Standardization of Acconitum Ferrox

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Abstract— Aconitum Ferrox is a highly poisonous herb found in almost all countries. It is widely used in India and Nepal in treatment of neuralgia, leprosy, fevers, cholera and rheumatism. Its tuberous roots are used as drug externally and rarely administered internally. It is one among the 'Panchavisha' referred in Sanskrit texts. The main constituents responsible for toxicity are diterpenoids and alkaloids. In case of processed drug the aconite alkaloids are hydrolysed to benzyl aconine and aconine derivatives. The article is an attempt to summarise the various aspects of A.Ferrox and standardise the processed and unprocessed drug by phytochemical study.

Index Terms— *Crystillium, Dola yenta*, *Napelline, Sodhana, Vatsanabha*

1 INTRODUCTION

THERE exists a misbelief that only modern medicine has side effects and traditional Ayurvedic medicine is absolutely harmless. *A. Ferrox* belongs to Svedajanma family (Ayurvedic classification) is highly toxic drug. It is a perennial plant having tuberous roots, ovate five lobbed sharp toothed leaves, follicle fruits and black rough surfaced seeds. *Aconitum* species are the rich sources of diterpene alkaloids and flavonoids. Chinese group have reviewed the structure of diterpenoid alkaloids from *Aconitum* species [1]. It also consists of free fatty acids, polysaccharides and different classes of alkaloids [2].The acute toxicity of alcoholic extracts of *Aconitum* species tubers appeared to be directly related to the alkaloid content of the tubers. *Aconitum* species are usually used by mixing with other plants rather than a single component. The most common aconite based medicinal plant *Vatsanabha* (*A. Ferrox* Wall.) is used in Ayurveda as an antipyretic, analgesic, anti-rheumatic, appetizer and digestive. Externally, it is applied to reduce pain and inflammation. It is applied by rubbing with oil and it stimulates tip of sensory nerve fibers. In therapeutic dose, it acts as an appetizer [3], [4].

2 REVIEW OF LTERATURE

2.1 Chemical constituents

The major alkaloids present are 14-*O*-acetylsenbusin A, aconitine, pseudaconine, bikhaconitine, 14-*O*-Benzoyl-Lipopseudaconitine, Lipobikhaconitine, Lipopseudaconitine, Veratroylbikhaconine[5] , diacetyl pseudaconitine, veratry pseudaconitine[6], chamaconitine[7], aconine, pseudaconitine[8] veratryl gamma aconine, and di-Ac–Y-aconitine. The toxicity of *Aconitum* mainly derives from the diester diterpene alkaloids including aconitine, mesaconitine and hypaconitine. Processed tubers show lower level of toxic alkaloidal content

as compared to unprocessed tubers[9] .The process involves the hydrolysis of aconite alkaloids to less toxic benzyl aconine and aconine derivatives[10].

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Pharmacological studies reveal purification of aconite after *Sodhana* [11], [12]. The tuber of *Vatsanabha* contains 0.4–0.8% diterpene alkaloids and the concentration of aconite in the fresh plant is between 0.3% and 2.0% in tubers and 0.2% and 1.2% in the leaves. The highest concentration of aconite is found in the winter [13]. Aconitine and related compounds exhibit antipyretic, anti-inflammatory, analgesic and anti-rheumatic properties in experimental models and certain species of aconite have antitumor activities and regulate neurological disorders [14].

2.2 Ayurveda of A. Ferrox

Over dosage of Aconitum species results in a tingling or burning sensation in the fingers and toes first, followed by sweats and chills, a generalized paraesthesia, a feeling of roughness and dryness in the mouth, numbness and a feeling of intense cold. Later there were violent vomiting, colicky diarrhoea, skeletal muscle paralysis, cardiac rhythm disturbances and intense pain. The main causes of death in aconite poisoning were cardiovascular collapse and ventricular arrhythmias [3], [4].

In Ayurveda, the aconite toxicity is managed by giving Tankana bhasma (Borax) and ghee or mixture of turmeric juice, borax and ghee to the patient. Excessive cow milk is also drunk to patient for vomiting purpose. A strong coffee or a strong tea or tannic acid can be given to precipitate the alkaloid. A. *oreochryseum* is also used as the antidode of *Aconitum* poisoning [15]. Neermashi or its mixture with root of *Asparagus* is recommended as a preventive measure of aconite poisoning in Annapurna region [16], [17].

