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Research Article

QUALITATIVE PHYTOCHEMICAL SCREENING OF *ACONITUM FEROX* ROOTS

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A B S T R A C T

Plant based medicines have been used by mankind since ancient time. Herbs are the general way to support and maintain the body's physiology. According to the report of World Health Organization (WHO), over 80 % of the world population relies on the traditional system of medicine, largely plant based, to meet their primary health care. The present research was designed to evaluate the phytochemical potential of the roots of *Aconitum ferox*. The plant was collected then identified and authenticated by a botanist Dr. Zia UlHasan at Department of Botany, Safia Science College, Bhopal (MP) with a voucher Specimen no. 316/Bot/Safia/12. It was extracted out by the soxhlet apparatus using different solvents including water and ethanol. The chemical tests were evaluated for detection of different constituents including alkaloids, glycosides, tannins, resins, steroids, carbohydrates, flavonoids, proteins and amino acids. The result demonstrated the successful presence of above constituents. The further research is suggested to isolate the chemical constituents responsible for the desired activity.

INTRODUCTION

All over the world plants were used as main sources of medicine by for human kind. The rise of modern western medicine was initially accompanied by a decline in the practice of herbalism in all cultures and we started believing that synthetic chemical were the best medicines to treat illness and cure disease [1]. Plant based medicines have been used by mankind since time immemorial. According to the report of World Health Organization (WHO), over 80 % of the world population relies on the traditional system of medicine, largely plant based, to meet their primary health care [2] Medicinal plants have been used in the form of folklore medicine or traditional medicine and ethnic medicine (Indian Herbal Pharmacopoeia, 1999). Herbs are the general way to support and tone the body's system. As with any therapy, one should work with health care provider to diagnose the problem before commencement of treatment [3].In ancient times, Vaidhya used to treat patients on individual basis, and prepare drug according to the requirement of the patient. Herbalism is a traditional medicinal or folk medicine practice based on the use of plants and plant extracts [4]. In a recent study focused on patients with Alzheimer's disease, over half of the care givers acknowledged trying at least one alternative therapy to help the patient's memory. Vitamins, health foods, home remedies, and herbal medicines were the most popular choices cited by care givers [5].

The present research was designed to evaluate the phytochemical potential of the roots of *Acconitum ferox*.

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MATERIALS AND METHODS

Collection, Identification and Authentication of the plant

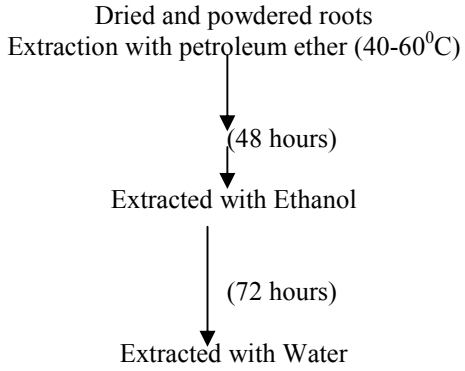
The plant was selected on the basis of morphology from Vindhya herbals BarkhedaPathani Bhopal (Madhya Pradesh). The plant was identified and authenticated by a botanist Dr. Zia UlHasan at Department of Botany, Safia Science College, Bhopal (MP) with a voucher Specimen no. 316/Bot/Safia/12.

Extraction (Soxhlet)

The roots were dried in shade. Then moderately coarse powder of the roots of *Aconitum ferox* were subjected to successive soxhlet extraction with different solvents in increasing order of polarity from nonpolar to polar. Successive soxhlet extraction was rapid and continuous and might be employed in sparingly soluble constituent due to repeated extraction, which cannot be done by either percolation or maceration methods. Due to various advantages offered by soxhlet extraction, this method was selected for present study, using different solvents-

1. The dried coarsely powdered drug was weighed 227.66 g and packed in Soxhlet apparatus and defatted with Petroleum Ether (40-60^{0}c) till complete defatted. Complete defatting ensured by placing a drop by thimble on the filter paper which did not exhibited any oily spot.
2. The defatted material was removed from the soxhlet apparatus and air dried to remove the last traces of petroleum ether. The defatted material was subjected to extraction by Ethanol as solvent by soxhlet apparatus and finally with water by maceration process. The completion of extract was confirmed by evaporating a few drops of the extract on the watch glass and ensuring that no residue remained after evaporating the solvent.

