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Research Paper Ayuveda

Scientific Evalution of Sodhana (Purification) process of Kupilu (Strychnos nux vomica) & its standardization

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KEYWORDS :

The quality assurances of traditional remedies rely upon good manufacturing practices with adequate batch analysis and standardized methods of preparation. It is essential to fix some standards for standardization of drug, so that gentility of the drug is established. Each and every drug has got its own physical and chemical characteristics which help for separating it from other closely related drug. Hence Pharmacognostical studies & Physiochemical studies of a particular sample by making use of various parameters help in standardizing the drug and authenticate it.

Standardization of any Ayurvedic drug depends specially on following three points:

- Standardization of Single drug
- Standardization of Processing
- Standardization of Final product
- It is very difficult task to standardize any drug in all above mentioned steps, because in Ayurveda there are various literatures from different time period which shows different opinion regarding particular drug, on its identification, processing & final product.
- We need to oversee this diversity in literature & make a uniform standard. which is beneficial for the successes of the AYUSH drugs (Ayurveda).

Kupilu is a drug which needed Sodhana (purification) before any therapeutic use, as it has chemical impurities.

There is various process of Sodhana given by the different Acharyas

गवां मूत्रे कुपीलुं तु स्थाप्येत् सप्तरात्रकम्।
तत् उद्धत्य गोदुग्धे दोलायन्त्रे विपाचयेत्।।
याममात्रं ततः कृत्वा त्वगंकुर विवर्जितम्।
नीरेण क्षालयित्वाऽथ रसयोगेशु योजयेत्।।
(रसामृत– परिषिश्ट–8)

A reference from Rasāitam Appendix 8, page 285 was selected. This reference is also quoted in "Āyurvedic Formulatory of India"

Selection of drug-:
Kupīlu's healthy seeds are taken without any pest's infection;that must have some weight, smooth & not too old.

Instruments- A Mortar and Pestle, Dolāyantra, Container.

Material used -: Gaumutra, Gaudugdha, dry healthy Kupīlu's seeds

Quantity of material taken-:

- Weight of Kupīlu's seeds - 2.25 Kg.
- Fresh Gaumutra per day- 3 lit.
- Gaudugdha - 5lit

1st Step - Kupīlu's seeds should be kept in Gaumutra (Cow's urine) for seven nights. The Gaumutra (Cow's urine) is changed every day. Its colour, pH & sp gravity changes are monitored everyday.

2nd Step- Kupīlu's seeds are then removed from Gaumūtra (Cow's urine) & subjected to swedana with Gaudugdha (Cow's milk) by Dolā yantra method for one Yāma (3 hours).

3rd Step-Dehusked the Kupīlu's seeds outer covering & inside Ankura (Sprout). Than washed with water & powdered it.

Grinding of the Śodhita Kupīlu's seeds:

Kupīlu's seeds are too hard & sticky to grind. So after Śodhana it is necessary to grind it immediately, because after Śodhana it become soft. Practically this difficulty is also faced, in the current work.

4th Step -After Grinding of the wet seeds of Kupilu, the powder is dried & filled in to the 100 mg. empty capsules.

Changes in drug weight after different steps of the process-:

Initial weight of the drug	After Śodhana in Gaumutra	After Śodhana in Gaudugdha	After dehusking	after grinding & dry powder
2.25 kg	3.56 kg	3.00 kg	2.00 kg	1.5 kg

Analytical data of Pure & Impure Kupīlu Seeds:

Physicochemical tests	Data of Pure Kupīlu seeds	Data of Impure Kupīlu seeds	Standard (AyurvedicPharmacopiea of India)
Water soluble extractive	12%	15.2%	Not less than 12%
Alcohol soluble extractive	12.53%	8.4%	Not less than 4%
Ether soluble extractive	0.36%	0.52%	Not mentioned
Test for Iron	0.298%	0.9306	Not mentioned
Test for Nickel	0.0908%	0.0183%	Not mentioned
Moisture content	13.4%	10%	Not mentioned
pH Value	6.89 (1:5)	6.95 (1:5)	Not mentioned
Foreign matter	0%	0%	Not more than 1%
Total Ash	0.23%	0.51%	Not more than 2%
Acid insoluble ash	0.05%	0.09%	Not more than .2%

Thin Layer Chromatographic Study:
The three sample extracts are taken:

- Alcoholic extract of pure Kupīlu
- Alcoholic extract of impure Kupīlu
- Strychnine as a standard

1. In Tolulene: Ethyl-acetate: Di-ethyl-amine = 7 : 2 : 1:
At the site of alcoholic extract of Impure Kupīlu, two prominent orange spots are visualized by Ultra-violet chamber & Dragendorff reagent-:
(Rf value = *Distance of each spot/ total distance travelled by the front of mobile phase)*.
Total distance travelled by the front of mobile phase=16.2 cm

SN	Spots	Distance of the spots	Rf value
1	1st spot	72 cm	7.2/16.2= 0.44
2	2nd spot	10.2 cm	10.2/16.2= 0.63

- The alkaloid "Strychnine" sample shown Rf similar to 2nd spot Rf of Impure Kupīlu i.e. 0.63.
- In Alcoholic extract of Pure Kupīlu no prominent spot is seen.

