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PHYTOCHEMICAL SCREENING OF ACONITUM FERROX ROOTS

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ABSTRACT
Aconitum ferrox is a medicinal plant native to India that belongs to the Ranunculaceae family. A. Ferrox is reported to have a variety of medicinal properties. Since ancient times, this plant has employed several formulations in India's traditional treatment system, Ayurveda. It has been used to treat urinary infections, diarrhea, and inflammation in patients. It's also been utilized to promote hepatoprotective activity and as an expectorant. Alkaloids, carbohydrates, proteins and amino acids, saponins, glycosides, quinones, flavonoids, terpenoids, and other compounds have been discovered in various plant portions, according to chemical investigations. The therapeutic characteristics of A. Ferrox, as well as their phytochemistry and pharmacognosy, are discussed in this study. Scientific data on the plant was gathered from various sources, including electronic sources (Google scholar, Pubmed) and some old Ayurvedic and ethnopharmacology textbooks. The research also includes a review of the literature on A. ferrox, as well as the most relevant pharmacological and other results on this drug. This article should be helpful to new researchers who are starting a study on the plant A. ferrox and will serve as a beneficial tool for them.

Keywords: Aconitum Ferrox, Soxhlet Apparatus, Phytochemical Screening.

INTRODUCTION

Traditional medicine encompasses health practices, approaches, knowledge, and beliefs that include plant, animal, and mineral-based medicines, spiritual therapies, manual techniques, and exercises, which are used singly or in combination to treat, diagnose, and prevent illnesses, as well as to maintain well-being. Traditional medicine has grown in popularity in Cameroon during the last decade, owing in part to the country's long-term unsustainable economic position. The therapeutic approach to alternative traditional medicine as a possibility for a concerted search for new chemical entities has been prompted by the high cost of pharmaceuticals and the rise in drug resistance to prevalent ailments such as malaria, bacterial infections, and other sexually transmitted diseases (NCE). The World Health Organization (WHO) has established a strategic framework for the practice and development of TM in Cameroon in partnership with the Cameroon government [1]. Aconitum ferrox (A. Ferrox) is an ayurvedic medicinal plant that is utilized as the major ingredient in several Ayurvedic formulas in India. Aconitum species are also commonly utilized in Chinese and Bhutanese herbal medicine. In Indian English, this plant is known as atees and atis root; in Sanskrit, it is known as ativisha, shuklakanda, aruna, and vishada; in Urdu, it is known as atees; in Hindi, it is known as atis and atvika; in Bengali, it is known as ataish; in Telugu, it is known as ati vasa [2]. The plant kingdom's 'Magnoliophyta' division includes A. Ferrox, which belongs to the Ranunculaceae family, and the Aconitum genus [3]. There are around 300 species of Aconitum worldwide, with species identified in India. The dried tuberous roots of A. ferrox Wall. ex. Royle, a perennial plant native to the western Himalayas and found in Kashmir, Uttarakhand, Sikkim, and Nepal at altitudes between 2,500 and 4,000 m, are used to make medicinal A. Ferrox. The majority of the species are highly toxic, earning them the title of "Queen of all Poisons," with numerous species having been utilized on the ends of hunting spikes and still being used today. As a result, this plant must be handled with caution [4, 5].

Antidiarrheal, expectorant, diuretic, hepatoprotective, antipyretic and analgesic, antioxidant, alexipharmic, anodyne, anti-atrabilious, anti-flatulent, anti-periodic, anti-phlegmatic, and carminative properties have been reported for A. Heterophyllum; it can also be used to 61 treat patients with reproductive disorders [6-8]. Figs. 1, 2 show pictures of the plant and its root.

All the parts of Aconitum ferrox plant are poisonous; the root part is more potent than other parts. Root part is mostly used for medicinal use. As the root part is poisonous but useful in the treatment of various diseases such as rheumatoid arthritis, fever, and hypertension and also acts as Rasayana.

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A tuberous-rooted, herbaceous perennial reaching 1.0 metre tall by 0.5 metres wide and tolerant of many soil types, Aconitum ferox forms the principal source of the Indian poison known variously as bikh, bish, and nabee. It contains large quantities of the extremely toxic alkaloid pseudaconitine (also known as nepaline, after Nepal) and is considered to be the most poisonous plant found in the Himalaya and one of the most poisonous in the world.

The symptoms of poisoning usually appear 45 minutes to an hour after the consumption of a toxic dose and consist of numbness of the mouth and throat and vomiting. Respiration slows, with blood pressure falling synchronously, while the heart rate slows to 30-40 beats per minute. Consciousness characteristically remains unclouded until the end, which consists usually of death by asphyxiation, although occasionally of death due to cardiac arrest.

