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CHEMOPROFILING OF HOMOEOPATHIC DRUG *COFFEA CRUDA*

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ABSTRACT
Chemoprofiling of homoeopathic drug/tincture (HT) represents a comprehensive approach for evaluation of quality, purity, safety and efficacy of HT. According to the phytoequivalence concept the full HT can be seen as the active compound, because the several constituents act together being responsible for its therapeutic effect. *Coffea arabica* (Coffee) is the healthiest beverages in the world is commonly called *coffea cruda* in homoeopathic system of medicine and frequently used in the treatment of insomnia, headaches, toothaches and labor pains. The main active constituents of *Coffea cruda* are caffeine, trigonelline, chlorogenic acid, quinic acid, tannic acid, tannin, fixed oil and proteins. Present study reveals the moisture content (3.683 %), Total Ash (2.66 %), Alcohol (6.48 %), Water extractive values (21.69 %), total solids (0.48 %), Wt/ml (0.82 g) and Alcohol content (90.0 %). In UV spectroscopy λmax. at 280 nm shows presence of caffeine in HT. HPTLC analysis of in house HT, caffeine standard and three market sample were performed by using chloroform methanol (9:1, v/v) as mobile phase. Under UV light (254 nm), spot of caffeine was observed at Rf. 0.56 in above samples. However excess amount of caffeine content found in In-house HT rather than the market samples.

KEYWORDS: *Coffea arabica*, Homoeopathic drug, Chemoprofiling, H.P.T.L.C fingerprint.

INTRODUCTION
The coffee tree belongs to the Rubiaceae family, genus Coffea. Although more than 80 coffee species have been identified worldwide.[1] *Coffea arabica*, native of Ethiopia[2], is known to be as one of the most important crops worldwide. In India it is cultivated Andhra Pradesh, Karnataka, Kerala and Tamil Nadu like states.

Coffea arabica (Coffee) is the healthiest beverage in the world and is commonly called *Coffea cruda* in homoeopathic system of medicine. The Main active constituents of *Coffea cruda* are caffeine, trigonelline, chlorogenic acid, quinic acid, citric acid, phenols, acetylaldehyde, malic acid ,tannic acid, tannin, potassium, fixed oil and proteins. Due to rich in active constituents it shows antioxidant[3], antiviral, antibacterial and anti-inflammatory like properties and used for the treatments of stress-related symptoms: hypersensitivity and nervous piercing etc. It is also commonly used for headaches, insomnia (sleeplessness due to an overactive mind)[4-7], used in the treatment of excessive pain, such as toothaches, labor pains and for skin wound healing treatments. Different parts of coffee plants have been used for therapeutic purpose, In Homeopathy it was discovered as a remedy centuries back and till now it is used for immense therapeutic purposes and help in curing a number of diverse health conditions. It is usually the coffee beans[8] that are used in homeopathy.

Coffea cruda (coffee) has both positive and negative effects on the human body caffeine blocks adenosine receptors in the brain. Adenosine has many functions, but one of them is as a neuromodulator that promotes sleep and regulates blood flow. In blocking adenosine receptors, caffeine increases wakefulness. Caffeine is metabolised into three different molecules in the liver these are theobromine which increases the flow of oxygen and nutrients to the brain.

MATERIALS AND METHODS
The seeds of *Coffea cruda* were collected by Center of Medicinal Plants Research in Homoeopathy (CMPRH), Emerald, Tamil Nadu, and was authenticated by the staff of the Center of Medicinal Plants Research in Homoeopathy (CMPRH), Emerald, Ooty. The voucher specimen has been deposited in the herbarium and in the laboratory of DDPR Central Research Insitute for Homoeopathy, Noida, Uttar Pradesh, India, for future reference. Authentic plant material was used to prepare

the Mother Tincture. Caffeine (C8H10N4O2, M. P. 235°C, purity >99% w/w by HPLC) was purchased from Sigma Aldrich, USA. The solvents ethanol, HPLC water, methanol and chloroform were of analytical grade purity (Merck Ltd., India).

