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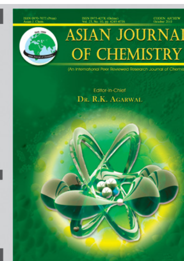


# Antioxidant Property of the Methanol Extracts of the Leaves and Rhizomes of *Hedychium spicatum* (Buch-Ham. Ex Smith)

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Antioxidant activity and phenolic contents of the methanolic extract of the leaves and rhizomes of *Hedychium spicatum* were evaluated using *in vitro* standard methods. Spectrophotometry was the bases of the determination of the total tannin, total phenol, total flavonoids, total flavonols and total proanthocyanidins, tannic acid, gallic acid, rutin, catechin equivalents were used for these parameter. The antioxidant activity of methanolic extract of the leaves and rhizomes of *Hedychium spicatum* was determined by DPPH radical scavenging assay. The results from this study showed that the antioxidant activity of leaves and rhizomes of *Hedychium spicatum* as determined by the total tannin, phenolics, flavonoids, flavonols and proanthocyanidins level present. The leaves extract has high free radical scavenging activity as shown in 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay than the rhizomes methanolic extract.

**Key Words: *Hedychium spicatum*, Antioxidant, Phenolics, Flavonoids, Flavonols, Tannins, Proanthocyanidins, Free radical scavenging activity *etc*.**

## INTRODUCTION

The concept of antioxidants is vastly catching up and latest research has shown that a number of herbal derivatives have excellent antioxidant action. Bacopa monnieri contains bacosides A and B and bacoside A is a strong antioxidant, which reduces several steps of free radical damage. *Coleus forskohlii* (forskolin), grape seed (proanthocyanidins), *Camellia sinensis* (polyphenols), *Huperzia serrata* (huperzine), *Pinus maritima* (Pycnogenol), *Borago officinalis* (gamma linoleic acid) and *Vinca minor* (Vinpocetine) are potential antioxidants. The plant is a biosynthetic laboratory, not only for chemical compounds, but also a multitude of compounds like glycosides, alkaloids *etc*. These exert physiological and therapeutic effect. The compounds that are responsible for medicinal property of the drug are usually secondary metabolites. A systematic study of a crude drug embraces through consideration of primary and secondary metabolites derived as a result of plant metabolism. The plant material is subjected to phytochemical screening for the detection of various plant constituents. With onset of scientific research in herbals, it is becoming clearer that the medicinal herbs have a potential in today's synthetic era, as numbers of medicines are becoming resistant. According to one estimate only 20 % of the plant flora has been studied and 60 % of synthetic medicines owe their origin to plants. Ancient knowledge coupled with scientific principles can come to the forefront and provide us with powerful remedies to eradicate the diseases[1]. Free radicals have been implicated in the remedy of several diseases such as liver chirrhosis, atherosclerosis, cancer, diabetes, *etc*. and compounds that can scavenge free radicals have great potential in ameliorating these disease processes[2-7]. Antioxidants thus play an important role to protect the human body against damage by reactive oxygen species[8,9]. Free radicals or reactive oxygen species (ROS) are produced *in vivo* from various biochemical reactions and also from the respiratory chain as a result of occasional leakage. These free radicals are the main culprits in lipid peroxidation[10]. Plants containing flavonoids have been reported to possess strong antioxidant properties[11,12]. Natural products from microorganisms have been the primary source of antibiotics, but with the increasing acceptance of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very important because these may serve as promising sources of novel antibiotic prototypes[13,14]. It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations[15]. In the present study, the methanol extracts of the leaves and stem of *Calpurnia aurea* were screened for antioxidant an antibacterial

properties using standard methods. The findings from this work may add to the overall value of the medicinal potential of the herb.

## EXPERIMENTAL

2,2-Diphenyl-1-picrylhydrazyl (DPPH) obtained from Himedia laboratory limted while tannic acid, gallic acid, rutin, catechin, ascorbic acid, ferric chloride, Folin-Ciocalteus's phenol reagent, sodium carbonate, aluminum chloride from sigma laboratory. All other chemicals used including the solvents, were of analytical grade.

The leaves and rhizomes of *Hedychium spicatum* collected in March 2010 from north sub tropical area Almora of India. The area falls within the altitude 3143 meter, longitudes 31º06″ and latitudes 77º0″. The plant were identified by their vernacular names and later validated at the department of pharmacognosy and ethanopharmacology, national botanical research institute, Lucknow and voucher specimens (Rhizome NBR/PH/227325, leaves NBR/PH/227323) deposited in Herbarium.