The marc extract was air dried before extracted with the next solvent. Ethanol extraction marc was dried and macerated with water for 24 h. The liquid extract was tarred conical flask. The solvent removed by distillation and last traces of solvent being removed under vacuum. The extract obtained with each solvent was weighed to a constant weight and percentage w/w basis was calculated [6].



Scheme: Successive solvent extraction procedure for *Aconitum ferox* root

Table 1 Percentage Yield

Parts	Solvent	Extract's Color	Yield (g)	% Yield
Root	Ethanol	Dark Brown	42.573	18.70
Root	Water	Brownish Black	87.31	47.51

Phytochemical's Screening Protocol

Determination of Alkaloids

Table 2 Identification test of Alkaloids

S.no.	Identification test	Procedure	Observation
1	Mayer's Test	Test solution + Mayer reagent (Potassium mercuric iodide solution)	White or yellow precipitate
2	Dragendorff's Test	Test solution + Dragendorff's reagent (Potassium iodide + bismuth nitrate)	Showed orange red precipitate
3	Wagner's Test	Test solution + Wagner's reagent (iodine solution)	Brown or reddish brown precipitate
4	Hager's Test	Test solution + Hager's reagent (saturated solution of picric acid)	Gives characteristic crystalline ppt.

Determination of Glycosides

Table 3 Identification Test for Glycosides

S.no.	Identification test	Procedure	Observation
1	Raymond's Test	Test solution + 1 ml of 50% ethanol + 0.1% solution of dinitrobenzene in ethanol + 2-3 drops of 20% sodium hydroxide solution	Appearance of violet color, which changes into violet.
2	Killer Killani Test	2 ml of extract + glacial acetic acid + one drop of 5% $FeCl_3$ + conc. H_2SO_4.	Reddish brown color appeared at the junction of the two liquid layers and upper layer appeared bluish green.

Determination of Carbohydrates

Table 4 Identification Tests for Carbohydrate

S.no.	Identification test	Procedure	Observation
1	Molisch's Test	2-3 ml. extract +few drops of α-naphthol solution (20% in ethyl alcohol) + 1 ml. conc. H_2SO_4 added along the side of the test tubes.	Violet ring was formed at the junction of two liquids.
2	Fehling's Test	Extract heated with dil. HCL +NaOH + Fehling's solution A & B	Brick red precipitate was formed
3	Benedict's Test	Extract +equal volume of Benedict's reagent. Heat for 5 min.	Solution appears Green, Yellow or Red

Determination of Tannins

Table 5 Identification Tests for Tannins

S.no.	Identification test	Procedure	Observation
1	Vanillin- HCl Test	Extract+ vanillin-HCl reagent (1 g vanillin + 10 ml. alcohol + 10 ml. conc. HCl)	Formation of pink or red color.
2	Gelatin Test	Extract solution + aqueous solution of gelatin	White buff color precipitate was formed.

Determination of Steroids

Table 6 Identification Test for Steroids

S.No.	Identification test	Procedure	Observation
1	Libermann- Burchard Test	2 ml. extract + Chloroform + 1- 2ml. acetic acid + 2 drops H_2SO_4 from the side of the test tube	First red, then blue and finally green color appeared.
2	Salkowski Reaction	2 ml. of extract +2 ml. chloroform + 2 ml. conc. H_2SO_4. Shake well.	Chloroform layer appeared red color and acid layer shows greenish fluorescence.

Determination of Proteins and Amino acids

Table 7 Identification of Proteins and Amino-acids

S.No.	Identification test	Procedure	Observation
1	Biuret Test	3 ml. of extract + 4% NaOH + 2-3 drops of 1% copper sulphate solution.	Presence of red/violet coloration
2	Precipitation test	Mix with absolute alcohol	White ppt.
3.	Ninhydrin Test	Extract + ninhydrin reagent in boiling water bath for 10 min.	Violet color appeared.

