

## EVALUATION OF ANTI-MALARIAL AND ANTIOXIDANT EFFECT OF *PARNASSIA NUBICOLA* METHANOLIC EXTRACT

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Received: 13 April 2012, Revised and Accepted: 20 May 2012

### ABSTRACT

Herbal medicine endow with realistic way for the handling of numerous diseases to facilitate them stubborn as well as inoperable in erstwhile system of medication. The endeavour of the swot is the methodical viewing of *Parnassia nubicola* methanolic extract among the intention of discovering innovative bioactive compounds for malaria disease in addition to create the systematic basis for the restorative activities of conventional plant medicines. There is an imperative necessitate to recognize novel anti-malarial drug targets for equally prophylaxis along with chemotherapy, owing to the rising dilemma of drug resistance to malaria parasites. The present study was to determine novel, effective plant-based extracts for the commotion adjacent to malaria. The flora was screen for the existence of phyto-chemicals i.e. reducing sugar, alkaloids, flavonoids, tannins, saponins, glycosides etc in addition to their effect on 2,2-Diphenyl-1-picryl-hydraxyl radical (DPPH) which was used to resolve free radical scavenging activity. Accordingly, in current investigation the particular Indian medical plants show substantial antioxidant activity aligned with radical scavenging assay. *P.nubicola* also shows compelling reticence of DPPH radical scavenging activity. The free radical scavenging property of this vegetation almost certainly contribute to the efficiency of the exceeding plant in malaria therapy.

**Keywords:** *Parnassia nubicola*, Antimalarial, Antioxidant activity, 2,2-Diphenyl-1- picrylhydrazyl, Phytochemical Screening

### INTRODUCTION

One of the primmest recorded disease in the world is Malaria. Every year near about 300 to 500 million latest cases are diagnosed along with just about 1.5 million individuals die of the disease and the widely held of them are children (Greenwood *et al.*, 2005). In various part of the world, the re-emerging of malaria is unpaid to the speedy augment of confrontation to mainly of the accessible anti-malarial drugs, in addition to the resistance of vectors to insecticides (Ridley, 2002; Zirhi *et al.*, 2005). in Around the world, in numerous endemic areas the drug resistant strains of *P. falciparum* have been found furthermore a lot of of conservative anti-malarial drugs have been associated with action collapse. Besides, the complicatedness of creating resourceful vaccines with also unfavourable side-effects of the active anti-malarial drugs draw attention to the urgent need for novel, well-tolerated anti-malarial drugs (Ridley, 2005) for both prophylaxis with the handling of malaria. The past reveal that the plants has all the time been consider as an significant foundation of medicine alongside malaria: together quinine and artemisinin has been imitative from conventional medicine with plant extracts. Artemisinin derivative are nowadays suggested by the WHO worldwide (Mutabingwa, 2005), in amalgamation with former drugs, like as lumefantrine, amodiaquine, mefloquine, sulphadoxine-pyrimethamine (SP), as the first-line treatment of malaria. This fact has buoyant the progressing search for new innate product-derived anti-malarial drugs. Numerous plants are utilize in conventional medicine for the dealing of malaria and/or fever in malaria-endemic countries. In addition, quite a lot of studies has been undertaken to appraise the inhibitory possessions of an assortment of plant extracts on *P. falciparum* (Tran *et al.*, 2003; Wanyoike *et al.*, 2004).

*Parnassia nubicola* belongs to family Parnassiaceae. It is also known as nirvanshi. It is a perennial herb which is simply notable by its retiring white flower borne on a slender stem 11-30 cm tall, among a single stalk less, stem-clasping ovate leaf arising from below the middle of the stem, and many stalked leaves at the base. The stems 3 or 4(or 5), (5- ) 13-40 cm, with 1 leaf near base or in proximal 1/4. Basal leaves 3-8; petiole 3-7(-13) cm; leaf blade abaxially greenish, adaxially deep green or brown-green, elliptic or ovate-oblong, rarely oblong, (2-)2.5-7.5 × (1.5-)2-3.8 cm, thick textured or thin and papery, base subcuneate, sometimes truncate, apex acute or shortly acuminate. Its root paste is taken to get relief from cuts & wounds and the juice of leaf is applied to treat eye problems and inflammation.

Various plant species has been utilize as long-established medicines although it is obligatory to ascertain the methodical basis for the remedial activities of habitual plant medicines as these might provide as the resource for the expansion of additional effective drugs. Considering the great potential of plant biodiversity and based on Indian traditional system (Jorjani, 1992; Khorasani, 1992), *Parnassia nubicola* was selected in this exploration. The plan of this study was to ascertain novel, efficient plant-based medicines for the action of malaria.

