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ANTIMALARIAL AND FREE RADICAL SCAVENGING ACTIVITIES OF AERIAL PARTS OF *POLYGONATUM VERTICILLATUM* (L.) ALL. AND IDENTIFICATION OF CHEMICAL CONSTITUENTS BY GC-MS

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Abstract

The present study was aimed to evaluate the aerial parts of *Polygonatum verticillatum* (L.) All. for its antimalarial and antioxidant activity. *In vitro* antimalarial activity was carried out against chloroquine resistant *Plasmodium falciparum* while free radical scavenging assay was performed against DPPH. Chemical identification of constituents was carried out on GC MS spectrometry. The crude extract demonstrated potent activity (IC_{50}: 14.75 µg/ml) which further increased upon fractionation. The maximum antiparasitic potency was noted for *n*-hexane fraction (IC_{50}: 4.86 µg/ml) followed by chloroform (IC_{50}: 5.71 µg/ml). However, the remaining fractions were insignificant in the assay. The extracts of the plant illustrated marked scavenging activity against stable free radical, DPPH. The most potent antioxidant was crude extract (IC_{50}: 122 µg/ml) followed by ethyl acetate (IC_{50}: 137 µg/ml) that strongly augmented the antimalarial potential of the plant. GC MS spectrometry was used to explore the chemical composition of *n*-hexane fraction that can be attributed to the current antimalarial activity. Based on our findings, aerial parts of the plant could be a significant natural healing agent against resistant *P. falciparum*.

Introduction

Malaria is still a killing disease in different parts of the world especially third world countries. It is accountable for the death of almost 1 million individuals each year; mostly children below 5 years i.e., 85% (Anon., 2011). Like other developing countries of the world, similar pathetic condition has been observed in Pakistan. According to the survey of Ministry of Health, the total number of confirmed *Plasmodium falciparum* cases reported throughout the country was 31,407 during 2002 (Anon., 2005). In the current scenario, the most alarming issue is the resistance of malarial parasites to the available synthetic drugs like resistance of *P. falciparum* to chloroquine. This is also a major threat to the WHO program for malaria control "Roll Back Malaria". Over the decades, medicinal plants and various compounds isolated or derived from these have been used in the treatment of malaria (Chiyaka *et al*., 2009). Malaria is the single most important ailment that has been effectively treated with herbal products for the last many years. The classical compounds used in the management of malaria such as quinine and artemisinin, were either directly derived from plants or developed using chemical structures of plant based compounds (Tangmouo *et al*., 2010; Adebayo & Krettli, 2011; Sichaem *et al*., 2011). Obviously, medicinal plants still have immense potential to be explored for the effective management of various malarial strains including those resistant to available therapies.

P. verticillatum (L.) All., locally named as Nooreallam. The genus, *Polygonatum* consists of approximately 57 species of the family Convallariaceae (Khan *et al*., 2012a). The formulation of fresh rhizome of *P. verticillatum* has been used in the treatment of pain, pyrexia, burning sensation, for phthisis and also recommend as diuretic in combination with other plants and for the attenuation of painful urination (Khan *et al*., 2012a). Several pharmacological activities of the plant have been validated (Saeed *et al*., 2010a; Saeed *et al*., 2010b; Khan *et al*., 2010, Khan *et al*., 2011a, Khan *et al*., 2012a, Khan *et al*., 2012b, Khan *et al*., 2012c, Khan *et al*., 2012d, Khan *et al*., 2012e, Khan *et al*., 2012f, Khan *et al*., 2012g, Khan *et al*.,2013). The current study was designed to evaluate the antimalarial and antioxidant activities of the aerial parts of *Polygonatum verticillatum* followed by GC MS analysis for the detection of chemical components.

Materials and methods

Plant material: *P. verticillatum* (L.) All. was collected from District Swat of Khyber Pakhtunkhawa province, Pakistan, in July-Aug 2007. The botanical identification of the plant material was made by the Taxonomy Department of PCSIR Laboratories, Peshawar and a specimen with catalogue No: 9970 (PES) was deposited there in the herbarium.