2.3 Studies conducted

Studies on two species of Aconitum(*A.heterophyllum Wall. ex Royle and A.balfourii Stapf*) exhibits that the quantity of atisine and toxic aconitine was also found high (0.35% & 0.27% respectively) in greenhouse than naturally grown plants (0.19% & 0.16% respectively). It was noticed that the plant height, leaf number, and average length of tubers were high in plants grown inside greenhouse in contrast to natural habitat [18].A case of hypotension and bradycardia due to aconite poisoning caused by overdosing of an Ayurvedic medicine (*Mahashankha Vati*), was studied recently. The recommended dose of aconite is only 15 mg, while in this case consumed 70mg [19]. The pharmacological activity of *A. Ferrox*, on the cardiovascular system of isolated frog studied to explain some of its reported local uses and adverse effects. *In vivo* and *in vitro* studies of the effects of A.Ferrox on the cardiovascular system of the anaesthetized frog showed positive inotropic (Positive inotropic effect) and negative chronotropic (Negative chronotropic effect) effects and half maximal effective concentration (EC50) value has been calculated on its heart [20].

3 MATERIALS AND METHODS

3.1 Materials

The drug is obtained from the local Ayurvedic medical shop at Thiruvananthapuram as raw tuberous roots and identified by Dr.L.G.Radhika, Senior Research Officer, Drug Standardisation Unit, Government Ayurveda College, Thiruvananthapuram, Kerala, India. The drug is processed and purified under the guidance of Dr. Meena.C.V, Research Officer, Drug Standardisation Unit, Government Ayurveda College, Thiruvananthapuram, Kerala, India.

3.2 Purification

Using cow's urine- The crushed drug washed in pure water is soaked in cow's urine kept under sunlight for 3 consecutive days. The cow's urine has to be replaced every day carefully without wastage of drug. In sunny days cow's urine may get evaporated hence make sure that the drug is fully soaked in cow's urine. After 3 days the drug is separated from it and washed in boiled water.

Using milk-The "dola yantra" Technique is used. The drug wrapped in a clean cloth in the form of "kizhi" is tied on a small stick such that it touches the surface of milk taken in a pot. The pot having holes on opposite side is more useful as the stick can be placed through the hole so that the pot can be closed using earthen lid. The pot is maintained at low flame for 3 hours. The milk is poured frequently so as to maintain the contact between milk and drug in kizhi. After 3 hours the drug is separated from it and washed in boiled water

3.3 Moisture Content

Moisture content is determined by Dean and Stark's apparatus.10g of powdered drug in an R.B flask and adds Xylene to cover the drug. The DS apparatus fixed on a R.B flask is connected to water condenser.It is heated in an electric mantle for at least. 1 hr. The moisture in the drug gets evaporated and gets condensed, collected in the graduated tube of the apparatus. The heating is continued till the water level remains constant. Note the level and percentage of moisture content can be calculated using the formulae.

Percentage of moisture content= (Volume)*100/Weight of the drug

3.4 Volatile oil content

The volatile content is determined by distilling the drug with distilled water. Oil content is determined by Clevenger apparatus. 10g of powdered drug in an R.B flask and add 100 ml distilled water to the drug. Connect the R.B flask to Clevenger apparatus which is connected to water condenser. It is heated in an electric mantle for at least 1 hr. The distillate is automatically separated and returned to the distilling flask, collected in the graduated tube of the apparatus. Note the level and percentage of oil content can be calculated using the formulae.

Percentage of oil content= (Volume)*100/Weight of the drug

3.5 Total ash

The residue remaining after incineration is the ash content which simply represents inorganic salts naturally occurring in the drug or adhering to it or deliberately added to it as form adulteration. The drugs are absorbed with sand, oil, chalk powder etc. Ash value is a criterion to judge the identity or purity of crude drugs. Total ash usually contains carbonates, phosphates, silicates etc. Incinerate about 2g of the accurately weighed powdered drug in a previously weighed crucible until free from Carbon. Cool and weight the crucible. The heating and cooling is continued to get concordant values. The percentage of ash with reference to the drug is calculated.

3.5.1 Acid insoluble ash

Boil the ash for 5min with 25 ml of 2N HCl. Collect the insoluble matter on an ash less filter paper. Wash it with hot water for several times until free from chloride and ignite to constant weight. The heating and cooling is continued to get concordant values. The percentage of acid insoluble ash with reference to the drug is calculated.

