*Pak. J. Bot.*, DOI: http://dx.doi.org/10.30848/PJB2022-2(43)

# ANTIOXIDANT ACTIVITY OF *SERIPHIDIUM LEUCOTRICHUM*: AN ANTI MALARIAL PLANT FROM MINAPIN VALLEY, DISTRICT HUNZA-NAGAR, PAKISTAN

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**Abstract**

An ethnomedicinal survey was conducted in 2013 in Minapin Valley, which is an administratively controlled area of Central Karakorum National Park (CKNP), Gilgit and Baltistan. *Seriphidium leucotrichum* (Krasch. ex Ladyg.) K. Bremer & Humphries ex R. Ling belonging to family Asteraceae was collected during the survey. According to local inhabitants this species was being used as a remedy of malaria fever. Therefore the species was evaluated in order to verify the statement. Folin-Ciocalteu reagent (FCR) method was used to estimate the Total Phenolic Contents (TPC) of *S. leucotrichum* and DPPH radical scavenging method was used to examine its antioxidant activity. Consequently, 64.5 mg/100 g phenolic contents were calculated from *S. leucotrichum.* Similarly, it also showed high potential of scavenging activity ranging from 68.18±1.0 to 81.78 ±1.0 µg/ mL .These values were also compared with ascorbic acid which was used as standard. In this connection $IC_{50}$ values were calculated and were found higher in *Ascorbic acid* than *S. leucotrichum.* The present study is the first report on the free radical scavenging properties of *S. leucotrichum* that might contribute to the possible role of this species against the activity of Plasmodium parasites.

**Key words:** Minapin, *Seriphidium leucotrichum*, Malaria, Radical scavenging property.

## Introduction

Life of human beings has always been in danger due to the various infectious diseases since its existence on the earth. However, with the passage of time, treatments of many diseases have been discovered through antibiotics and antiviral agents while some of the microorganisms develop resistance to antibiotics (Kim *et al*., 2014). Among such infections, malaria is also included. It is a mosquito-borne infection which is caused by single-celled parasitic organisms belonging to the genus plasmodium. It is a widespread infection which causes lethal affects on the humans and other animals. A large population is infected every year by this disease, especially, infants up to 5 years are severely infected. Five species of the genus plasmodium including, *P. falciparum P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi* are responsible for causing malarial infection in the humans and animals. Among them four species including, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi* are not considered as much harmful as *P. falciparum* (Tuteja, 2007; Lee *et al.*, 2011). These species mainly cause infection in the tropical areas which are supposed to be most promising places for their growth. However, the impact of the infection also reaches to the temperate areas (Khattak *et al*., 2013). Furthermore, it is also revealed that infection has major negative economic effects also on the regions where it is spread widely. For instance, malaria was considered, a major factor in the slow economic growth of the southern states of America during the late 19th and early 20th centuries (Humphreys, 2001). On the other hand it is still considered major reason of deaths in the most part of Africa and Asia. In this connection, Pakistan, India and Bangladesh are considered most promising areas for its growth in the Asia. However, *P. falciparum* and other mixed species do not spread infections commonly in Pakistan. It is mainly influenced by the infection of *P. vivax* (Khattak *et al.*, 2013). According to William & Meek (2011) and Anon., (2013) infection spreads near to the border areas frequently in Pakistan such as, Kakar *et al*., (2010) highlighted the regions near to Afghanistan and Iran in his work. Anon., (2013) reported that these areas do not have facilities for prevention and the treatment of malaria due to poor economic stability. For instance, Caraballo, (1996) had already revealed that malarial parasite can be recognized normally by the microscopic study of the blood. Similarly, diagnosis of the illness can also be possible through antigen based rapid tests but these facilities are not commonly available in these regions, in spite of the fact that a high amount is allocated on the subject of Malaria Action Plan (Anon., 2013). However, despite of all the disadvantages people living in these regions are dependent on their surrounding natural resources especially certain plant species play a fundamental role in healing the various ailments (Farnsworth *et al.*, 1985). In this connection, Ali (2008) has described the significance of the flora of Pakistan related to its medicinal value while Ali & Qaiser (1986) have already discussed at length the geographical characteristics of the vegetation in Pakistan that is floristically diverse. According to Stewart (1972), among the floristically diverse regions of Pakistan, Gilgit-Baltistan is a most promising place.

