

Full Length Research Paper

Analgesic activity of medicinally important leaf of *Diplazium esculentum*

Sonia Chawla^{1*}, Saurabh Chawla², Veerma Ram², Alok Semwal¹ and Ramandeep Singh¹

¹Department of Pharmacy, Himachal Institute of Pharmacy, Paonta Sahib-173025, H.P. India.

²Department of Pharmacy, Sardar Bhagwan Singh Post Graduate Institute of Biomedical Sciences and Research, Dehradun-248161, U.K. India.

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The use of medicinal plants in modern medicine suffers from the fact that though hundred of plants are used in the world to prevent or to cure disease, scientific evidence in term of modern medicine is lacking in most cases. However, today it is necessary to provide scientific proof as to whether or not it is justified to use a plant or its active constituents. Pain and inflammation are considered one of the oldest known diseases of mankind and affects a large population of the world, unsatisfactory progress and lack of proper scientific evidences has enriched this disease worldwide. *Diplazium esculentum* is one of the most popular fern which is also used for medicinal purposes. Various parts of the *D. esculentum* are used for numerous purposes. However, its analgesic activity is still a mystery for the scientific world. So this study includes successive extraction and pharmacological evaluation of the analgesic activity of various extracts obtained from *D. esculentum*. To investigate the analgesic activity, 'acetic acid induced writhing' method in mice was used. The various extracts obtained were subjected to analgesic activity. The finding of this study highlighted several novels and important aspects of the extracts derived from *D. Esculentum*.

Key words: *Diplazium esculentum*, analgesic, writhing method, successive extraction.

INTRODUCTION

From the ancient time, plants are used as a vital source of food, medicines and other necessities of life. There are so many higher plants of edible economic values, especially the pteridophytes. They have the prominent part of the earth's vegetation. These pteridophytes are used in various medication systems like homoeopathy, Ayurvedic Charak, Sushrut and Unani medicines and they also provide food, insecticides and ornamentations

(Mannan et al., 2008). Generally, ferns are vascular plants and therefore known as vascular cryptogams. The growths of ferns were preferred in shady, moist habitat with moderate temperatures. Mostly they are found in the rain forests, both tropical and temperate, but also occur throughout a very diverse range of habitats from high altitude and latitude to arctic-alpine situations, to the heart of the tropical rain forests, and from sub-desert

*Corresponding author. E-mail: ssonia.khurana@gmail.com.

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Table 1. Extractive values of powdered leaf of *D. esculentum*.

Solvents	No. of h for extraction (h)	Extractive value (g)
DEPEE (Extract I)	25	2
DECE (Extract II)	12	2.35
DEEE (Extract III)	0	1.89
DEAE (Extract IV)	12	3.58

DEPEE (Extract I)- *D. esculentum* petroleum ether extract, DECE (Extract II)- *D. esculentum* chloroform extract, DEEE (Extract III)- *D. esculentum* ethanolic extract, DEAE (Extract IV)- *D. esculentum* aqueous extract.

scrub of continental interiors to the rock of the sea coast and mangrove swamps (Khare, 1996; Pande and Pande, 2003).

Diplazium esculentum is one of the most popular fern which is also used for medicinal purposes. It is generally found at low altitudes of Kumaon Himalayas, growing abundantly along with water channels. Various parts of the *D. esculentum* are used for numerous purposes. Leaves of *D. esculentum* are used as vegetables; dried rhizomes are used as an insecticide, decoction of this plant is used in the treatment of cough and sometimes as a tonic (Shankar and Khare, 1985; Kaushik et al., 2012; Sudha et al., 1999).

From the current literature review it is evident that *D. esculentum* is the most commonly consumed fern throughout Asia and Oceania. However, the conclusive analgesic study of its leaves has not been performed yet.

MATERIALS AND METHODS

Drugs and chemicals

All the other chemical and reagents used in this study are analytical grade, and used without further purification.

Collection of plant material

The fresh plant material was collected during the month of December to January from different areas of Dehradun city, and was authenticated by Dr. H C Pande, Scientist, Botanical Survey of India., Dehradun (Uttarakhand) India. The fresh samples were dried at room temperature (25°C to 35°C) for 7 to 10 days. The leaves were crushed and weight and preserved in air tight container.

Extraction of plant materials

The dried plant materials (Leaves) were extracted successively with various solvents. These solvents were used for dissolving the different component present in the plant material, based on their differential polarity. Solvents in order of increasing polarity were used; petroleum ether, chloroform, ethanol and distilled water. All the extracts thus obtained and kept in a desiccator for future use.

Extractive values from the leaves of *D. esculentum* are given in Table 1.

Phytochemical screening

The various extracts of leaves of *D. esculentum* (Figure 1) were subjected to qualitative chemical examination for the presence or absence of alkaloids, carbohydrates, flavanoids, proteins, saponins and tannins, phenolic compounds and glycosides. The results of preliminary phytochemical screening are as shown in Table 2 (Tiwari et al., 2011; Goyal et al., 2012).