Plant extraction and fractionation: The air dried aerial parts of the plant (10 kg) were, chopped into small pieces and powdered. The extraction of plant material was carried out by soaking the powder in methanol at ambient temperature for 14 days. The methanolic extract was filtered through filter paper and the marc obtained was again macerated with methanol. The same process of extraction was repeated 3 times and the combined filtrates were concentrated under vacuum at low temperature (40°C) using rotary evaporator (Khan *et al*., 2009). Finally, a crude methanolic extract (2.410 kg) was obtained. The crude extract (1.8 kg) was dissolved in distilled water and sequentially partitioned with various solvents to obtain *n*-hexane (275 g), chloroform fraction (295 g), ethyl acetate fraction (210 g), *n*-butanol fraction (317g) and aqueous fraction (445 g).

***In vitro* antimalarial activity:** The *in-vitro* antimalarial activity of the crude extract of aerial parts of *P. verticillatum* and its subsequent solvent fractions were executed using previously established methods (Makler *et al*., 1993; Khan *et al*., 2012). Briefly, cultures of *P. falciparum* were maintained in human erythrocytes of infected patients. Stock solutions of crude extract and solvent fractions (1 mg/ml) were prepared in DMSO (0.1%) which was subsequently diluted with supplemented RPMI-1640 medium. Negative controls contained an equal concentration of DMSO. The total volume (200 µL) was placed into the wells of 96-well microtiter plates with the diluted extract and the suspension of *P. falciparum*-infected RBCs (0.5% hematocrit with 1% parasitemia). The plates were incubated in candle jar with 5% CO_2 at 37ºC for 72 h.After which, a blood smear was taken from each well, and parasitemia counted. The parasitemia for each well was acquired and LD_{50} was estimated using of EZfit computer program. All tests were performed in triplicate. Positive controls contained 1 mM chloroquine diphosphate (Sigma).

1,1-diphenyl-2-picrylhidrazyl (DPPH) scavenging activity: The crude methanol extract and successive solvent fractions were tested for potential antioxidant activity on the ground of scavenging action of the stable DPPH free radical (Neelam *et al*., 2012). For preparation of DPPH stock solution of, 5 ml was dissolved in 2 ml of ethanol. It was reserved in the dark at ambient temperature. Various dilutions of the extracts were made in ethanol and were aliquoatted into a 96-well micro titer plate (Molecular Devices, USA). The reaction mixture was heated in Elisa at 37°C for 30 min and the absorbance was taken at λ 517 nm. Percentage inhibition of radical scavenging capacity was established by relating the results to control. Ethanol was used as negative control while ascorbic acid (Sigma USA) was used as reference control. All the analysis was executed in triplicate. The concentration of the compound that results 50% scavenging on DPPH was estimated as IC_{50}. All the used chemicals were of analytical standard.

GC-MS analysis: Gas chromatography followed by GC Mass of *n*-hexane fraction of aerial parts of the plant was analyzed applying gas chromatography attached with flame ionization detector (FID) (Qayum *et al*., 2012; Falodun *et al*., 2009). GC analysis was performed on the Shimadzu GC17-A system. GC-MS was supported by Joel JMS-600H GC and Joel JMS HX 110 quadruple mass spectrometer. Less polar capillary column, DB-5 (Optima-5) was used, coated in fused silica having the dimensions 30 mm, 0.25 mm internal diameter and 0.25 mm coating thickness.

Test sample (1.0 µl) was injected in AOC-20i auto-sampler into the GC system at 250°C in split mode being the split ratio as 40:1. The initial GC temperature was tuned to 50°C for 60 sec and 80°C for 3 min ramped with 10°C/min until the final temperature 300°C was achieved. The carrier gas, nitrogen was passed at a velocity of 35 cm/Sec while inlet pressure during the experiment was adjusted to be 99.31 KPa. The detector was adjusted at 280°C utilizing hydrogen gas (carrier) at the flow rate of 55 ml per min while the air flow rate was 400 ml/min. The mass spectrometer was set in the EI mode with 70 eV (ionization energy) while the GC experimental conditions were unchanged. As a carrier gas, helium was used at an operating temperature of 250°C. The qualitative naming of the compounds was done on the comparison/matching of their relative retention times (RT) and mass spectra with the data available in mass spectral search databases (NIST 1998 and GC-MS Library Shimadzu, 1996). In case of quantitative analysis of individual components (percent composition), the relative concentration of the peak area of each constituent was calculated against the total peak area.