*Seriphidium leucotrichum* (Krasch. ex Ladyg.) K. Bremer & Humphries ex R. Ling of the family Asteraceae is a fairly common species of Minapin Valley, Central Karakorum National Park (CKNP). This species is usually used by the native inhabitants to cure Malaria disease. Therefore, in order to confirm the efficacy of *S. leucotrichum* the present study was undertaken. Total phenolic constituents (TOC) and *In vitro* antioxidant activities were studied.

**Study area**: Gilgit–Baltistan exist at unique geographical location, occupying around 72,496 square kilometers area bordering China, Afghanistan and Indian held Kashmir in between longitude 72°-75° north and latitude 35°- 37°east. According to a report (Anon., 2016), from the department of Archeology, Govt. Pakistan, around 10,557.73 km 2 area of this region is occupied by the Central Karakorum National Park (CKNP) in between Skardu and Gilgit, It is the highest national park in the world, situated at the highest altitude ranging from 2,000 m. a.s.l. to over 8,000 m a.s.l., including K2, the second highest peak in the world. In addition, the world's largest glaciers, outside the Polar Regions are also found in the region. Area is a paradigm of natural beauty and important resources comprising diverse flora and fauna. Administratively, it was declared as national park and named as Central Karakoram National Park (CKNP) in 1993. The park falls into four administrative districts including district Hunza-Nagar of Gilgit-Baltistan. It is the biggest park in Pakistan and is considered to be the hotspot of biodiversity. A number of plant species belonging to angiosperms, gymnosperms and pteridophytes are known to be of therapeutic value among the indigenous people of the region. Minapin valley, our study area is included in the district Hunza-Nagar which is located in the south of the park. Minapin valley an administratively controlled area of Central Karakoram National Park (CKNP) is located at 35° 54'N latitude and 75° 31' E longitude in between extreme mountainous area of Skardu and Gilgit (Fig. 1). Minapin valley comprises of lush green vegetation and can be accessed from three different routes (i) Rakaposhi base camp, (ii) Diran base camp and (iii) Suymar village. For instance trek no 18 goes towards Minapin leading to Rakaposhi base camp. Trek no 19 leads to Minapin and ends in Diran base camp and trek no 44 goes to Suymar village.


Fig. 1. Encircled area (Minapin) in the map is collection locality.

## Materials and Methods

For biological assays including total phenolic contents (TPC) and antioxidant activity (AA) 5 kg of green aerial parts of *S. leucotrichum* were harvested from the natural habitat (Khayadar Minapin hills) during the ethnomedicinal survey of Minapin valley in the month of July, 2013. Species was identified with the help of Flora of Pakistan (Ghafoor, 2002). The harvested sample was dried at room temperature with good ventilation. Dehydrated material (aerial part) of the species was chopped into small pieces to make its fine powder (1000 g) for the purpose of extraction. The voucher specimen (1101) was deposited in the herbarium, Center for Plant Conservation, University of Karachi (KUH).

**Extraction:** Extraction was carried out after two days interval through cold method, using methanol solvent to obtain crude extract. The method was repeated twice. Solvent was removed from the extract under reduced pressure. Consequently, 35.2 g crude extract of *S. leucotrichum* of dark-green colour was obtained. Chlorophyll was removed from the crude extract using *n*-hexane solvent before making their fractionations. Fractionation process was carried out using three solvents i.e., n-hexane, chloroform and methanol to compare their polarity. The polarity was in the order: n-hexane (2g) < chloroform (4.4 g)< methanol (20.3g) while remaining was soluble in aqueous. Therefore, methanolic extract was selected to carry out the antioxidant activity.

**Estimation of total phenolic content:** Folin-Ciocalteu reagent (FCR) method of Slinkard & Singleton (1977) was followed to determine the phenolic contents. Half ml of test sample was mixed with 0.75 mL of the Folin–Ciocalteu reagent in the test tube. This liquor fusion was kept for 5 minutes at room temperature, 0.75 mL of sodium carbonate (Na2CO3) was added to the mixture and shaken gently and after 1 .5 hours absorbance was recorded with Spectrophotometer at 725 nm. Consequently, a bend was observed in contrast to gallic acid which was used as standard reference (range of concentration from 0.01 to 0.05 mg/mL).