MATERIALS AND METHODS

Collection of Herb

The roots of the plant were collected in the month of July 2022 form different parts/district of Uttarakhand State.

Identification and Authentication of Collected Plant

Plant parts were identified and authentication from Botanical Survey of India, Shibpur, Howrah, West Bengal.

Washing and Shade Drying

Plant samples gathered for preparing undergo a washing process to remove contamination from particles that adhere, such as dust and other contaminants.

There are 3 washing methods:-

1."Wash by machine in the bag,"
2."Beaker soak" tap or deionized water by hand washing,
3."Rinse the colander" with tap or deionized water.

Samples are carried out in soft mode through at least one full wash / rinse / spin cycle. Not more than 15 are processed at one moment. Some moment after washing plants dried at room temperature [24±5], the temperature rises 50-60. The temperature should not be increased by more than 60 ° C as well as volatile active constituents also evaporate above the 60 ° C enzymes and proteins are denatured. Dried plant material until they were free of moisture and subjected to various parameters of physical assessment.

Grinding and Sieving

Samples of plant tissue were reduced to the particle size of 0.5 to 1.0 mm to ensure homogeneity and facilitate the destruction of organic matter. Using the suitable Wiley Mill 22 mesh sieve, specimens were passed through a 1.0 mm screen [22 meshes]. However, a 40 mess screen should be used if the sample aliquot to be tested is < 0.5 gm.

Samples are finely ground to achieve homogeneous powder using a stainless steel screen Cyclone Udy Mill to pass through a 22 mess sieve. Large specimens were first grounded by a conventional Beater Cross grinder and then decreased to a manageable size by quartering. The Cyclone Udy Mill or Intermediate Wiley Mill then ground these.

Extraction Procedure

General extraction methods for medicinal plants include maceration, infusion, percolation, digestion, decoction, warm constant extraction (Soxhlet), aqueous-alcoholic fermentation extraction, counter-current extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical liquid extraction and distillation methods (water distillation, steam distillation, phyphylaxis). Hydrolytic maceration followed by distillation, expression and effleurage (cold fat extraction) may be used for aromatic crops, hydro-water and steam distillation. Headspace trapping, strong phase micro extraction, protoplast extraction, micro distillation are some of the recent extraction techniques for aromatic plants.

The Aconitum Ferrox tuberous roots were washed, shade dried and grinded to coarse powder. Approximately 700 gm of dried powder were extracted successively with decreasing polarity range such as petroleum ether, ethyl acetate, ethanol, and water at temperature ranges between 40-60 ° C using constant heating Soxhlet apparatus. For 15 cycles, the extract was continued. The extract was finally filtered and concentrated to dry weight.

Physicochemical Investigations

Samples of plant powder of Aconitum Ferrox were subjected for determinations of physicochemical parameters such as loss on drying, ash values, pH value in 1% aqueous and methanolic extractive values were carried out according to the methods recommended by World Health Organization [9, 10]

Determination of pH range

The pH of different formulations in 1% w/v (1gm:100ml) of water soluble portions of plant powder of Aconitum Ferrox were determined using standard simple glass electrode pH meter**.**

Loss on drying / Moisture Content (Gravimetric determination)

Separately place about 1.0 gm of whole plant powder of Aconitum Ferrox in an accurately weighed moisture disc. For the estimation of loss on drying, it was dried at 105˚C for 5 hours in an oven, cooled in desiccator for 30 minutes, and weighed without delay. The loss of weight was calculated as content in mg per gram of air-dried material.

Determination of hot water and methanol-extractable matter

Separately place about 5.0 gm of whole plant powder of the Aconitum Ferrox, in an accurately weighed, glass stoppered conical flask. For the estimation of hot water-extractable matter, 100 ml of distilled water was

added to the flask and weighed to obtain the total including the flask. The contents were shaken well and allowed to stand for 1 hour. A reflux condenser was attached to the flask and boiled gently for 1 hour; cooled and weighed. The flask was readjusted to the original total weight with distilled water and it was shaken well and filtered rapidly through a dry filter. Then 25ml of the filtrate was transferred to an accurately weighed, tarred flat-bottomed dish (petri disc) and evaporated to dryness on a water bath. Finally, it was dried at 105 ˚C for 6 hours in oven, cooled in desiccator for 30 minutes, and weighed without delay. Same procedure was followed using ethanol instead of distilled water to determine extractable matter in ethanol. The extractable matter was calculated as the content of in mg per gram of air-dried material.