Results and Discussion

Antimalarial bioassay: As presented in Table 1, the crude extract and its less polar fractions exhibited notable antimalarial activity against *P. falciparum*. The maximum potency was exhibited by the *n*-hexane fraction (IC_{50}: 4.86 µg/ml), followed by the chloroform fraction (IC_{50}: 5.71 µg/ml) while the crude extract was comparatively least potent (IC_{50}: 21.67 µg/ml). However, the polar fractions such as ethyl acetate, *n*-butanol and aqueous were found inactive in the assay.

Table 1. *In vitro* antimalarial activity of the crude methanol extract and fractions of the aerial parts of *Polygonatum verticillatum* against *Plasmodium falciparum*.

Test organism	Extracts/Fractions	IC_{50} (µg/ml)
Plasmodium falciparum	Crude methanol extract	14.75
	Hexane	4.86
	Chloroform	5.71
	Ethyl acetate	>25
	n-Butanol	>25
	Aqueous	>25
	Chloroquine diphosphate	0.025

Tested Sample was 1 mg. Incubation period was 72 h at 37 ^{0}C. Positive control was Chloroquine diphosphate while DMSO as Negative control.

Antioxidant assay: According to the results of antioxidant assay (Table 2) the aerial parts of the plant had promising antioxidant activity (Fig. 1). The highest free radical scavenging activity was shown by the crude extract (IC_{50}: 122 µg/ml) followed by ethyl acetate (IC_{50}: 137 µg/ml) and *n*-butanol (IC_{50}: 167 µg/ml) fractions.

Table 2. The IC_{50} values of the crude methanol extract and fractions of the aerial parts of *Polygonatum verticillatum*.

Test organism	Extracts/Fractions	IC_{50} (µg/ml)
1,1-diphenyl-2-picrylhidrazyl (DPPH)	Crude extract	122 ± 3.55
	n-Hexane	NA
	Chloroform	190 ± 2.88
	Ethyl acetate	137 ± 5.46
	n-Butanol	167 ± 2.88
	Aqueous	194 ± 4.04
	Vitamin-C	24 ± 1.73

IC_{50} values are the mean ± S.E.M. of three assays

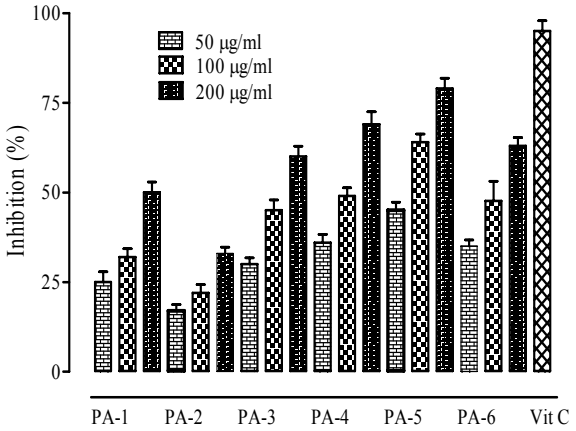

Fig. 1. 1,1-diphenyl-2-picrylhidrazyl (DPPH) antagonistic potential of Aerial parts. A-1 = Crude extract; A-2 = *n*-hexane; A-3= Chloroform; A-4 = Ethyl acetate; A-5 = *n*-butanol; A-6 = Aqueous. Standard drug = Vitamin C. Negative control = Ethanol. Symbols represent mean ± S.E.M. (n = 3).

GC MS spectrometry: The results of GC MS spectrometry demonstrated that the oily components of the aerial parts were α-Bulnesene (1.5648%), Linalyl acetate (0.4535%), Eicosadienoic (0.3702%), Pentacosane (0.3319%), Piperitone (0.3091%), Docasane (0.1720%), and Calarene (0.1321%) (Fig. 2, Table 3).