**Evaluation of *In vitro* antioxidant activity: AA** The antioxidant activity of plant extract was determined using 1,1- diphenyl-2-picrylhydrazyl (DPPH) according to the procedure of Kim *et al*., (2002) and the inhibition percentage of DPPH free radical was calculated using the following equation:

$$\text{DPPH radical scavenging (\%)} = \frac{\text{(Absorbance of the control (blank) - Absorbance of test sample)}}{\text{Absorbance of the control (blank)}} \text{x } 100$$

where A test = Absorbance in the presence of extract or positive control and A cont = Absorbance of negative control.

The lower value of $IC_{50}$ represents higher antioxidant activity (Moyo *et al*., 2013; Ndhlala *et al*., 2013). Therefore $IC_{50}$ of each sample was also calculated to understand their 50 percent inhibition capacity against DPPH radical formation. The values were calculated on the graph by plotting extract concentrations vs. percentage inhibition of DPPH radical using Microsoft Excel. Each experiment was repeated in triplicate and the averages of the three values were used to calculate $IC_{50}$. Standard deviation was also calculated for each concentration from the three values.

## Results

**Total phenolic content (TPC):** Phenolic compounds found in the plants have redox properties which reveal their antioxidants behavior (Shoib & Shahid., 2015). In the present study methanolic extract of *S. leucotrichum* showed significant high value of total phenolic content in contrast to the extracts of chloroform and *n*-hexane (Table 1) therefore the higher value of TPC in methanolic extract is supposed to be responsible for antioxidant activity of *S. leucotrichum*. This activity is reported for the first time.

**Table 1. Total phenolic contents in different samples extract of *S. leucotrichum*.**

| Sample extracts | Total phenolic contents mg 100/g |
|---|---|
| *n*-Hexane (C6H14) | 35.2 ± 0.01 |
| Chloroform (CHCl3) | 43.5 ± 0.01 |
| Methanol (CH3OH) | 64.2 ± 0.01 |

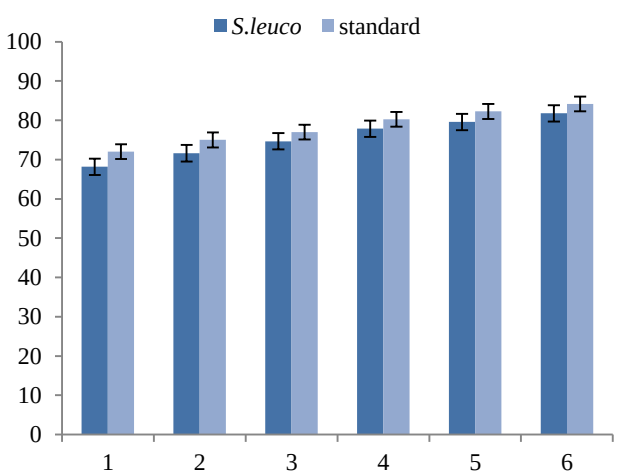


Fig. 2. Percentage inhibition at various concentrations (800-2000 µg/Ml) of methanol extract of aerial parts of *S. leucotrichum.*

**Evaluation of *In vitro* antioxidant activity:** (DPPH) ($C_{18}H_{13}N_5O_6$) is a commonly employed method in antioxidant studies of plant extracts or specific compounds over a short period since it provides information on the reactivity of the extracts with a stable free radical. The efficacy of antioxidants is usually associated with the inhibiting ability of oxidative damage by scavenging free radicals while free radical scavenging capability is associated with the strength of phenolic contents of the species (Ferreira *et al*., 2010; Sytar *et al*., 2018).

The present study revealed the radical scavenging capability (RSC) of *S. leucotrichum* at various concentrations of methonolic extracts including 800, 1000, 1500, 1800 and 2000,2500 ug/mL. The rate of inhibition was increased with the increase of

concentration (Fig. 2). *S. leucotrichum* showed significantly high activity but comparatively lesser than the standard. However, at various concentrations these differences were not found significant except at lower concentration (800 ug/ml), a slight significant difference could be seen (Fig. 2).

**Table 2. The $IC_{50}$ values of methanol extracts of *S. leucotrichum* and the standard.**

| S. No. | Sample | $IC_{50}$ µg /mL |
|---|---|---|
| 1. | *S. leucotrichum* | 996.6 ± 4.43 |
| 2. | Standard | 948.26 ± 0.88 |

**Statistical analysis**

The result of DPPH radical scavenging capacity of *S. leucotrichum* and Ascorbic acid were analyzed statistically using t-test. The variances of various concentrations of methanol extract of the species (Fig. 2) were found to be non- significant. According to the rule of t-test, the calculated values were obtained at 4 degree of freedom (df) lesser then the tabulated value. Thus, the analysis also indicated that the method of experimental work was almost correct while the minor errors can be associated to the laboratory treatment as described by Bautista *et al*., (2016).