Physicochemical studies

Moisture content was determined by loss on drying method. Total ash, water-soluble ash, foreign matter and acid-insoluble ash parameters were performed as per methods recommended in Homoeopathic Pharmacopeia of India.[9]

Determination of physical constants (raw drug standardisation)

Loss on drying

Loss on drying is the loss of mass expressed as percentage w/w. The test for loss on drying determines both water and volatile matter in the crude drug by IR balance. Moisture is an inevitable component of crude drug, which must be eliminated as far as possible. An accurately weighed quantity of 2 g of powdered drug was taken in a porcelain dish. The porcelain dish was kept open in a vacuum oven, and the sample maintained at a constant temperature of 100°C. Then, it was cooled in a desiccator at room temperature.[10] The procedure was repeated till constant weight on repeated weighing is observed. Percentage loss on drying was calculated using the following formula.

$$\% \text{ Loss on drying} = \frac{\text{Loss in weight of the sample}}{\text{Weight of the sample}} \times 100$$

Determination of foreign matter

Weigh 100–500 g of the plant material understudy and spread it out in a thin-layer. Inspect the sample with the unaided eye or with the use of a 6x lens and separate the foreign organic matter manually as completely as possible. Weigh the sorted foreign matter and determine the percentage of foreign matter from the weight of the drug taken.

Ash value

Ash value is helpful in determining the quality and purity of a crude drug, especially in the powdered form. On incineration, crude drugs normally leave a quantity of ash as residue usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The total ash of a crude drug reflects the care taken in its preparation of non-dissipation of non-volatile elements. A higher limit of acid-insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is very high.

Determination of total Ash value

Accurately 2 g of the powdered drug in a silica crucible, previously ignited and weighed. Incinerate by gradually increasing the heat to temperatures not exceeding 450°C for 4 h, until free from carbon, crucible is cooled and weighed. Calculate the percentage of ash with reference to air-dried drug using the following formula.

$$\% \text{ Total ash value} = \frac{\text{Weight of crude drug taken}}{\text{Weight of total ash}} \times 100$$

Determination of acid-insoluble ash value

Boil the ash for 10 min with 25 ml of 2M HCl. Filter and collect the insoluble matter on ashless filter paper, wash with hot water and ignite in a crucible at a temperature not exceeding 450°C for 4 h. Cool in a dessicator and weigh. Calculate the percentage of acid-insoluble ash with reference to the air-dried drug using following formula.

$$\% \text{ Acid insoluble ash value} = \frac{\text{Weight of acid insoluble ash}}{\text{Weight of the crude drug taken}} \times 100$$

The results obtained with reference to air-dried drug are tabulated and observations are recorded in Table 1.

Determination of water soluble extractive value

Accurately weigh 2 gm air dried drug, coarsely powdered with 100 ml of water of the specific strength in a conical flask for twenty four hours shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly taking precautions against loss of water evaporate 25 ml of the filterate to dryness in a tared flat bottomed petri dish, dry at 105°C and weigh. Calculate the percentage of water-soluble extractive value with reference to the air dried drug.

$$\% \text{ Water-soluble extractive value} = \frac{\text{Weight difference of the drug}}{\text{Weight of the crude drug taken}} \times \frac{100 \times 100}{25}$$

Determination of alcohol soluble extractive value

Accurately weigh 2 gm air dried drug, coarsely powdered with 100 ml of absolute alcohol of the specific strength in a conical flask for twenty four hours shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly taking precautions against loss of alcohol, evaporate 25 ml of the filterate to dryness in a tared flat bottomed petri dish, dry at 105°C and weigh. Calculate the percentage of alcohol-soluble extractive value with reference to the air dried drug.[11]

$$\% \text{ Alcohol-soluble extractive value} = \frac{\text{Weight difference of the drug}}{\text{Weight of the crude drug taken}} \times \frac{100 \times 100}{25}$$

Phytochemical analysis

Phytochemical tests were conducted on the seeds of *Coffea cruda* to identify various phytochemicals present in the plant material (A.K. Gupta et al., 2008).[12-14] The various tests conducted are given below and the observations are recorded in Table 2.

1. Test for tannins (lead acetate test): To the test solution, a few drops of 10% lead acetate were added. Precipitate was formed, indicates the presence of tannins.

2. Test for Glycosides (Sodium hydroxide reagent test): Dissolve a small amount of alcoholic extract in 1 ml water and add sodium hydroxide solution. A yellow color indicates the presence of glycosides.

3. Test for triterpenoids (Lieberman Burchardt test): To the test solution, add a few drops of acetic acid and one ml concentrated sulphuric acid gives deep red color at the junction of 2 layers.

4. Test for flavonoids

• Sulphuric acid (H_2SO_4 test): The test solution was treated with concentrated H2 SO4. formation of orange colour indicates the presence of flavonoids.