Determination of total ash

Two grams of the whole plant powder of Aconitum Ferrox, was placed in previously ignited (350˚C for 1 hour) and tarred crucible accurately weighed. Dried material was spread in an even layer in the crucible and material ignited by gradually increasing the heat to 550˚C for 5 hours in a muffle furnace until it is white, indicating the absence of carbon. Cooled in a desiccator and weighed. Total ash content was calculated in mg per gram of air-dried material.

Determination of acid-insoluble ash

25 ml of hydrochloric acid TS was added to the crucible containing the total ash covered with a watch-glass and boiled gently for 5 minutes. The watch-glass was rinsed with 5 ml of hot water and this liquid added to the crucible. The insoluble matter was collected on an ash less filter paper (Whatmann 41) and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred to the original crucible, ignited by gradually increasing the heat to 550˚C for 3 hours in a muffle furnace to constant weight. Allowed the residue to cool in a suitable desiccator for 30 minutes, and then weighed without delay. Acid-insoluble ash content was calculated as mg per gram of air dried material.

Determination of Water-Soluble Ash

25 ml of water was added to the crucible containing the total ash, covered with a watch glass and boiled for 5 minutes. Insoluble matter was collected on an ash less filter-paper. Washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450˚C in a muffle furnace. Allowed the residue to a cool in a suitable desiccator for 30 minutes, and then weighed without delay. The weight of the residue was subtracted in mg from the weight of total ash. Water – soluble ash content was calculated as mg per gram of air-dried material.

Determination of Sulfated Ash

Ignited suitable crucible (silica) at 550˚C to 650˚C for 30 minutes cooled the crucible in a desiccator (silica gel) and weighed it accurately. 1 gram of the plant powder of the Aconitum Ferrox, was placed in a previously dried ignited crucible, ignited gently at first, until the substance was thoroughly white. Cooled and moistened the sample with a small amount (usually 1 ml) of sulfuric acid TS, heated gently at a temperature as low as practicable until the sample thoroughly charred. After cooling, moistened the residue with small amount (usually 1 ml) of sulfuric acid, heated gently until the white fumes were no longer evolved, and ignited at 800˚C ± 25˚C until the residue is completely incinerated. Ensure the flames were not produced at any time during the procedure. Cooled the crucible in desiccators, weighed accurately. This was repeated until the sample reached a constant weight and calculated the percentage of residue.

Table 1: Percentage Yield of Different Solvent Extracts of Aconitum Ferrox

Plant Name	Extracts	Color and consistency	% Yield (w/w)
Aconitum Ferrox	Pet. Ether	Brownish yellow and sticky	1.95%
	Ethyl Acetate	Brown sticky	3.65%
	Ethanol	Brown and semisolid	7.45%
	Aqueous	Dark Brown	5.82%

Table 2: Physicochemical parameters of Aconitum Ferrox..

Sr. No.	Parameters	Pet. Ether	Ethyl Acetate	Ethanol	Aqueous
1	pH range	8.56± 0.01	6.56± 0.01	4.56± 0.01	8.16± 0.01
2	Loss on drying	7.41 ± 0.10	4.05 ± 0.10	6.25 ± 0.10	3.15 ± 0.10
3	Methanol Soluble extractive value	18.26 ±0.40	18.98 ±0.40	15.26 ±0.40	17.15 ±0.40
4	Water Soluble extractive value	29.63 ± 0.50	28.02 ± 0.50	22.63 ± 0.50	29.14 ± 0.50
5	Total Ash Value	9.62 ± 1.10	8.20 ± 1.10	5.62 ± 1.10	7.15 ± 1.10
6	Water Soluble Ash	5.15 ± 0.05	4.23 ± 0.05	2.15 ± 0.05	3.96 ± 0.05
7	Acid Soluble Ash	3.45 ± 0.20	2.36 ± 0.20	1.45 ± 0.20	2.90 ± 0.20
8	Sulphated Ash	2.12 ± 0.30	2.59 ± 0.30	1.05 ± 0.30	2.89 ± 0.30

The above extracts were undergone to identification of constituents by phytochemical tests.