3.5.2 Water insoluble ash

Boil the ash for 5 min with 25 ml of distilled water.Proceed as above. Here we get the percentage of water insoluble ash with reference to the drug is calculated.

3.6 Extractives

3.6.1 Water soluble extractives

2.5g of the dried sample is weighed accurately and transfer it into R.B flask. Add 50 ml chloroform water and keep it for 24 hours with occasional shaking. Filter and collect the filtrate in a clean previously weighed beaker. Evaporate to dryness. Heating and weighing is continued to get constant weight. Calculate the percentage of water soluble extract with reference of the air dried drug.

3.6.2 Alcohol soluble extractives

0.5g of the dried sample is weighed accurately and transfers it into R.B flask. Add 10 ml alcohol and keep it for 24 hours with occasional shaking. Proceed as above. Here we get the percentage of water soluble extract with reference of the air dried drug.

3.7 Fiber content

Weigh accurately 3g of the drug and transfer to thimble and to a Soxhlet apparatus and extracted with petroleum ether. When the extraction was completed, the drug was transferred to a dry 500 ml beaker. Add 200 ml 0.255 N H_2SO_4. Put a glass rod and cover with a watch glass boiled for 30 minutes. After boiling, filter the solution with ordinary filter paper in a 500 ml conical flask. Wash with hot water until it is free from sulphate ions. Then transfer the residue into a 500ml beaker. Add 200 ml 0.313 N NaOH solution into the residue, put a glass rod and cover with a watch glass. Boiled the so-

lution for 30 minutes then filter the solution through a Budhner funnel with suction by using weighed Whattmann no: 41 filter paper. The residue was washed with hot water until it is free from alkali. Dry the residue at 100°C placing the funnel in the oven. The transferred the filter paper with residue into a crucible and weighed it. Heat to ash and also take the weight. Difference in weight gives the weight of residue and found the percentage.

Percentage of fiber content= (Volume)*100/Weight of the drug

3.8 Sugar content

Reducing sugar reduces Fehling's solution to cuprous oxide (Cu_2O).

$$2KMnO_4+3\ H2SO_4 \longrightarrow K_2SO_4+MnSO_4+3H_2O+5(O)$$

$$Cu_2O+Fe_2(SO_4)_3 + H2SO_4 \longrightarrow 2CuSO_4+FeSO_4+H_2O$$

$$2FeSO_4 + H2SO_4 + (O) \longrightarrow Fe_2(SO_4)_3 +H_2O$$

5g of the dried sample is weighed accurately and transfer it into a R.B flask. Add 100 ml distilled water and reflux for 1 hr. After cooling the solution filtered it into a conical flask and 2 ml of lead acetate solution is added. Filter the mixture into a conical flask using Whattmann no: 41 filter paper. Add one spatula of sodium oxalate and again filtered into another conical flask. A slight excess of sodium oxalate is added to remove the excess lead acetate. Collect the filtrate quantitatively.

3.8.1 To find reducing sugar content

20 ml of the filtered solution is pipetted into a 250 ml beaker. To this solution added 50ml Fehling's solution, prepared by mixing 25 ml Fehling's A and Fehling's B and 30 ml DW. The solution is then heated to boiling, by keeping the beaker covered with watch glass. The hot solution is filtered through a sintered G_4 crucible. The precipitated cuprous oxide is collected. The filtering flask is washed with water and the crucible with the adapter is attached in the flask. A hot solution of 25 ml ferric alum and 20 ml 4N sulphuric acid are added into the G4 crucible, the crucible is washed with DW and the washings were collected along the filtrate. The reducing solution is titrated against standardized $KMnO_4$ solution. From the titre value, the weight of cuprous oxide can be calculated and the actual weight of the reducing sugar can be obtained from the table of standard value.

3.8.2 To find total sugar content

20 ml of the filtered solution is pipetted into a 250 ml beaker and is hydrolyzed by adding 2ml of 6N HCl. Boiled for 5 minutes. Cooled and neutralized with anhydrous Na_2CO_3. The experiment is proceede as above.

3.8.3 To find non-reducing sugar

The non-reducing sugar is obtained by subtracting reducing sugar from the total sugar.