5. Test for phenolic compounds (ferric chloride test): To the test solution, few drops of ferric chloride test reagent were added. An intense green, purple, blue or black colour developed is taken as an evidence for the presence of tannins.

6. Tests for alkaloids

1. Dragendorff's test - To 2–3 ml of the filtrate, add a few drops of Dragendorff's reagent. Observe for formation of orange-brown precipitate.

2. Mayer's test - To 2–3 ml of the filtrate, add a few drops of Mayer's reagent. Observe for formation of precipitate.

3. Hager's test - To 2–3 ml of the filtrate, add a few drops of Hager's reagent. Observe for formation of yellow precipitate.

4. Wagner's test - To 2–3 ml of the filtrate, add a few drops of Wagner's reagent. Observe formation of reddish brown precipitate.

The results of phytochemical tests carried out are recorded in Table 2.

Preparation of in-house mother tinctures

100 g of coarsely powdered *Coffea cruda* seed were taken, strong Alcohol in sufficient quantity added to make 1000 ml of mother tincture using the percolation method (as per Homoeopathic Pharmacopoeia of India).[15] This tincture was transferred to a suitable glass container and stored for further study.

Standardisation of mother tincture

Standardisation of mother tincture was conducted to identify the organoleptic and physicochemical properties of mother tincture (Banerjee, D.D. 2006, Augmented Textbook of Homoeopathic Pharmacy: B.Jain Publishers).[15]

1. Organoleptic properties

- Appearance: Clear liquid
- Colour: brownish
- Odour: Characteristic

2. Physicochemical properties

- Sediments: Nil
- Weight per ml: 0.820 g
- Alcohol content: 90%
- pH value: 6.56
- Total solid: 0.480 g

High-performance thin layer chromatography study of *Coffea cruda*

HPTLC analysis of *Coffea cruda was* done by HPTLC as mentioned below.

Preparation of standard *Caffeine*

Dissolve 10 mg of caffeine in 10 ml absolute alcohol in volumetric flask and kept it in sonicator for 10 minute for sonication.

Preparation of sample

Evaporate 25 mL of Mother Tincture and three market sample on water bath to remove *alcohol* and extract with 3x20 mL of *chloroform*. Combine and concentrate *chloroform* layer to 2 mL. Carry out TLC of *chloroform* extract of Mother Tincture (A) and three market sample (C, D and E) on silica gel 60 F_{254} pre- coated plate using *Methanol: chloroform* (9:1, v/v) as mobile phase for TLC studies.

Chromatographic conditions

Instrument

HPTLC system equipped with a sample applicator device CAMAG Linomat 5. CAMAG Twin Trough Chamber, Camag TLC Scanner and integration software (visionCATS).

HPTLC Plate	: Silica gel 60 F254 pre- coated plate (Merck) 20 × 10 cm.
Mobile Phase	: Chloroform: Methanol (9:1, v/v).
Development Chamber	: 10X10, Twin-trough chamber
Plate thickness	: 0.2 mm
Plate size	: 100 x 100 mm
Syringe size	: 100 µl syringe
Application	: Position Y: 8.0 mm, length: 8.0 mm, width: 0.0 mm
Table speed	: 150 nl/s
Distance from bottom	: 10 mm
Volume applied	: 6 µl
Pre dosage volume	: 0.20 ul
Distance between tracks	: 10 mm

Development distance : 80 mm
Wavelength : 254 nm and 366 nm.
Visualizing reagent used : Iodine

U.V. spectrophotometeric studies

Spectrophotometer set at range 190 to 1100 nm, samples and standard were put in cuvette. Before analysis cuvettes were washed with ethanol and analysis were performed on Specord 200 plus spectrophotometer and Analytical Jena win aspect software was used for the UV analysis.

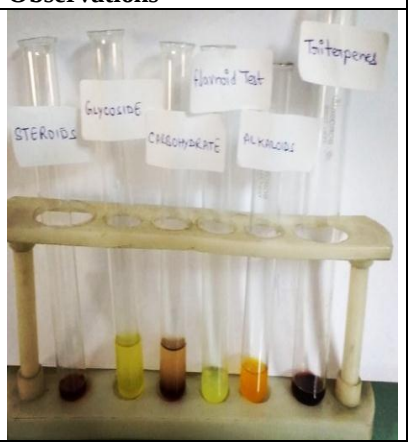
Samples (in house and market) used for U.V analysis were prepared by mixing one part of Mother tincture and ninety nine parts of absolute alcohol (1:99) and filtered through membrane filter prior to U.V. analysis.