Pakistan is very rich in the wealth of medicinal plants that are scattered throughout the country especially in the province of Khyber Pukhtonkhawa and Northern areas. Numerous herbalists are using these on the base of long empirical learning without any scientific evidence (Saeed *et al*., 2010c; Jahan *et al*., 2010; Neelam and Khan, 2012). Such traditional heritage that enriches our ethnopharmacology needs scientific validation in the light of modern technologies for the discovery of new effective therapeutic modalities.

The results of our investigations demonstrated marked antimalarial activity of the aerial parts of the plant against *P. falciparum* by crude extract and less polar fractions, while except *n*-hexane, all other fractions were effective against DPPH in free radical scavenging assay without any cytotoxicity (Khan *et al*., 2012d).

The antimalarial activity of hexane fraction or oily components is already reported in literature (Saiin *et al*., 2003; Ratsimbason *et al*., 2009; Khan *et al*., 2011b). It can be assumed that the current antimalarial activity is due to the presence of these detected components.

Table 3. Qualitative and quantitative composition of *n*-hexane fraction of Aerial parts of *Polygonatum verticillatum*.

P. No.	Compound	R.T (min)	Molecular weight	Concentration (%)
2	Docasane	19.298	310.3 [$C_{22}H_{46}$]	0.1720
6	Pentacosane	23.490	352.4 [$C_{25}H_{52}$]	0.3319
9	Linalyl acetate	25.788	196.2 [$C_{10}H_{20}O_2$]	0.4535
10	α-Bulnesene	27.048	204.3 [$C_{15}H_{24}$]	1.5648
12	Eicosadienoic	28.844	308.3 [$C_{20}H_{36}O_2$]	0.3702
23	Piperitone	37.119	152.2 [$C_{10}H_{16}O_6$]	0.3051
25	Calarene	38.216	204.2 [$C_{15}H_{24}$]	0.1321

N/D = Not determined. Data bases used for the elucidation of constituents was performed were: GC-MS Library of Shimadzu Class-5000, ver 2.0 (1996). NIST Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library, ver. 16d (06/24/1998). Gaithersburg, MD, USA

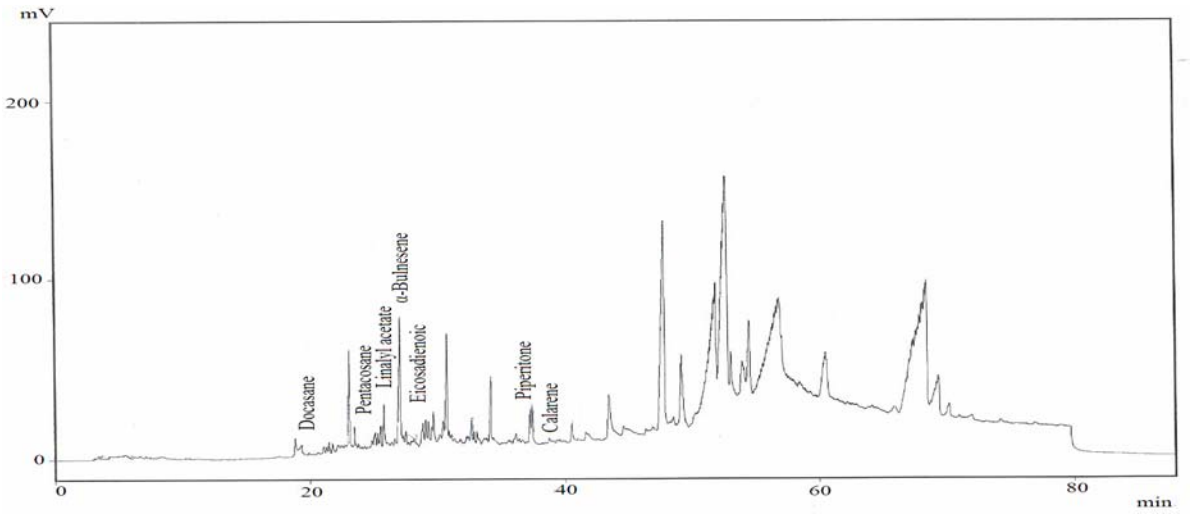

Fig. 2. Gas chromatogram of *n*-hexane fraction of Aerial parts.

