

QUALITY CONTROL OF CRUDE DRUGS

Chemical Evaluation



By

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CHEMICAL EVALUATION

- It comprises of **different chemical tests and assays**.
- Isolation, purification and identification of active constituents are chemical methods of evaluation.
 - **Resin:** Sulphated ash, acid value
 - **Balsam:** Acid, saponification and ester values
 - **Volatile oil:** Acetyl and ester values
- Preliminary phytochemical investigation is a part of chemical evaluation.
- Qualitative chemical tests are useful in detection of adulteration.



QUALITATIVE CHEMICAL TESTS

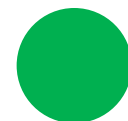
○ Tests for Alkaloids

Dragendorff's test (Potassium bismuth iodide solution): Orange red colour precipitate

Mayer's test (Potassium mercuric iodide reagent): Cream colour precipitate

Wagner's test (Iodine-potassium iodine): Reddish-brown precipitate

Hager's reagent (Saturated solution of iodine): Yellow precipitate



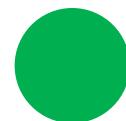
- **Tests for tannins**

- **Ferric chloride reagent**

A 5% w/v solution of ferric chloride in 90% alcohol was prepared. Few drops of this solution were added to a little of the drug filtrate. If **dark green or deep blue** colour is obtained, tannins are present.

- **Lead acetate test**

A 10% w/v solution of basic lead acetate in distilled water was added to the test filtrate. If **precipitate** is obtained, tannins are present.



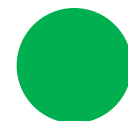
- **Tests for flavonoids (Shinoda test)**

A small quantity to test residue was dissolved in 5 ml ethanol (95% v/v) and reacted with few drops of concentrated hydrochloric acid and 0.5 g of magnesium metal. The **pink, crimson or magenta colour** is developed within a minute or two, if flavonoids are present.

- **Tests for amino acids**

- **Ninhydrin test**

The Ninhydrin reagent is 0.1% w/v solution of ninhydrine in n-butanol. A little of this reagent was added to the test extract. A **violet or purple** colour is developed if amino acids are present.



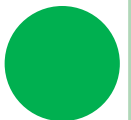
- **Tests for proteins**

- **Biuret test:**

A few mg of the residue was taken in water and 1ml of 4% sodium hydroxide solution was added to it. A drop of 1% solution of copper sulphate followed this. **Violet or pink** colour is formed if proteins are present.

- **Xanthoproteic test**

A little residue was taken with 2 ml of water and 0.5 ml of concentrated nitric acid was added to it. **Yellow colour** is obtained if proteins are present.



- **Tests for sugars**
- **Fehling's solution test**

The Fehling's solution was prepared as follows:

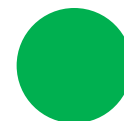
Solution A:

- Copper sulphate - 34.64 g.
- Sulphuric acid - 0.5 ml
- Distilled water to - 500 ml

Sodium B:

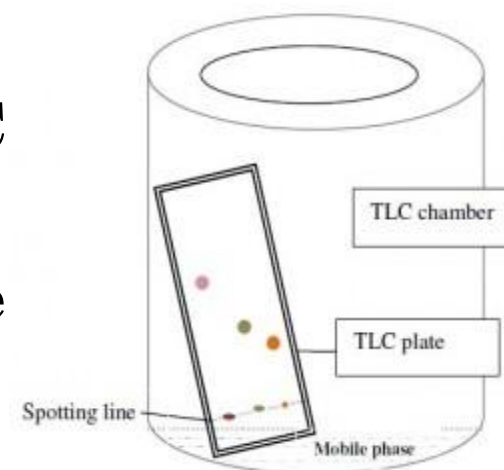
- Sodium potassium tartarate - 176 g
- Sodium hydroxide - 77 g
- Distilled water to - 500 ml

- The two solutions were mixed in equal volumes immediately before use. Drug is treated with mixture and warmed. If a **red precipitate** of cuprous oxide is obtained, reducing sugars are present.



