Res 2010; 2 Supp 6: 86-91.
17. Sutar AC, Sohoni DP, Banavaliker MM, Blyani MK. Indian drugs 2002; 39: 434 – 434.
18. Chung KT, Wong TY, Huang YW, Lin Y. Crit Rev food sci nutr 1998; 38: 421 – 464.
19. Agarwal PK. Carbon-13 NMR of flavonoids. Elsevier, New York. 1989.


Source of support: Nil, Conflict of interest: None Declared