3.9 Qualitative Chemical Analysis

10g of accurately weighed drug taken in a R.B flask and add alcohol to cover the drug. Connect the R.B flask to water condenser. It is heated in an electric mantle for at least 1 hr. The content in the R.B flask is filtered to conical flask and the filtrate is used as extract of the drug for qualitative chemical analysis.

3.9.1 Detection of steroids (Liberman-Burchard Test)

The steroids are determined by evaporating the extract in a watch glass and to the residue added acetic anhydride and concentrated H_2SO_4 acid through the sides. A play of colors from yellow, green, brown to black indicates the presence of steroids.

3.9.2 Detection of phenols

The residue of the extract is dissolved in alcohols and added neutral ferric chloride. A deep blue or violet color indicates the presence of phenolic compounds.

3.9.3 Detection of flavonoids

The residue of the extract is dissolved in alcohol. Magnesium ribbon and con.HCl are added to it. A reddish brown, magenta or pink color indicates the presence of flavonoids.

3.9.4 Detection of alkaloids

The residue of the extract is stirred with a few drops of dil.HCl and filtered. To filtrate added Mayer's reagent. A white precipitate indicates the presence of alkaloids.

With the Drangedroff's reagent it gives an orange brown precipitate and with Wagener's reagent gives reddish brown precipitate.

3.10 TLC and AAS

Thin Layer Chromatography [21] (TLC) and Atomic Absorption Spectroscopy [22] (AAS) were also conducted. TLC carried out with Toluene-Butanol (1:1) Hexane-Butanol (4:1) Toluene- Acetic acid (7:3) Chloroform –Methanol (9.5:0.5). AAS were studied for Zinc, Copper and Nickel.

4 RESULTS

4.1 Phytochemical Parameters

Phytochemical parameter	Raw Drug(Unprocessed)	Purified with cow's urine	Purified with cow's milk
Appearance and Smell	Brownish colour with intolerable 'Ruksha' smell	Brownish colour with smell of cow's urine	Blackish colour with smell of milk
Moisture Content	15.99%	21.98%	23.89%
Volatile Oil	-	-	-
Water Soluble Extractive	21.96%	13.21%	12.40%
Alcohol Soluble Extractive	4.66%	5.34%	5.12%
Total ash	2.285%	3.100%	2.765%
Acid insoluble ash	0.86%	0.57%	0.56%
Water insoluble ash	1.46%	1.28%	1.69%
Fibre content	4.56%	4.55%	4.49%
Reducing sugar	2.99	0.54	5.74
Total sugar	3.51	0.82	6.63
Non-reducing sugar	0.52	0.28	0.89

Table 1. Phytochemical parameters of processed and unprocessed *A.Ferrox*

All the data obtained from the phytochemical analysis is summarized in the table given above. This is done in order to compare the various factors at a glance.

4.2 Qualitative Chemical Analysis

	Raw Drug (Unprocessed)	Purified with cow's urine	Purified with cow's milk
Test for steroids	Presence	Presence	Presence
Test for alkaloids	Presence	Traces	Traces
Test for phenols	Absence	Absence	Absence
Test for flavonoids	Absence	Absence	Absence

Table 2. Qualitative Analysis result of processed and unprocessed *A.Ferrox*

4.3 Concentration of Metals –AAS analysis

Drug	Raw Drug(Unprocessed)	Purified with cow's urine	Purified with cow's milk
Conc. of Zinc (ppm)	0.4398	0.2293	0.0820
Conc. of Copper (ppm)	0.4931	0.2466	0.0604
Conc. of Nickel (ppm)	0.5471	0.3275	0.6768

Table 3. AAS Analysis result of processed and unprocessed *A.Ferrox*

4.4 TLC Analysis

TLC analysis was carried out with both Iodine chamber and under U.V light.

Table 3. R_f value obtained from processed and unprocessed

System	Drug	U.V	I_2 chamber	R_f value (Retention factor)
Toluene-Butanol (1:1)	Raw Drug(Unprocessed)	Green	-	0.2100
		-	Brown	0.8403
	Purified with cow's urine	-	Brown	0.8403
	Purified with cow's milk	-	Brown	0.8403
Hexane-Butanol (4:1)	Raw Drug(Unprocessed)	Green	-	0.7692
		-	Brown	0.0982
	Purified with cow's urine	-	Brown	0.0982
	Purified with cow's milk	-	Brown	0.0982
Toluene- Acetic acid (7:3)	Raw Drug(Unprocessed)	Blue	-	0.8691
		-	Brown	0.3268
	Purified with cow's urine	-	-	-
	Purified with cow's milk	Blue	-	0.8691
		-	Brown	0.3268
Chloroform –Methanol (9.5:0.5)	Raw Drug(Unprocessed)	-	Brown	0.7589
		-	Brown	0.6250
		-	Brown	0.3571
	Purified with cow's urine	-	Brown	0.6250
	Purified with cow's milk	-	Brown	0.6250