THIN LAYER CHROMATOGRAPHY (TLC)

- Based on adsorption chromatography
- Adsorbent: Silica gel G (3 mm thick)
- Activation: Heating the plate at 105°C for 30 minutes in an oven
- Solvent system: Depend on the nature of chemical constituents.
- For volatile oil
Toluene: ethyl acetate (97:3)



$$R_f = \frac{\text{Distance travelled by component}}{\text{Solvent front}}$$



HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

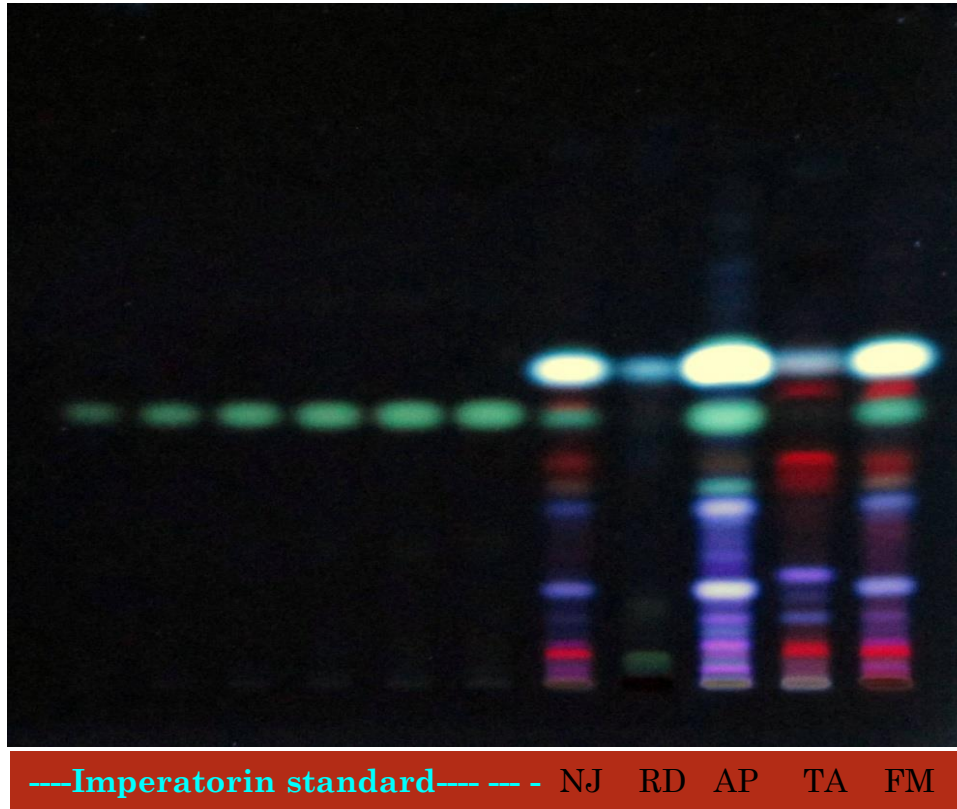
CAMAG HPTLC

HPTLC plate: silica gel G F₂₅₄

Sample, 10 mg/ml; Standard, 1 mg/ml;

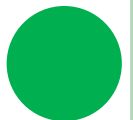
Mobile phase: toluene - ethyl acetate, (90:10)

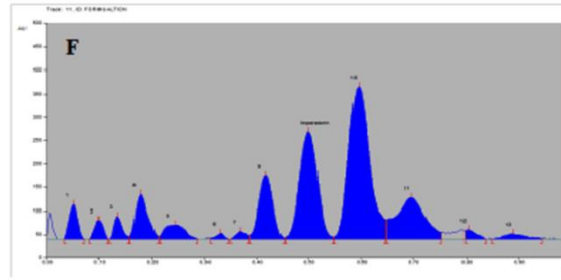
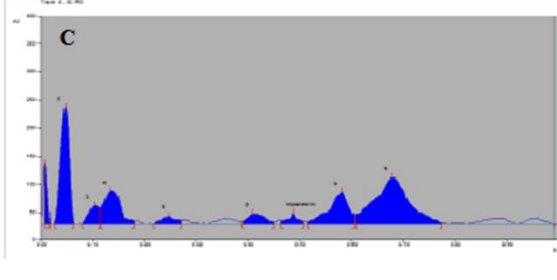
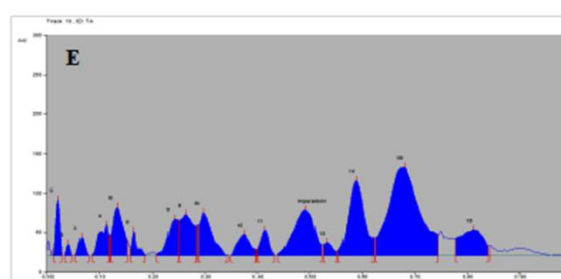
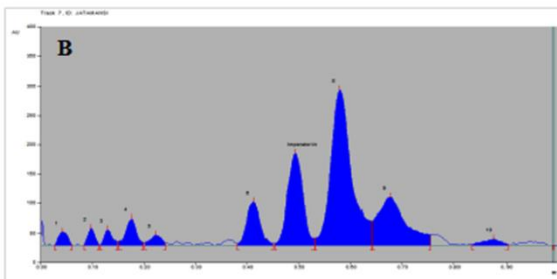
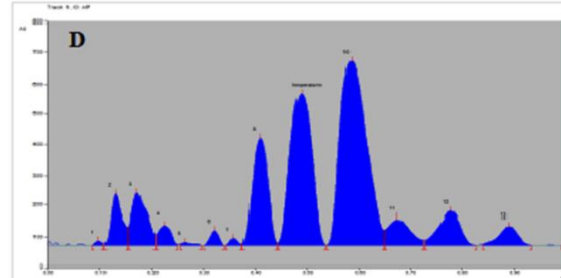
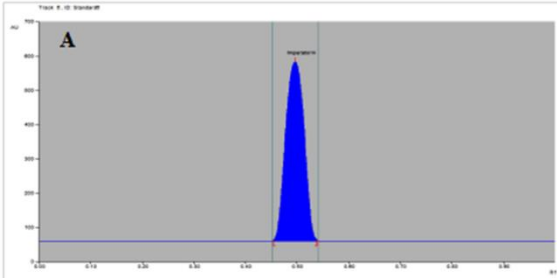
Scanning: 313 nm