2. In Chloroform Methanol solution

one prominent orange spot is seen, at the site of alcoholic extract of Impure Kupīlu & the alkaloid "Strychnine" sample.

Distance of the spot = 11.5 cm.

Total distance travelled by the front of mobile phase= 16.2 cm

Rf value of the spot = 11.5/16.2= 0.71

In Alcoholic extract of pure Kupīlu no prominent spot is seen

HPTLC (High Performance Thin-Layer Chromatography):

Samples:

- Pure Kupīlu (Visamusti pure)
- Impure Kupīlu (Visamusti Impure)
- Seed bark of Kupīlu (Visamusti Bk)
- Strychnine (Standard)

Rf of strychnine is 0.44; size 1.0; deviation 10.0% conc- 1mg/ml.

Bands are formed by "Camag Linomat 5" with "Nitrogen Cylinder"

There are 12 Tracks in different appl. Volume i.e.-:

Track 1	Visamusti pure	5µl
Track 2	Standard	1µl
Track 3	Visamusti Impure	5µl
Track 4	Visamusti Bk	5µl
Track 5	Visamusti pure	7µl
Track 6	Standard	2µl
Track 7	Visamusti Impure	7µl
Track 8	Visamusti Bk	7µl
Track 9	Visamusti pure	9µl
Track 10	Standard	2µl
Track 11	Visamusti Impure	9µl
Track 12	Visamusti Bk	9µl

Mobile Phase : Tolulene: Ethyl-acetate: Di-ethyl-amine = 7 : 2 : 1

After dip in the mobile phase separation is done.

Quantitative Analysis:

- In above 12 tracks different concentration of extract is taken in three ways. At track 1, 3,4 there is 5µl conc. & for Strychnine 1µl. where as in track 5,7,8 there is 7µl conc. & for Strychnine 2µl. In 9, 11, 12 track there is 9µl conc. & for Strychnine 2µl.
- The graph of track 9,10,11,12 is shown, where in all 4 samples Strychnine graph is seen. Where as in track 1 & 5 which are the tracks of Pure Kupīlu no strychnine graph is seen.
- **Tracks of Pure Kupīlu extracts:**

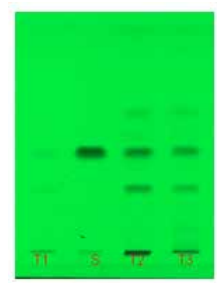
In track 1 (Pure Kupīlu= 5µl) there is no Rf with respect no Strychnine. In track 5 (Pure Kupīlu=7 µl) Height is 69.11.& in track 9 (Pure Kupīlu= 9µl) height is 92.06.

- **Tracks of Impure Kupīlu extracts:**

Track 3, 7, 11 are the tracks of Impure, where height are respectively 340.05, 444.02, 514.02.

- **Tracks of Kupīlu seed Bark extract:**

Track 4,8,12 are the track of Kupīlu seed bark, where height are respectively 238.14, 319.88, 387.49.



UV-254nm Spray-Dragendorff's Reagent
(* T1 = pure Kupīlu extract, S= Strychnine,

*T2 =Impure Kupīlu extract, *T3 = Seed Bark)

Conclusion-

- In Purification Gaumutra & Gaudugdha is taken & found that the pH & colour of Gaumutra is changed every day comes to constant value at last day (7th day).
- The grinding Kupīlu is too difficult.
- Physiochemically it is found that pH, Total ash, acid insoluble ash, water soluble extractive, ether soluble extractive, iron & nickel of pure Kupilu seeds is decreased than impure.
- The moisture content alcohol soluble ash is increased than impure Kupīlu seeds.
- TLC is done in Tolulene: Ethyl-acetate: Di-ethyl-amine = 7 : 2 : 1 mobile phase where Rf of impure Kupīlu is 0.44, 0.63 which are similar to Rf of standard mentioned in API. In Impure Kupīlu there is no spot is seen.
- By HPTLC presence of strychnine in pure, impure & seed bark of Kupīlu is determined.
- The impure & seed bark contains more strychnine than pure Kupīlu.