**Extract preparation:** Both leaves and rhizomes were dried at room temperature to constant weights. The dried plant materials were separately grounds to powder. Hundred grams of powdered leaves and rhizomes were macerated with I L of methanol separately for 48 h on an orbital shaker. Extracts were filtered using Buchner funnel and Whatman No. 1 filter paper. The filtrate was dried under reduced pressure at 35 ºC using a rotary evaporator. The percentage yield for leaves was 3.75 %, while that of the rhizome was 4.38 %.

**Determination of total tannis[16]:** Extracted 2 g powdered plant material with 100 mL of distilled water by boiling on water bath for 6-8 h, filtered and made up the volume to 100 mL volumetric flask. Took 1 mL aliquot of it, added 5 mL folin reagent, 10 mL saturated sodium carbonate and made up the volume to 100 mL in volumetric flask. The instrument was calibrating through blank and took the corresponding absorbance of different samples, total tannin content calculated by using $y = 0.091x + 0.065$, $r^2 = 0.988$, at 760 nm where y was the absorbance tannic acid equivalent (mg/mL).

**Determination of total phenolics[16]:** Stock solution of gallic acid (1 mg/mL) was prepared aliquot prepared. The sample stock solution (1 mg/mL) was prepared and take 500 µL in three 250 mL volumetric flask. The instrument was calibrated through blank and took the corresponding absorbance of different samples, total phenolics were calculated using $y = 131.8x + 0.044$, $r^2 = 0.997$ at 765 nm, where y was the absorbance and was gallic acid equivalent (mg/mL).

**Determination of total flavonoids:** Total flavonoids were estimated using the method of Ordon ez *et al.*[17]. To 0.5 mL of sample, 0.5 mL of 2 % methanol solution of $AlCl_3$ was added. After 1 h at room temperature, the absorbance was measured at 420 nm. A yellow colour indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 0.01 mg/mL total flavonoid content were calculated as rutin (mg/mL) using the following equation based on the calibration curve: $y = 165.1x + 0.069$, $r^2 = 0.998$, where y was the absorbance and was the rutin equivalent (mg/mL).

**Determination of total flavonols:** Total flavonols in the plant extracts were estimated using the method of Kumaran and Karunakaran[18]. To 1 mL of sample, 2 mL of 2 % methanolic solution of $AlCl_3$ and 3 mL of 5 % sodium acetate solution were added. The absorbance at 440 nm was read after 2.5 h at 20 ºC. Extract sample were evaluated at a final concentration of 0.05 mg/mL. Total flavonol content was calculated as rutin (mg/mL) using the following equation based on the calibration curve: $y = 27.97x + 0.0208$, $r^2 = 0.997$, where y was the absorbance and was the rutin equivalent (mg/mL).

**Determination of total proanthocyanidins:** Total proanthocyanidins in the plant extracts were estimated using the method of Sun *et al.*[19]. To 0.4 mL of sample, 3 mL of 4 % methanolic solution of vanillin and 1.5 mL concentrated hydrochloric acid were added. The absorbance at 500 nm was read after 15 min at room temprature. Extract sample were evaluated at a final concentration of 0.01 mg/mL. Total proanthocyanidin content was calculated as catechin (mg/mL) equivalent using the following equation based on the calibration curve: $y = 4.8x + 0.036$, $r^2 = 0.974$, where y was the absorbance and was the rutin equivalent (mg/mL).

**Determination of antioxidant activity:** DPPH radical scavenging assay the effect of extracts on DPPH radical was estimated using the method of Liyana-Pathirana and Shahidi[20]. A solution of 0.135 mM DPPH in methanol was prepared and 1 mL of this solution was mixed with 1 mL of extract in methanol containing 0.02-0.1 mg of the extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 0.5 h the absorbance of mixture was measured spectrophotometrically at 517 nm while ascorbic acid and rutin were used as reference standards. The ability to scavenge DPPH radicals was calculated by the following equation:

DPPH radical scavenging activity (%) = $[(Abs_{control} - Abs_{sample}) /(Abs_{control})] \times 100$; where, $Abs_{control}$ is the absorbance of DPPH radical + methanol; $Abs_{sample}$ is the absorbance of DPPH radical + sample extract/reference.

## RESULTS AND DISCUSSION

**Total tannins, phenolics, flavonoids, proanthocyanidins content:** Result obtained in the present study revealed that the level of these phenolics compounds in the methanol extracts of the leaves and rhizomes of *Hedychium spicatum* were found considerable. The leaves extract higher levels of total tannin, phenols and flavonols than the rhizome extract. On the other hand, rhizome extract possessed higher levels of flavonoids and proanthocyanidins (Table-1, Fig. 1).