### MATERIALS AND METHODS

#### Collection

Authentic samples: Various market samples of *Parnassia nubicola* were procured from Chunnilal Attar Ayurvedic Store, Ghat Gate and Jaipur in the month of March, 2010.

#### Identification

All the samples were authenticated and were given identification number. The identification was as follows:

These samples were authenticated and submitted in Ethnomedicinal Herbarium, Centre of Excellence funded by DST, MGiaS, Jaipur (Rajasthan).

#### Processing of plant materials

During the course of the study each sample was screened for its foreign matter and milled, before use.

#### Experimental details

Present studies were performed on *Parnassia nubicola* for the following studies

1. Phytochemical test of plant extract
2. Antioxidant Potentials of Methanolic extract of plant

#### Phytochemical screening

Phytochemical screening was performed using standard procedure:

#### Test for Reducing Sugars (Fehlings Test)

The aqueous ethanol extract (0.5gm in 5 ml of water) was added to boiling fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction.

### Test for Terpenoides (Salkowski Test)

To 0.5 gm each of the extract was added to 2ml of chloroform. Concentrated sulphuric acid (3ml) was carefully added to form a layer. Reddish brown coloration of the interface indicates the presence of terpenoides.

### Test for Flavonoides

4ml of extract solution was treated with 1.5ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated Hydrochloride acid was added and red colour was observed for flavonoids and orange color for flavons.

### Test for Tannins

About 0.5 g of the extract was boiled in 10ml 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 a blue-black coloration.

### Test for Saponins

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

### Test for Alkaloids

Alkaloids solutions produce white yellowish precipitate when a few drops of Mayer's reagents are added. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent.

The alcoholic extract was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of mayer's reagent. The sample was then observed for the turbidity or yellow precipitation.

### Antioxidant Activity

#### Preparation of test extracts

All the test plant sample and their adulterants were milled and refluxed in ethanol for 36 h, filtered, concentrated to dryness *in vacuo*. A portion of ethanolic extract was further successively extracted in pet. ether, benzene, chloroform, alcohol and water, concentrated and stored at minimum temperature, until used.

#### Preparation of DPPH

DPPH (2, 2'-diphenyl-1-picrylhydrazyl, C<sub>18</sub>H<sub>12</sub>N<sub>5</sub>O<sub>6</sub>; Hi media) 0.8 mg was dissolved in 10 ml methanol to obtain a concentration of 0.08 mg/ml for antioxidative (qualitative and quantitative) assay.

### Qualitative assay

Each successive extract (10 mg) was dissolved in 10 ml of its suitable solvent to get a concentration of 1 mg/ml and from this, 0.25 $\mu$ l was taken with the help of micropipette, applied on silica gel G coated plates. These circular spots were sprayed with DPPH solution, allowed to stand for 30 min. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced, and the changes in colour (from deep- violet to light- yellow on white) were recorded at 517 nm on a UV spectrophotometer (Varian Cary PCB 150, Water Peltier System).

### Quantitative assay

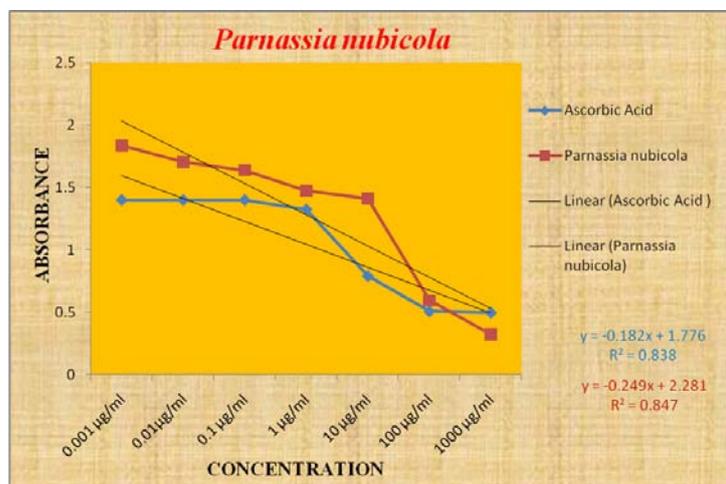
A concentration of 1 mg/ml of ethanolic extract of each test sample was prepared to obtain different concentrations (10<sup>2</sup> $\mu$ g to 10<sup>-3</sup>  $\mu$ g/ml). Each diluted solution (2.5 ml each) was mixed with DPPH (2.5ml). The samples were kept in the dark for 15 min at room temperature and then the decrease in absorption was measured. Absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured. The UV absorbance was recorded at 517 nm. The experiment was done in triplicate and the average absorption was noted for each concentration. Data were processed using EXCEL and concentration that cause 50% reduction in absorbance (RC<sub>50</sub>) was calculated. The same procedure was also followed for the standards- quercetin and ascorbic acid.

