# Preliminary Phytochemical Analysis of *H. perforatum* L. from Kashmir Himalayas

**Mohammad Yaseen Mir[*1], Azra N. Kamili[1], Qazi P. Hassan[2], Sabreena Rafi[1] and Javid A. Parray[1]**

**[1]Department of Environmental Science and Centre of Research for Development, University of Kashmir, Srinagar-190006, J & K, India**
**[2]Indian Institute of Integrative Medicine (CSIR), Sanat Nagar, Srinagar, J & K, India**
**[*]Corresponding author: yaseencord36@gmail.com**

## Abstract

*H. perforatum* is one of the important medicinal plants. It is reported to have anticancerous and antiviral properties. Northwest Himalayas is a rich source of this medicinal plant. Since only few studies are reported from our region with respect to this plant. Therefore this study was carried out for the investigation of preliminary phytochemicals in *H. perforatum.* From our studies it was analyzed that *H. perforatum* contains most of the primary and secondary metabolites. However steroids and glycosides tests were found to be negative.

**Keywords:** Photochemicals, *H. perforatum*, anticancerous properties, antiviral properties

## Introduction

Medicinal herbs are important sources of bioactive compounds that are utilized for the commercial production of drugs. Furthermore these drugs are less expensive, easily available, safe, efficient and rarely have side effects. The medicinal herbs provide important clues and leads to modern drug design. These compounds are synthesized by primary or secondary metabolism within living organisms (Ganie *et al.*, 2012). Secondary metabolites are chemically as well as taxonomically extremely diverse compounds with obscure function. The commercialty important bioactive compounds found in plants are alkaloids, tannins, flavonoids, carbohydrates and phenolic compounds. The pharmacological attributes that are associated with these bioactive compounds are anti-inflammatory, hepatoprotective, antiviral, antimicrobial, antioxidant, antitumoral and wound-healing activities (Karioti and Bilia 2010). *H. perforatum* L. (common St. John's Wort), a herbaceous flowering plant species of family hypericaceae. The genus *Hypericum* comprises over 450 species of herbaceous perennials, evergreen and deciduous shrubs as well as trees (Victor *et al.*, 2014). The herbaceous perennial *H. perforatum* is native to Europe, Asia and North Africa but has been introduced into many temperate regions of the world, including North and South America, South Africa, Australia and New Zealand (Bruni and Sacchetti, 2009). In India, it grows in Himalayas at higher altitude and in the hills of central parts. It's growth is most prolific in areas where rainfall exceeds 760 mm per annum. The presence of different types of secretory structures, including dark glands, translucent glands and various secretory canals, is characteristic to *H. perforatum* (Karioti and Bilia 2010)

## Materials and Methods

### *Selection/collection of plant material*

The whole plants of *Hypericum perforatum* L. were collected from Zaberwan Hills, Srinagar, Kashmir (34°3'58"N: 74°53'35"E). Plants were indentified at Centre for Biodiversity and Taxonomy, University of Kashmir, under voucher specimen no.2032-KASH Herbarium.

### *Preparation of plant extract*

The plants were shade dried at room temperature. The dried plant material was grinded to a coarse powdered form and extracted exhaustively with methanol at $45^0$C temperature in a Soxhlet extractor. The extracts were concentrated in vacuum rotary evaporator and dried for further analysis.

### *Qualitative phytochemical analysis*

### *Proteins (Xanthoproteic Test)*

The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins (Tiwari *et al.*, 2011).

### *Aminoacids*

To the extract, 0.25% (w/v) ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid (Mir *et al.*, 2015).

### *Tannins*

About 0.5 g of the dried powdered samples were boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration (Jigna and Sumitra, 2007).

### *Saponins*

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed constituent with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion (Harborne, 1984).

### *Flavonoids*

5 ml of dilute ammonia solution was added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated $H_2SO_4$. A yellow colouration observed in each extract indicated the presence of flavonoids (Harbrone, 1984).

### *Steroids*

Two ml of acetic anhydride was added to 0.5 g methonlic extract of each sample with 2 ml $H_2SO_4$. The colour changed from violet to blue or green in some samples indicating the presence of steroids (Sofowara, 1993).

***Cardiac glycosides (Keller-Killani test)***

Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer (Harborne, 1984).

***Phenols (Ferric Chloride Test)***

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols (Tiwari *et al.*, 2011).

***Carbohydrates (Molisch's Test)***

Extracts were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates (Tiwari *et al.*, 2011).

***Alkaloids (Dragendroff's Test)***

Plant extracts were treated with dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids (Harborne, 1984).