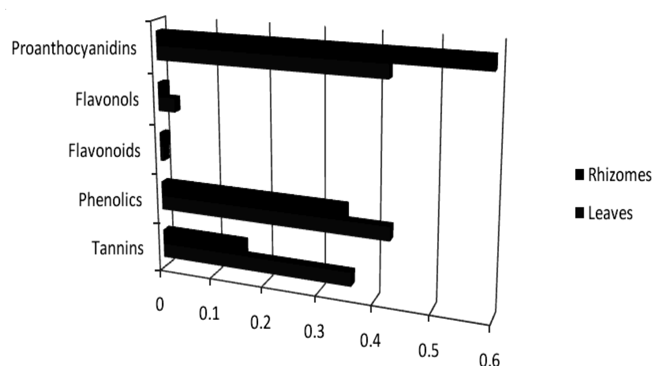


Fig.1. Pollyphenolic compounds present in *Hedychium spicatum*

TABLE-1
POLYPHENOL CONTENTS OF THE METHANOL EXTRACTS OF THE LEAVES AND RHIZOMES OF *HEDYCHIUM SPICATUM.* (n = 3, X ± SDV)

| Polyphenols | Leaves | Rhizomes |
|---|---|---|
| Total tannins[a] | 0.3597 ± 0.0095 | 0.1593 ± 0.1 |
| Total phenolics[b] | 0.427 ± 0.000141 | 0.348 ± 0.0000707 |
| Total flavonoids[c] | 0.0068 ± 0.00 | 0.0099 ± 0.0000354 |
| Total flavonols[d] | 0.0322 ± 0.000219 | 0.0118 ± 0.000304 |
| Proanthocyanidins[e] | 0.422 ± 0.0000707 | 0.591 ± 0.000495 |

a: expressed as percent gm tannic acid equivalent in dried plant material; b: expressed as percent gm gallic acid equivalent in dried plant material; c: expressed as percent gm rutin equivalent in dried plant material; d: expressed as percent gm rutin equivalent in dried plant material; e: expressed as percent gm catechin equivalent in dried plant material

**DPPH radical scavenging activity:** Fig. 2 shows the dose-response curve of DPPH radical scavenging activity of the methanolic extract of the leaf and rhizome of *Hedychium spicatum* compared with ascorbic acid and rutin. It was observed that extract of the leaves had higher activity than the rhizome. At a concentration of 0.1 mg/mL the scavenging activity of the leaves reached 46.10 %, while at the same concentration, that of the rhizome was 16.91 %.

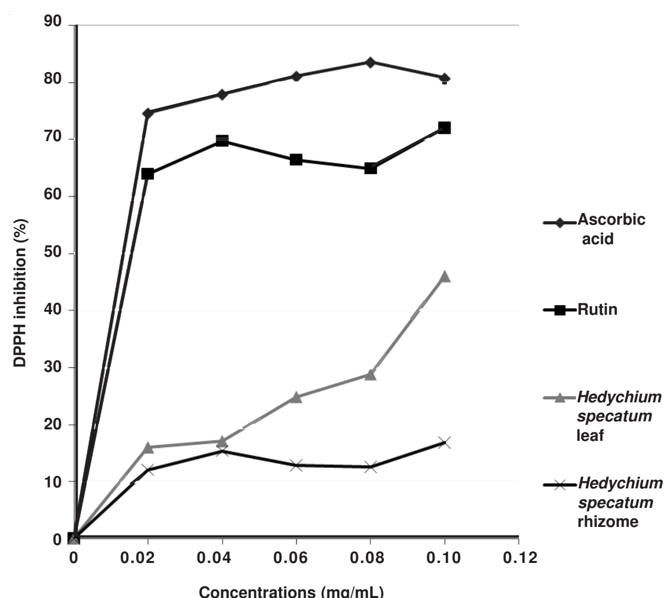


Fig. 2. DPPH scavenging activities of the methanolic extracts of the leaves and rhizomes of *Hedychium spicatum*

**Total tannins, phenolics, flavonoids, proanthocyanidins content:** Polyphenolics are the major compounds with antioxidant activity. This activity is believed to be mainly due to their redox properties[2], which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. The result obtained in the present study revealed that the level of these phenolics compounds in the methanolic extract of the leaf and rhizome of *Hedychium spicatum* were considerable. The result strongly suggests that polyphenolics are important component of this plant and some of its pharmacological effect could be attributed to the presence of these valuable constituents.

**DPPH radical scavenging activity:** The effect of antioxidants on DPPH is due to their hydrogen donating ability[2]. The DPPH scavenging ability of the extracts were significantly less than those of ascorbic acid and rutin, the study shows that the methanolic extracts of the plant *Hedychium spicatum* leaf and rhizomes have the proton donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

## Conclusion

The result from this study indicate that the leaves and rhizome extract of *Hedychium spicatum* posses antioxidant properties and could serve as free radical inhibitors or scavenger or acting possibly as primary antioxidants. Recently lot of attention is being devoted to natural sources of antioxidant; the data obtained in this study might suggest a possible use of *Hedychium spicatum* as a source of natural antioxidant.

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