Imperatorin standard from Sigma Aldrich, New Delhi, India.

Naquvi *et al.*, *Asian Journal of Traditional Medicines*, 2014, 9(1): 20-30.





HPTLC chromatogram of

(A) Standard imperatorin,

(B) Methanolic extract NJ,

(C) Methanolic extract of RD,

(D) Methanolic extract of AP,

(E) Methanolic extract of TA and

(F) Safoof-e-Muhazzil

Imperatorin content in the samples and formulation

Test samples	Imperatorin content (Mean \pm SD, % w/w)
<i>N. jatamansi</i> DC. (NJ)	1.58 \pm 0.03
<i>Rosa damascena</i> Mill. (RD)	0.44 \pm 0.03
<i>Apium graveolens</i> L. (AG)	3.05 \pm 0.02
<i>Trachyspermum ammi</i> L. (TA)	1.23 \pm 0.01
<i>Safoof-e-Muhazzil</i>	2.16 \pm 0.02

OTHER ANALYTICAL TECHNIQUES

- High Performance Liquid Chromatography, **HPLC**
- Gas Liquid Chromatography, **GLC**
- Column Chromatography, **CC**
- Ultra-violet and Visible spectrophotometry
- Infra-Red Spectroscopy, **IR**
- Nuclear Magnetic Resonance Spectroscopy, **NMR**
- Mass Spectroscopy



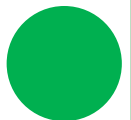
HEAVY METAL ANALYSIS

- The medicinal plant materials can be contaminated with **Arsenic, Lead, Mercury, and Cadmium**. As these components even in trace amounts are dangerous, they have to be removed from the herbal drugs. The heavy metals are analyzed by using technique of **Atomic Absorption Spectrophotometry**.
- Limit for Arsenic (As): 10 mg/ kg (= 10.0 ppm)
- Limit for Cadmium (Cd): 0.3 mg/ kg (= 0.3 ppm)
- Limit for Lead (Pb): 10 mg/ kg (= 10.0 ppm)
- Limit for Mercury (Hg): 1 mg/ kg (= 1.0 ppm)



DETERMINATION OF AFLATOXINS

- Aflatoxins are the mycotoxin from *Aspergillus flavus* and *Aspergillus parasiticus* having the chemical formula $C_{17}H_{12}O_6$. This is predicted to cause **hepatic carcinoma** in human beings. The plant species may be contaminated with this toxin.
- The test for aflatoxins as prescribed by WHO for the herbal drugs is designed to detect the presence of B_1 , B_2 , G_1 , and G_2 . The method used to detect is presence is simple TLC developments. The presence of aflatoxins is detected by **blue fluorescence** spot detected in UV light at 365 nm.



PESTICIDES RESIDUES

- Pesticides are simple substances or mixtures used to eliminate undesirable vegetable and animal life in agricultural and urban ecosystems.
- Pesticides can be **classified** according to their chemical composition, function and mode of action in organisms.
- **Chlorinated hydrocarbons and related pesticides:** Aldrin, BHC, DDT, DDD, DDE, Endrin, Lindane etc.
- **Chlorinated phenoxyalkanoic acid herbicides:** 2,4- D & 2,4,5- T.
- **Organophosphorous pesticides:** Malathion, Carbophenothion, Parathion etc.
- **Carbonate insecticides:** Carbaryl (Carbaril).
- Chromatography (mostly **column and gas**) is recommended as the principal method for the determination of pesticides residues.



THANK YOU