Conclusion

In conclusion, the aerial parts of the plant offered an outstanding natural source of antimalarial components. The composition of the most potent fraction, hexane was explored by GC MS spectrometry analysis. The aerial parts also showed promising antioxidant activity that further augmented the antimalarial potential of the plant. This study has provided a strong foundation for the uses of the plant even in crude form. However, further detailed studies are warranted to discover new clinically effective antimalarials and to cope with the drastic current issue of resistance.

References

Adebayo, J.O and A.U. Krettli. 2011. Potential antimalarials from Nigerian plants: A review. *Journal of Ethnopharmacology,* 133: 289-302.

Anonymous. 2005. MOH. Annual report of Director General Health (2002−2003), Bio-Statistic Section /PHC Cell. Government of Pakistan, Islamabad, Pakistan, 13-14.

Anonymous. International Federation of Red Cross and Red Crescent Societies. 2011. http://www.ifrc.org/ what/health/ diseases/ malaria/malariaday.asp? gclid=CMfxqf6e8a YCFYVjfAodsnS8GA.

Chiyaka, C., W. Garira and S. Dube. 2009. Effects of treatment and drug resistance on the transmission dynamics of malaria in endemic areas. *Theoretical Population Biology*, 75:14-29.

Falodun, A., R. Siraj and M.I. Choudhary. 2009. GC-MS Analysis of Insecticidal Leaf Essential Oil of Pyrenacantha Staudtii Hutch and Dalz (Icacinaceae). *Tropical Journal of Pharmaceutical Research*, 8:139-143.

Jahan, N., M.A. Mehjabeen., M. Zia-ul-Haq S.M. Alam and M. Qureshi. 2010. Antimicrobial screening of some medicinal plants H of Pakistan. *Pakistan Journal Botany* 43: 4281-4284.

Khan, H,. M. Saeed, A.H. Gilani, M.H. Mehmood, Najeeb-ur-Rehman, N. Muhammad, M. Abbas and Ikram-ul-Haq. 2012a. Bronchodilator activity of Aerial parts of *Polygonatum verticillatum* augmented by anti-inflammatory activity: attenuation of Ca2+ channels and lipoxygenase. *Phytotherapy Research* [Accepted. DOI:10.1002/ptr.4860].

Khan, H., M. Saeed and N. Muhammad. 2012e. Pharmacological and phytochemical updates of *Polygonatum*. *Phytopharmacology*, 3: 286-308.

Khan, H., M. Saeed, A.H. Gilani, M. Naveed, Ikram-ul-Haq, N. Ashraf, Najeeb-ur-Rehman and A. Haleemi. 2012b. Antipyretic and anticonvulsant activity of *Polygonatum verticillatum*: comparison of rhizomes and aerial parts. Phytotherapy Research [Accepted DOI: 10.1002/ptr. 4721].

Khan, H., M. Saeed, A.H. Gilani, M.A. Khan, A. Dar and I. Khan. 2010. The antinociceptive activity of *Polygonatum verticillatum* rhizomes in pain models. *Journal of Ethnopharmacology* 127: 521-527.

Khan, H., M. Saeed, A.H. Gilani, M.A. Khan, I. Khan and N. Ashraf. 2011. Anti-nociceptive activity of aerial parts of *Polygonatum verticillatum*: Attenuation of both peripheral and central pain mediators *Phytotherapy Research*, 25: 1024-1030.

Khan, H., M. Saeed, M.A. Khan, I. Khan, M. Ahmad, N. Muhammad and A. Khan. 2012d. Antimalarial and free radical scavenging activities of rhizomes of *Polygonatum verticillatum* supported by isolated metabolites. *Medicinal Chemistry Research* 21: 1278-1282.

Khan, H., M. Saeed, M.A. Khan, Izhar-ul-Haq, N. Muhammad and R. Ghaffar. 2012c. Isolation of long chain esters from the rhizome of *Polygonatum verticillatum* with potent tyrosinase inhibition. *Medicinal Chemistry Research* DOI 10.1007/s00044-012-0194-8.