**Results and Discussion**

By performing the general chemical tests on the crude methanolic extract of *Hypericum perforatum*. It was analyzed that major bioactive compounds (**Table 1**) were present in extracts particularly alkaloids, carbohydrates, tannins, flavonoids, phenolics, saponins, proteins and aminoacids. Furthermore tests for glycosides and steroids were found to be negative. Our results are in line with preliminary phytochemical analysis of Iraqi species of *Hypericum perforatum* (Victor *et al*., 2014). Karioti and Bilia (2010) and Zobayed *et al.*, (2006) reported about 10 classes of bioactive compounds in *H. perforatum*. The concentration of these bioactive compounds vary in plants because of genetic variability within species or due to different environmental growing conditions, processing and preparation of sample materials and harvesting time. Bagdonaite *et al.*, (2012) reported flavonoids found in *H. perforatum* ranges from 7% in stems to 12% in flowers and leaves. Flavonoids in *H. perforatum* include flavones (luteolin), flavonols (quercetin, kaempferol), biflavones (biapi-genin), amentoflavone, glycosides (rutin, hyperside, and isoquercitrin), myricetin, hyperin, oligomeric proanthocyanadins and miquelianin (Zubricka *et al.*, 2015). Patocka 2003 reported the presence tannins (ranging from 3% to 16%), xanthones (1.28 mg/100 g), phenolic compounds (caffeic acid, chlorogenic acid, and *p*-coumaric acid) and hyperfolin in *H. perforatum.*

**Table 1. Phytochemical analysis of methanol exracts of *H. perforatum***

| Bioactive compounds | Result |
|---|---|
| Alkaloid | + |
| Phenolics | + |
| Tannins | + |
| Glycosides | - |
| Flavonoids | + |
| Saponins | + |
| Steroids | - |
| Proteins | + |
| Aminoacids | + |
| Carbohydrates | + |

Presence (+) / Absence (-)

## Conclusion

In general *Hypericum perforatum* extracts have found to contain several bioactive compounds. However, further research is needed to be conducted for isolation of main compounds. Since flavonoids and phenolics were found in the samples therefore research pertaining to antioxidant activity needs to be done.

## Acknowledgement

The authors are thankful to Director, CORD, for providing necessary facilities and cooperation during this research work.

## References

Bagdonaite, E., Martonfi, P., Repcak, M. and Labokas, J. 2012. Variation in concentrations of major bioactive compounds in *Hypericum perforatum* L. from Lithuania. ***Ind. Crop. Prod*. 35:** 302-308.

Bruni, R. and Sacchetti, G. 2009. Factors affecting polyphenol biosynthesis in wild and field grown St.John'sWort (*Hypericum perforatum* L. Hypericaceae/Guttiferae). ***Molecules*. 14**: 682–725.

Ganie, S. A., Jan, A., Muzaffar, S., Zargar, B. A., Hamid, R. and Zargar, M.A. 2012. Radical scavenging and antibacterial activity of *Arnebia benthamii* methanol extract. ***Asian Pac. J. Trop. Med*. 5 (10)**:766–772.

Harborne, J.B. 1984. ***Phytochemical Methods- A Guide to Modern Techniques of Plant Analysis***, 2nd Edition. Chapman and Hall, New York, 288 pp.

Jigna, P. and Sumitra, V. C. 2007. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plant. ***Turk. J. Biol*. 31**: 53-58.

Karioti, A. and Bilia, A. R. 2010. Hypericins as potential leads for new therapeutics**.** ***Int J. Mol Sci*. 11:** 562−594.

Mir, M. Y., Kamili, A. Z., Hassan, Q. P., Parry, J. A. and Janwari, S. 2015. Qualitative phytochemical analysis. ***Journal of Research and Development*. 15**: 60-65.

Patocka, J. 2003. The chemistry, pharmacology and toxicology of the biologically active constituents of the herb *Hypericum perforatum* L. ***J. Appl. Biomed*. 1**:61-70.

Sofowora, A. 1993. Medicinal plants and Traditional medicine in Africa. ***Spectrum Book Ltd, Ibadan, Nigeria*.** 289 pp.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G. and Kaur, H. 2011. Phytochemical screening and extraction: A Review. ***Internationale Pharmaceutica Sciencia*. 1**: 98-106.

Victor, B. G. Wafaa, M. A. and Hamed, S. 2014. Preliminary phytochemical screening and evaluation of in vitro oxidant activity of Iraqi species of *Hypericum perforatum* aerial part. ***Int. Res. J.Pharm*. 5**: 369-373.

Zobayed, S. M. A., Afreen, F., Goto, E. and Kozai, T. 2006. Plant–environment interactions: accumulation of hypericin in dark glands of *Hypericum perforatum*. ***Ann Bot*. 98**: 793−804.

Zubrická, D., Mišianiková, A., Henzelyová, J., Valletta, A., De Angelis, G., D'Auria, F. D., Simonetti, G., Pasqua, G. and Cellarova, E. 2015. Xanthones from roots, hairy roots and cell suspension cultures of selected *Hypericum* species and their antifungal activity against *Candida albicans*. ***Plant Cell Rep.* 34:** 1953–1962. 10.1007/s00299-015-1842-5.