A.Ferrox

5 DISCUSSIONS

5.1 Phytochemical Parameters

Moisture content of raw material increased when purified by cow's urine and cow's milk. The excess of water content absorbed to the tuberous root is from purifying medium, as roots are the highly water absorbing part of a plant. **Volatile oil** was not present in the samples. Comparing the values obtained **water soluble extractives** decreased while **alcohol soluble extracts** increased during the purification process. Water soluble extractive mainly represents the percentage of organic constituents such as tannins, sugar, plant acids, mucilage and glycosides.

The ash mainly represents the inorganic salt present in the drug. Ash value regarded as general criteria to ascertain purity of any drug. **Acid insoluble ash**, mainly gives the percentages of sand and impurities that remain insoluble in dil. HCl and it was found to be 0.86, 0.57, and 0.56 respectively. Lower the value of acid insoluble ash, the higher the purity. **Water insoluble ash** mainly gives the percentage of organic matter present in ash and it is found to be 1.46, 1.28, and 1.369 respectively.

The **fiber content** values of the samples are given in the above table. Fiber content remains more or less same after purification. **Total sugar** is the sum total of reducing and non-reducing sugar in a plant part. It was found to be 3.51, 0.82 and 6.63 respectively for the three samples. The total sugar in sample purified by cow's milk is increased while cow's urine reduces the sugar content. The reducing sugar was found to be 2.99, 5.74 and non- reducing sugar 0.52, 0.28 and 0.89 respectively.

5.2 Qualitative Chemical Analysis

The steroid is present in all the three samples more or less similar. But it is clear that the amount of alkaloid is varying, as it is evident from the color of the precipitate obtained by Drangedroff's reagent. It is also clear that it does not contain any phenols and flavonoids.

5.3 Concentration of Metals –AAS analysis

The ash mainly represents the inorganic salt present in the drug. Ash value regarded as general criteria to ascertain purity of any drug. Acid insoluble ash, mainly gives the percentages of sand and impurities that remain insoluble in dil. HCl and it was found to be 0.86, 0.57, and 0.56 respectively. Lower the value of acid insoluble ash, the higher the purity. Water insoluble ash mainly gives the percentage of organic matter present in ash and it is found to be 1.46, 1.28, and 1.369 respectively.

5.4 TLC Analysis

From the overall analysis of TLC unprocessed drug produces a green spot under U.V light in Toluene- Butanol (1:1) and Hexane- Butanol (4:1) system. A blue spot is found for unprocessed drug and drug purified with cow's milk in Toluene –Acetic acid (7:3) system while no spot under U.V light for Chloroform – Methanol (9.5:05) systems. The 3 samples gave spots with same R_f value in Iodine showing the presence of single component unchanged during purification in Toluene-Butanol (1:1), Hexane – Butanol (4:1), Toluene-Acetic (7:3) system. Toluene-Butanol (1:1) system gave the R_f value 0.8403 and Toluene- HAc (7:3) system gave 0.3268.During the TLC analysis in the system Chloroform-Methanol (9.5:0.5) there was no spot in U.V light but gave 3 different spots in Iodine for unprocessed drug and 1 spot each for both processed drug having the R_f value 0.6250. All the three samples gave same spot at R_f value 0.6250 along with two additional spots at 0.7589, 0.3571.

6 CONCLUSIONS

For any medicine to be effective, the authenticity of the drug that goes into preparations has to be properly identified. This article is a serious effort to characterise the drug chemically. The phytochemical parameters and TLC analysis obtained from this study could be used effectively for the identification of the drug. Here it is evident that during Shodhana,(1) elimination of harmful matter from the drug,(2) addition of useful matter to the drug, (3)modification of undesirable properties of the drug, (4)enhancement of therapeutically action of the drug. Though more work has to be carried out and more specific and decisive parameters obtained from sophisticated techniques, according to the principle that a standard fixed should be that followed easily in any simple lab, the values presented here would support further investigations of *A.Ferrox*.

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