Khan, H., M. Saeed, N. Muhammad, R. Gaffar, F. Gul and N. Raziq. 2013. Lipoxygenase and urease inhibition of the aerial parts of the *Polygonatum verticillatum*. *Toxicology and Industrial Health* [Acceptet].

Khan, H., M. Saeed, N. Muhammad, R. Ghaffar, S.A. Khan and S. Hassan. 2012f. Antimicrobial activities of rhizomes of *Polygonatum verticillatum*: attributed to its total flavonoidal and phenolic contents. *Pakistan Journal of Pharmaceutical Sciences*, 25: 463-467.

Khan, H., Saeed M, Muhammad N, Khan F, Ibrar M, Hassan S, Shah WA (2012g) Comprehensive nutrients analysis of rhizomes of *Polygonatum verticillatum*. *Pakistan Journal of Pharmaceutical Sciences*, 25: 871-875.

Khan, M., H. Khan., Khan, S., Mahmood, T., Khan, P. and Jabar, A. 2009. Anti-inflammatory, analgesic and antipyretic activities of *Physalis minima* Linn. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 24, 632-637.

Khan, S.S, V.U. Ahmad, N. Saba and R.B. Tareen. 2011. *Eremurus persicus*, a new source of medicinally important compounds. *Pakistan Journal of Botany*, 43:2311-2313.

Makler, M.T., J.M. Ries, J.A. Williams, J.E. Bancroft, R.C. Piper, B.L. Gibbins and D.J. Hinrichs. 1993. Parasite Lactate Dehydrogenase as an Assay for *Plasmodium falciparum* Drug Sensitivity. *American Journal of Tropical Medicine and Hygiene*, 48: 739-741.

Neelam, S. and Z.U. Khan. 2012. Antioxidant activity of Galium aparine L. from Punjab, Pakistan. *Pakistan Journal of Botany* 44: 251-253.

Qayum, M., M. Nisar, M.R. Shah, Zia-ul-Haq, W.A. Kaleem and I.K. Marwat. 2012. Biological screening of oils from *Impatiens bicolor* Royle. *Pakistan Journal of Botany*, 44: 355-359.

Ratsimbason, M., L. Ranarivelo, H.R. Juliani and J.E. Simon. 2009. Antiplasmodial activity of twenty essential oils from malagasy aromatic plants. African natural plant products: New discoveries and challenges in chemistry and quality. *ACS Symposium Series*, 1021: 209-215.

Saeed, M., H. Khan, M.A. Khan, F. Khan, S.A. Khan and N. Muhammad. 2010a. Quantification of various metals accumulation and cytotoxic profile of aerial parts of *Polygonatum verticillatum*. *Pakistan Journal of Botnay*, 42: 3995-4002.

Saeed, M., H. Khan, M.A. Khan, S.U. Simjee, N. Muhammad and S.A. Khan. 2010b. Phytotoxic, insecticidal and leishmanicidal activities of aerial parts of *Polygonatum verticillatum*. *African Journal of Biotechnology*, 9: 1241-1244.

Saeed, M., N. Muhammad, H. Khan and S.A. Khan. 2010c. Analysis of toxic heavy metals in Pakistani herbal products. *Journal of Chemical Society Pakistan*, 32: 471-475.

Saiin, C., R. Rattanajak, S. Kamchonwongpaisan, K. Ingkaninan, K. Sukontason, A. Baramee and B. Sirithunyalug. 2003. Isolation and *In vitro* antimalarial activity of hexane extract from Thai Picrasma javanica B 1 stembark. *Southeast Asian Journal of Tropical Medicine and Public Health*, 34: 51-55.

Sichaem, J., S. Surapinit, P. Siripong, S. Khumkratok, J. Jong-aramruang and S. Tip-pyang. 2011. Two new cytotoxic isomeric indole alkaloids from the roots of Nauclea orientalis. *Fitoterapia*, 81: 830-833.

Tangmouo, J.G, R. Ho, A. Matheeussen, A.M. Lannang, J. Komguem, B.B. Messi, L. Maes and K. Hostettmann. 2010. Antimalarial activity of extract and norbergenin derivatives from the stem bark of Diospyros sanza minika A. Chevalier (Ebenaceae). *Phytotherapy Research*, 24: 1676-1679.

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