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RESEARCH ARTICLE

Pharmacognostical and antioxidant activity investigations on *Vernonia anthelmintica* Wild fruits

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ABSTRACT

Vernonia anthelmintica Willd. (Asteraceae) is an annual herb used conventionally for the management of diabetes, fever, wound, malaria, cancer, acne and stomach disorder. As the plant is of ethno-pharmacological relevance and has been used in a number of polyherbal formulations, therefore, pharmacognostical studies were carried out to confirm the identity and quality of *V. anthelmintica* fruits. Also, the plant has been used as a remedial measure for skin ailments and oxidative stress also triggers skin ailments, therefore, antioxidant potential of the plant was explored using DPPH assay. Methanol extract of the plant showed maximum DPPH scavenging effect (90.4%) at a concentration of 90 µg/ml, whereas the standard drug - ascorbic acid showed maximum DPPH scavenging effect (94.8%) at 40 µg/ml.

Keywords: Asteraceae, DPPH, Pharmacognostical, *V. anthelmintica*.

INTRODUCTION

Vernonia anthelmintica Willd. (Purple Fleabane, or kalijiri) is an annual herb of Asteraceae family, widely spread throughout Africa and Asia.[1,2] The seed powder of *V. anthelmintica* is used as an anthelmintic, diuretic, tonic, purgative and to treat snake bites.[3,4] In Chinese system of medicine, mature fruit is used to manage skin disorders like psoriasis and leucoderma.[5] In India, leaf powder is used for the management of skin diseases[6] and clinically, it has been found effective against vircarcika eczema.[7] Several scientific reports suggest that the plant possesses a multitude of pharmacological activities, including antimicrobial, anti-inflammatory, antivitiligo, antidiabetic, anthelmintic, anticancer, anti-nociceptive and hepatoprotective activity.[8-14] Although the plant is of traditional and pharmacological relevance yet it has not been much explored. Thus, the objective of present research was to evaluate pharmacognostical parameters and antioxidant activity of *V. anthelmintica* fruits.

MATERIALS AND METHODS

Chemicals and instruments

2,2-Diphenyl-1-picrylhydrazyl (DPPH) and ascorbic acid were obtained from Sigma (St. Louis, MO, USA). All other chemicals used in the protocol were of analytical grade. Digital Microscope (Leica DM 4000B), Rotavapor (Rotavapor® R-210/R-215, Buchi), Spectrophotometer (Perkin Elmer Spectrum RX1 FTIR Spectrometer) were used.

Plant material and extract preparation

The identity of *V. anthelmintica* fruits collected from Punjab and Haryana, India was confirmed by Dr. Sunita Garg, Chief Scientist, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi (NISCAIR/RHMD/Consult/2014/2520/99, dated 22-09-2014). Dried, coarsely powdered fruits (250 g) were extracted with methanol (MeOH) using soxhlet extraction method. The solvent was recovered under reduced pressure using rotary evaporator and the dried MeOH extract (22.30% w/w, on dry weight basis) was stored at -4 ºC.

Pharmacognostical evaluation

Organoleptic evaluation

It was carried out to study the gross morphology, color, odor and taste of *V. anthelmintica* fruits (single seeded achene) as described by WHO.[15]

Microscopic evaluation

The fruits (single seeded achene) were taken and free hand sections were cut with the help of new sharp Wilkinson blade. The various tissues were

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observed under microscope using safranin and fast green stains.[16] Photomicrographs were taken using compound microscope (Leica DM 4000 B LED, Germany) at 40 X magnification.

Physico-chemical evaluation

Physico chemical evaluation (loss on drying, total ash value, acid insoluble ash, water soluble ash, extractive value, and foreign organic matter) was performed according to the official methods prescribed.[15,17] All parameters were determined in triplicate.

Phytochemical screening

Phytochemical screening of *V. anthelmintica* methanolic extract for different classes of phytoconstituents was done according standard protocols.[18]

Antioxidant activity evaluation

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used to determine the free radical scavenging potential of *V. anthelmintica* fruit extracts.[19,20] About, 1 ml of methanolic solution of DPPH (0.1 mmol/l) was incubated with varying concentrations of MeOH extracts of *V. anthelmintica* fruits. The mixture was mixed with vortex shaker and incubated at 30ºC for 30 min. Absorbance was read against a blank at 517 nm. Ascorbic acid was used as standard. The DPPH radical scavenging activity was measured as:

$$\% \text{ Scavenging effect} = \frac{\text{Absorption of control solution - Absorption of test solution}}{\text{Absorption of control solution}}$$

RESULTS AND DISCUSSION

Standardization is an essential measure for quality control, purity determination and sample identification.[21] It ensures that the material is of reasonable consistency.[22,23] Pharmacognostic studies are one of the cheapest and simplest methods to start with, for establishing the correct identity of the source materials. Plate 1 and table 1 report organoleptic features of *V. anthelmintica*.