Determination of Resins

Table 8 Identification Tests for Resins

S.No.	Identification test	Procedure	Observation
1	Color detection with ferric chloride	Extract + alcohol + few drops of $FeCl_3$ solution.	Green color appears
2	Turbidity Test	Extract solution (2 g of drug in methanol) +5 ml distilled water.	Turbidity appears

Determination of Flavonoids

Table 9 Identification Tests for Flavonoids

S.no.	Identification test	Procedure	Observation
1	Lead acetate test	Filter paper strip was dipped in the alcoholic solution of extract. Ammoniated with ammonia solution	Color changed from white to orange.
2	Shinoda Test	Extract + 5 ml. 95% alcohol + few drops of conc. HCl + 0.5 g magnesium turning.	Pink color observed

RESULTS AND DISCUSSION

Dried extract wastaken for the chemical detection of the constituents. Test for Alkaloids, Flavonoids, Tannins, Sterols, Phenolic compounds, Terpenoids, Carbohydrates etc.

Table 10 Qualitative estimation of phytochemical constituents

S.no.	Chemical test	Pet Ether	Ethanol	Aqueous
1.	**Carbohydrate**			
a.	Molish test	+	-	-
b.	Fehling test	+	-	-
c.	Pholoroglucinol test	++	-	-
d.	Tollen's test	-	-	-
e.	Cobalt chloride	+	-	-
f.	Tannic acid test	-	-	-
2.	**Protein**			
a.	Biuret test	++	+	-
b.	Millon's test	-	-	-
c.	Xanthroprotic test	+	-	-
3.	**Amino Acid**			
a.	Nihydrin test	-	-	-
b.	Cysteine test	-	-	-
4.	**Fats And Oils**			
a.	Filter paper test	+	+	++
5.	**Steroid**			
a.	Salkowski reaction	+	-	-
b.	Libermann-Burchard reaction	+	+	-
c.	Libermann's reaction	+	-	-
6.	**Glycosides**			
a.	Legal's test	-	-	-
b.	Keller-Killani test	-	-	+
c.	Borntrager's test	-	-	+
d.	Foam test	-	+	+
e.	Shinoda test	-	-	++
f.	Lead acetate test	+	-	++
7.	**Alkaloids**			
a.	Dragendorff's test	++	+	+
b.	Mayer's test	+	+	-
c.	Wagner's test	-	++	++
8.	**Phenolic Compounds**			
a.	5% $FeCl_3$ solution	+	+	++
b.	Lead acetate test	+	-	++
c.	Acetic acid solution	-	+	-
9.	**Volatile oil**			
a.	Sudan red	+	-	+
10.	**Tannic**			
A	Vanilline HCL	-	+	+
B	Gelatin	-	-	-
11.	**Resin**			
A	$Fecl_3$	-	+	++

In order to detectthe various constituents present in the different extracts of *Aconitum ferox*. Phytochemical screening was revealed for the presence of Alkaloids, Glycosides, Carbohydrates, Tannins, Resins, Flavonoids, Steroids, Proteins and Amino acids.

Above table depicts the findings of various contents of the plant. According to ancient literature, they are useful in vitiated conditions of pitta, ophthalmology, pruritis, cephalgia, stomatopathy, leprosy, ulcers, fever, vomiting, hiccough, insanity and galactorrhoea [7].

Aconitum is one of the most valuable and toxic drugs. The di-ester diterpenealkaloidal nature is responsible for its toxicity. But it can be utilized safely after processing. Assessment of *Aconitum* species needs to be carried out for their safely use [8].

CONCLUSION

It is one of the most researched plants of the 21th century having a huge number of valuable chemical constituents. This study suggests the isolation of constituents of the *Aconitum ferox* roots that could be used in the treatment of numerous ailments/diseases with the advantage of easily availability and cost-effectiveness.

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