Table 3: Phytochemical analysis of extracts

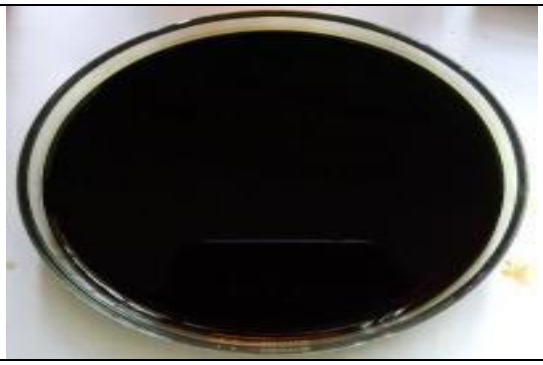
Sr. No.	Phytochemical	Name of Tests	Pet. Ether	Ethyl Acetate	Ethanol	Aqueous
1.	Alkaloids	Mayer's Test	-	-	+	-
		Wagner's Test	-	-	+	-
		Dragon draft's Test	-	-	+	-
		Hager's Test	-	-	+	-
2.	Glycoside	Modified Brontrager's Test	-	+	+	+
		Legal's Test	-	+	+	+
3	Tannins	Gelatin Test	-	-	-	-
4	Phenols	Ferric Chloride Test	-	+	+	+
5	Flavonoids	Alkaline Test	-	+	+	+
		Lead Acetate Test	-	+	+	+
6	Saponins	Froth's Test	-	-	+	+
		Foam Test	-	-	+	+
7	Steroids	Salkowaski Test	+	-	-	-
		Libermann Burchard's Test	+	-	-	-
8	Terpenoids		-	+	+	-
9	Phobatannins		-	-	-	-
10	Anthraquinones		-	-	-	-

Note: - +: Present, - : Absent

Figure 1: Aerial parts of Aconitum ferrox

Figure 2: Roots of Aconitum ferrox

Pet-Ether Extract of A.F



Ethyl Acetate extract of A.F

Ethanol Extract of A.F.

Aqueous Extract of A.F.

RESULTS & DISCUSSION

The Aconitum Ferrox tuberous roots were washed, shade dried and grinded to coarse powder. Approximately 700 gm of dried powder were extracted successively with decreasing polarity range such as petroleum ether, ethyl acetate, ethanol, and water at temperature ranges between 40-60 ° C using constant heating Soxhlet apparatus. For 15 cycles, the extract was continued. The extract was finally filtered and concentrated to dry weight.

Physicochemical Investigations

Physicochemical parameters were determined as per guidelines of WHO, air dried powdered sample of Aconitum Ferrox was subjected for determination of physicochemical parameters such as pH, Foreign organic matter, methanol soluble extractives, water soluble extractives, total ash content, acid insoluble ash, water soluble ash, loss on drying and % moisture content were determined. The average physicochemical parameters of Aconitum Ferrox are tabulated in table 2

CONCLUSION

The physico-chemical study is a major and reliable criterion of identification of plant drugs. The physic-chemical parameters are necessary for confirmation of the identity and the determination of quality and purity of the crude drugs. To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. Thus, in recent years there has been an emphasis on stardardization of medicinal plants, and evaluation of plant drugs pharmacognostical studies is still more reliable, accurate and inexpensive means. Physic-chemical studies on different plants have been done by various workers. According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is a first step towards establishing its identity and should be carried out before ant tests are undertaken.

The present work has proved that the extract of root *of Aconitum ferox* wall showed the presence of phytochemical constituents. Ethanol extract of root of *Aconitum ferox* wall are analyzed following conclusion were obtained from phytochemical study that plant contains Alkaloids, Saponins, Flavonoids, Phenol, Tannins, Terpenoid. The qualitative analysis of various extract of root of *Aconitum ferox* wall shows the presence of bioactive compounds. The results are summarized in the tables. The present study justified the claimed uses of this plant intraditional system of medicine to treat various infectious diseases caused by microbe.

The present result will form on the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds. Today, antibiotics are one of the most important weapons in fighting bacterial infections and have greatly benefited the health- related quality of human life. However, over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less and less effective not only because of many of them produce toxic reactions, but also due to drug emergence of drug resistance bacteria. Drugs derived from natural sources play a significant role in prevention treatment of human diseases. *Aconitum ferox* wall exhibited significant, antimicrobial activity and helpful as a future medicinal content.

The physico-chemical parameters help in judging the purity and quality of the drug. The powder was evaluated for its physico-chemical parameters like foreign matter, loss on drying, total ash, acid insoluble ash and different extractive values. The results of ethanolic extract of Aconitum Ferrox tuberous root were found to be satisfactory in physico-chemical parameters. The current study establishes not only physicochemical characterizations of plants but also phytochemical characters of all the plant extracts. All the plant extracts are found to be rich in flavonoid and saponins having wide spectrum of bioactivity. The plants studied here can be seen as a potential source of useful therapeutics. Further studies are going on these plant extracts in order to isolate, identify, characterize and elucidate the structures of bioactive compounds along with their pharmacological activity. In other words, the physicochemical features examined in the current study may serve as tool for identification of the plant for validation of raw material and for standardization of its formulations at herbal industrial level in coming days.

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