**Discussion**

Interest in the field of natural products as a source of innovation in drug discovery (Cockburn, 2007) is still growing worldwide and their immense diversity has been appreciated all over the world (Chin *et al.,* 2006). Therefore a number of plant species have been examined for their active principles to establish their therapeutic values as an alternative choice of synthetic medicine (Fabricant & Farnsworth, 2001; Zamarrud, 2012). The present study also revealed the antioxidant potential of *S leucotrichum* which was known to the indigenous people of Minapin village and its adjacent areas as a cure against the infection of *Plasmodium* parasites. Number of species belonging to the family Asteraceae are known for their medicinal value, including the well-known genus *Artemisia* for its several species having medicinal importance (Tan *et al*., 1998). For instance, *A. annua* has been studied by different workers such as Cai *et al*., (2004) and Ivanescu *et al*., (2010) who reported high concentrations of the phenols from this species. Iqbal *et al*., (2012) and Zhu *et al*., (2013) studied the radical scavenging capacity and antitumor activities of *A. annua*. In this connection, Ferreira *et al*., (2010) and Sytar *et al*., (2018) confirmed the radical scavenging capacity of the plant which was associated with the phenolic contents belonging to an important group of bioactiveion molecules. In view of the close resemblance of *S. leucotrichum* (sometimes treated under the genus *Artemisia*) with *Artemisia* species, *S. leucotrichum* was investigated for the first time to evaluate its antioxidant ability applying the DPPH radical scavenging and FCR method for calculating its total phenolic contents which contribute its radical scavenging property against plasmodium parasite. Total phenolic contents (64mg 100/g extract) and antioxidant activity were found to be quite high but weaker to some extent then ascorbic acid as observed during evaluation of antioxidant activity. The methanol extract of various concentrations of *S. leucotrichum*, exhibited high potential of radical scavenging activity ranging from 68.18±1.0% to 81.78±1.0 (Fig. 2) with the value of $IC_{50}$ = 982.31±1.0 µg/mL (Table 2) but weaker then the ascorbic acid (control) which exhibited high potential of radical scavenging capacity ranging from 72.05±1.0 to 84.15±1.0 (Fig. 2) with the value of $IC_{50}$ = 950.57±1.0 µg/mL (Table 2). Similarly, the TPC value was also observed higher but comparatively lesser than that of gallic acid (100) which was used as control (Table 1). However, in connection of qualitative analysis of phenolic contents, Tajuddin *et al*., (2018) have already reported the occurrence of caffeic acid, p-coumeric acid, gallic acid and an unknown compound-1 from *S. leucotrichum*. According to Rees & Harborne (1985), phenolic constituents behave as antioxidants and keep safe against the free-radical oxidation reactions which cause cellular damage therefore such constituents also act like artemisinin (Kim *et al.,* 2015) against the infection. Their regular use also create resistance against inflammatory activity in the body. For instant, caffeic acid is a hydroxycinnamic acid derivative and polyphenol with the potential of anti-oxidant (Son *et al*., 2002) anti-inflammatory (Da Cunha *et al*., 2004) and antineoplastic (Zeng *et al*., 2018) activities. As an antioxidant, they prevent oxidative stress and DNA damage by inducing free radicals. They target and inhibit the histone demethylase (HDM) oncoprotein gene amplified in squamous cell carcinoma and inhibit cancer cell proliferation. Similarly, p-coumaric acid is a common dietary phenol occurring in many fruits and vegetables (Luceri *et al*., 2007) and also exhibits antioxidant and anti-inflammatory properties (Kilic & Yesiloglu, 2013). According to Lou *et al*., (2012) it can also behave as antibiotic agent. Therefore, the phenolic contents and the related unknown constituents of *S. leucotrichum* might be responsible for inhibitory activity against the oxidative stress of *Plasmodium* parasite.

**Acknowledgements**

The first author is thankful to EVK2CNR of seed project of Italy/Pakistan for financial support for this study. We are also grateful to the laboratory staff of the Department of Chemistry, Karakoram International University, Gilgit-Baltistan, Pakistan for providing the support for chemical analyses.

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(Received for publication 5 September 2020)