### RESULTS AND DISCUSSION

In present viewing, attempts has been made to look for for methanolic extract which has the potentials as same as antioxidant agents as at the present days most of persons are suffering from malaria diseases and to cure such disease there is an urgent need of plant extract having potent antioxidant activity. The methanolic extracts of *P.nubicola* shows the antioxidant activity which is comparable to ascorbic acid. Throughout the present exploration it was showed that the maximum optical density comes out to be 1.837 nm which is at the concentration 10<sup>-3</sup>  $\mu$ g/ml and the smallest optical density is 0.322 nm which is at the concentration 10<sup>3</sup>  $\mu$ g/ml where as the other shows comparable O.D at different concentrations i.e. 1.704 nm at 10<sup>-2</sup> $\mu$ g/ml, 1.638 nm at 10<sup>-1</sup>  $\mu$ g/ml, 1.475 nm at 1 $\mu$ g/ml, 1.409 nm at 10<sup>1</sup>  $\mu$ g/ml, 0.595 nm at 10<sup>2</sup>  $\mu$ g/ml.

**Table 1: Showing optical density of *Parnassia nubicola* on different concentrations.**

Concentration ( $\mu$ g/ml)	O.D (nm)
0.001	1.837
0.01	1.704
0.1	1.638
1	1.475
10	1.409
100	0.595
1000	0.322



**Fig. 1: Graph showing Antioxidant Activity of *Parnassia nubicola* at different concentration.**

In the present investigations antioxidant activity of *Parnassia nubicola* shows significant activity aligned with the DPPH assay method where the regression line apparent shows the efficacy of it as it's has the potentials which are comparable to ascorbic acid. The antioxidant activity of *Parnassia nubicola* methanolic

extract using DPPH assay method shows substantial activity which is analogous to standard ascorbic acid. The straight line showed  $Y = -0.182x + 1.776$  & regression = 0.838 whereas, in above drug the straight line is  $Y = -0.249x + 2.281$  & regression = 0.847

**Table 2: Showing phytochemical screening results of *Parnassia nubicola*.**

<i>Parnassia nubicola</i>						
TEST	Reducing Sugar	Saponin	Tannin	Terpenoides	Flavonoides	Alkaloides
	-ve	-ve	-ve	-ve	-ve	+

The phytochemical screening of *P.nubicola* shows the occurrence of only alkaloids whereas it shows the absence of saponin, tannin, terpenoides, flavonoids and reducing sugar respectively. The viewing of the plants make only a few difference in the component of the hardened plants. The two drug show evidence of brawny antioxidant activity supplementary or in a smaller amount. The presence of alkaloids in this plant is probable to be conscientious for the free radical scavenging effects pragmatic.

#### CONCLUSION

On the whole, rising the worldwide broaden of multi-drug resistant malaria parasite shows that there is a necessitate for new-fangled chemotherapeutic agents to conflict malaria. Medical plants are worn in numerous countries to handle malaria disease and are considered to be not as much of toxic than allopathic hypoglycemic drugs whereas herbal medicines make available realistic means for the curling of malarial diseases. Through this swot, aiming to explore for novel anti-malarial drugs, the *in vitro* antioxidant activities of *Parnassia nubicola* was observed. The 2, 2-diphenyl -1-picryl hydrazyl (DPPH) radical was extensively used as the sculpt system to explore the scavenging activities of different naturally occurring compounds. DPPH radical is scavenged by antioxidants from side to side the contribution of proton, forming the concentrated DPPH. The methanolic extract of *Parnassia nubicola* can proficiently scavenge an assortment of reactive oxygen species or free radicals less than *in vitro* conditions. In the present work, the elevated antioxidant capacity experimental for methanolic extract of whole plant of *Parnassia nubicola* counsel that it plays a potential role in preventing infectious diseases in which free radicals are concerned. From the antioxidant results it can be concluded that the *Parnassia nubicola* methanolic extract has persuasive *in vitro* antioxidant potential which may ascribed to the presence of alkaloid. Additionally the Phytochemical screening of this plants shows the presence of large amount of alkaloids which states that this will exhibit the antioxidant activity by the side and it promotes a medication for cure of diseases. Accordingly, this kind of studies suggest that this

plant obtain antioxidant activities which can neutralize the oxidative damage induced by the malaria parasite and this may be further, their mode of accomplishment in malaria therapy.

#### ACKNOWLEDGEMENT

Author acknowledge with thanks the financial support from Department of Science and Technology, Government of Rajasthan, in the form of Centre with Potentials for Excellence in Biotechnology, sanction no F 7(17) (9) Wipro/Gaprio/2006/7358-46(31/10/2008).

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