Plate 1: *V. anthelmintica* fruits (single seeded achene)

Table 1: Organoleptic evaluation of *V. anthelmintica* fruits (single seeded achene)

Parameters	*V. anthelmintica*
Color	Dark brown
Odor	Characteristic
Taste	Bitter
Shape	Narrow oblong
Size	0.5 - 1 cm

Transverse section of fruit (Plate 2) shows a well demarcated pericarp, endosperm and testa. Epicarp consists of a layer of parenchymatous cells with abundant unicellular trichomes on the ridges. Mesocarp consists of compact transparent parenchyma in which vascular bundles are embedded, along with yellow coloured collenchyma and a wavy band of sclerenchymatous layer. Endocarp consists of thick walled cells, beneath pericarp lies the seed coat. The outer layer of seed coat is single layered consisting of beaker shaped cells while inner layer is made of thin transparent parenchymatous cells. Endosperm forms bulk of the seed coat and contains many aleurone grains and oil globules.

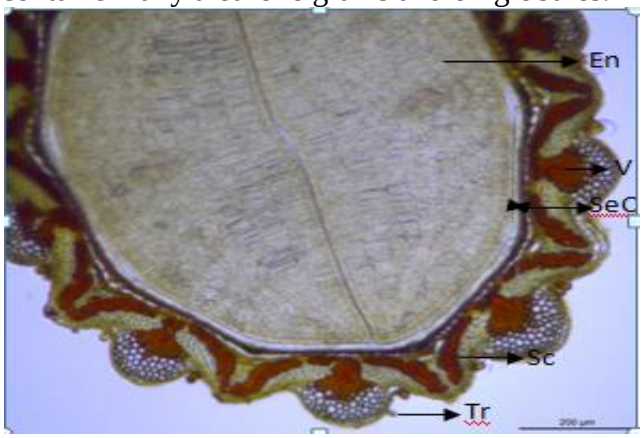

Plate 2: Transverse section of *V. anthelmintica* fruits (single seeded achene)

En-Endosperm; V-Vascular bundle; SeC-Seed coat; Sc-Sclerenchyma; Tr-Trichome

Physico-chemical standards are helpful in determining quality and purity of the crude drug.[24] Ash value (total ash, acid-insoluble and water-soluble ash), extractive value (water and alcohol soluble), moisture content, and foreign organic matter were evaluated. Ash value establishes the purity of a crude drug by determining presence or absence of inorganic matter or earthy matter or any other impurity. Extractive values determine the amount and nature of specific constituents soluble in a particular solvent. Moisture content was determined in order to detect the possibility of microbial growth. The results of physicochemical parameters are summarized in Table 2.

Table 2: Physico-chemical parameters of *V. anthelmintica*

Parameters	Mean values % (w/w)
Total ash	6.32±0.01

Acid-insoluble ash	1.32±0.20
Water soluble ash	2.06±0.08
Water soluble extractive value	7.39±0.01
Alcohol soluble extractive value	19.01±0.23
Moisture content	5.01±0.03
Foreign organic matter	Nil
All values are expressed as Mean±SD; n=3	

Preliminary phytochemical screening of *V. anthelmintica* methanol extracts tested positive for glycosides, triterpenoids, flavonoids, tannins and carbohydrates. Oxidative stress causes damage to cell membrane lipids or proteins and thereafter releases proinflammatory cytokines which induce skin diseases like acne, psoriasis, eczema.[25,26] Since the plant has been conventionally used in the management of skin ailments therefore its antioxidant activity was assessed by determining percentage inhibition of DPPH. Ascorbic acid was used as standard. Methanol extract of the plant showed maximum DPPH scavenging effect (90.4%) at a concentration of 90 µg/ml, whereas the standard drug - ascorbic acid showed maximum DPPH scavenging effect (94.8%) at 40 µg/ml. Increase in antioxidant activity was observed in concentration dependant manner as shown in table 3.

Table 3: Results of antioxidant activity of various extracts of *V. anthelmintica.*

Treatment	Concentration (µg/ml)	Mean^n % inhibition of DPPH radical ± S.D.
Ascorbic acid	1	22.83±1.44
	2	37.83±2.91
	4	50.40±2.81
	6	61.26±2.00
	8	70.73±1.47
	10	85.38±2.36
	20	92.97±1.83
	40	94.80±3.44
MeOH extract	10	14.89±1.01
	30	39.72±0.88
	50	58.37±0.69
	70	81.41±1.71
	90	90.40±0.14
n=3; S.D.- Standard deviation		

Since *Vernonia anthelmintica* Willd. is one of the ingredients of polyherbal formulations available in market for treating skin ailments[5,27,28] therefore it was thought worthwhile to carry out pharmacognostical investigations inorder to establish identity, purity and quality of the plant. Furthermore, antioxidant study was also carried out as generation of reactive oxygen species (ROS) has interplay in causing skin ailments.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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