Determination of the analgesic activity

Six groups of albino mice weighing 20 to 30 g, of both sex and each group containing 5 mice were chosen for the study. One group was kept as control and another group was treated with a standard drug (aspirin) (Singh et al., 2013). The method employed was that of 'acetic acid induced writhing' method in mice. The standard and extracts were administered intraperitoneally at a dose of 25 mg/kg. All the test extracts/standard was dissolved in distilled water. The control group of mice was administered 1 ml/100 g of normal saline intraperitoneally of body weight. Next, the animals were placed individually under a glass jar for observation. The trunk twists response; abdominal contractions and extension of hind limbs were recorded as writhing responses for a period of 10 min. The second group of animals were injected with aspirin (25 mg/kg). Fifteen minutes later, they were administered acetic acid solution 1 ml/100 g intraperitoneally. Rest groups of animal were injected test extracts I to IV at a level of 25 mg/kg intraperitoneally. Fifteen minutes later, they were administered acetic acid solution. The onsets of writhing responses were recorded during 10 min. The mean writhing scores were calculated in control, aspirin and test extracts treated groups. Observations and results are summarized in Table 3.

RESULT AND DISCUSSION

Phytochemical investigations

All the four extracts (*D. esculentum* aqueous extract (DEAE) (Extract I), *D. esculentum* petroleum ether extract (DEPEE) (Extract II), *D. esculentum* chloroform extract (DECE) (Extract III), *D. esculentum* ethanolic extract (DEEE) (Extract IV)) were found to have protein, steroids, triterpenoids, flavones and flavonoids.

Pharmacological investigations

The analgesic activity was evaluated following the 'acetic acid induced writhing' method in mice using aspirin as the standard (Kadam and Mahadik, 2010). For comparison, paired "t" test was carried out. The results giving mean of writhing response together with level of significance are given in Table 3. All the values are means of five readings. Values in parentheses indicate standard deviation. All the compounds and standard were administered at a dose level of 25 mg/kg intraperitoneally (Kadam and Mahadik, 2010). Based on the level of significance, the extract tested could be rated as:

Table 2. Preliminary phytochemical screening of powdered leaf of *D. esculentum*.

Plant constituent	Extracts			
	DEPEE (Extract I)	DECE (Extract II)	DEEE (Extract III)	DEAE (Extract IV)
Tests				
Alkaloids				
Hager's reagent	-ve	-ve	-ve	-ve
Wagner's reagent	-ve	-ve	-ve	-ve
Mayer's reagent	-ve	-ve	-ve	-ve
Dragendorff's reagent	-ve	-ve	-ve	-ve
Phenolic compounds and tannins				
Vanillin- hydrochloride test	-ve	-ve	-ve	+ve
Flavonoids				
Ferric chloride test	+ve	+ve	+ve	+ve
Zinc hydrochloride acid reduction test	+ve	+ve	+ve	+ve
Alkaline test	+ve	+ve	+ve	+ve
Proteins				
Biuret test	-ve	-ve	+ve	-ve
Million's test	-ve	-ve	+ve	-ve
Amino acids				
Ninhydrin test	-ve	-ve	+ve	-ve
Glycosides and carbohydrates				
Fehlling test	-ve	-ve	+ve	+ve
Molish's test	-ve	-ve	+ve	+ve
Barfod's test	-ve	-ve	+ve	+ve
Benedict's test	-ve	-ve	+ve	+ve
Legal's test	-ve	-ve	+ve	+ve
Triterpenoids				
Salkowaski test	+ve	-ve	-ve	-ve
Libermann- Burchard's test	+ve	-ve	-ve	-ve

Table 3. Analgesic activity of extracts (I to IV) obtained from the powdered leaf of *D. esculentum*.

Drug and extracts	Mean writhing response in 10 min with level of significance
Control	27.6 (5.59)
Standard	15.0 (3.60) p<0.020
DEPEE (Extract I)	14.8 (3.03) p<0.010
DECE (Extract II)	16.2 (2.77) p<0.015
DEEE (Extract III)	13.8 (2.77) p<0.017
DEAE (Extract IV)	14.4 (2.70) p<0.004

(DEAE(Extract IV)> DEPEE (Extract I), DECE (Extract II) and DEEE (Extract III).



Figure 1. Leaf of *D. esculentum*.

Conclusion

Conclusively, in present study, aqueous extract of *D. esculentum* was found to be potent analgesic. In accordance to the present study, it has been observed that *D. esculentum* has marked beneficial effects against centrally, peripherally and inflammatory pain models. This protective action may be attributed towards the presence of flavanoid and sterols. It is worthwhile to use *D. esculentum* as drug, and further studies should be initiated to establish exact mechanism of action and elaborative phytochemical investigations to find out which active constituents is responsible for analgesic activity. These reports may serve as a foot step in the research of potent

analgesic drug.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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