RESULTS

Table 1: Results of test of raw material

Name of test	Result (%)
Foreign matter	Not >2.00
Loss on drying	Not >3.68
Total ash	Not >2.66
Acid insoluble ash	Not > 0.70
Water soluble extractive	Not < 21.69
Alcohol soluble Extractive	Not <6.48

Table 2: Results of Phytochemical tests.

Name of phytochemical	Result	Observations
Tannins (lead acetate test)	Positive	
Flavonoids (alkaline reagent test)	Positive	
Triterpenoid (Lieberman Burchardt test)	Positive	
Alkaloids Mayer's test Hager's test Wagner's test Dragendorff's test	 Positive Positive Positive Positive	
Glycosides (Sodium hydroxide reagent test)	Positive	
Phenolic compounds (ferric chloride test):	Positive	

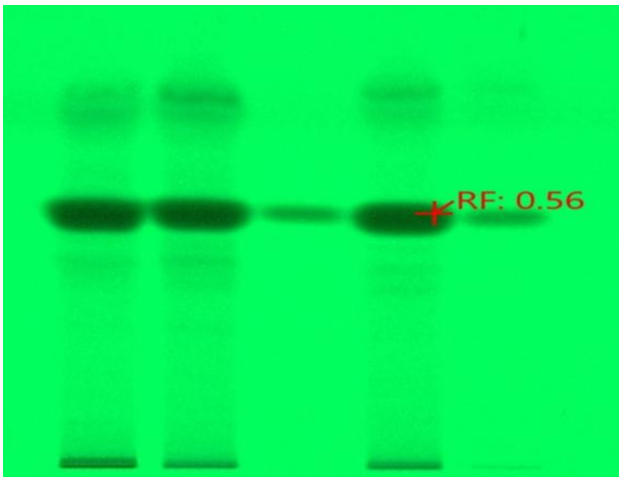


Figure 1: High-performance thin-layer chromatography fingerprints of A, B, C, D, and E i.e. inhouse HT (*Coffea cruda*), standard caffeine and commercial market samples under UV 254 nm.

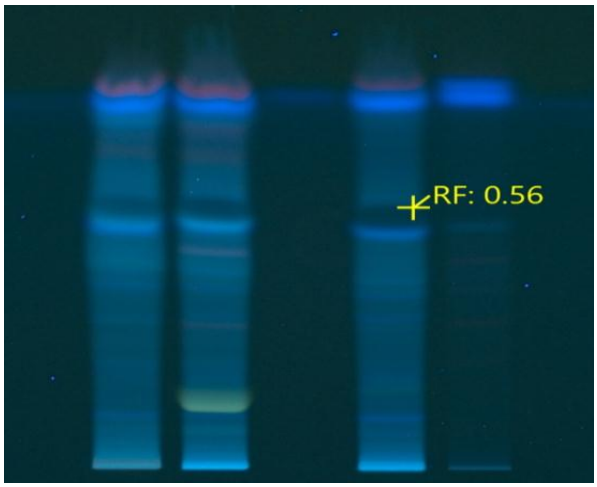


Figure 2: High-performance thin-layer chromatography fingerprints of A, B, C, D, and E i.e. inhouse HT (*Coffea cruda*), standard caffeine and commercial market samples under UV 366 nm.

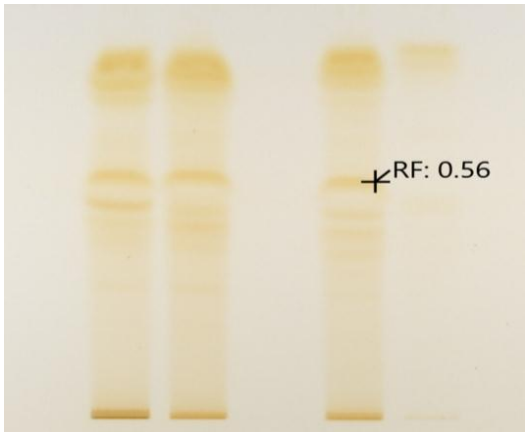


Figure 3: High-performance thin-layer chromatography fingerprints of A, B, C, D, and E i.e. inhouse HT(*Coffea cruda*), standard caffeine and commercial market samples after derivatisation.

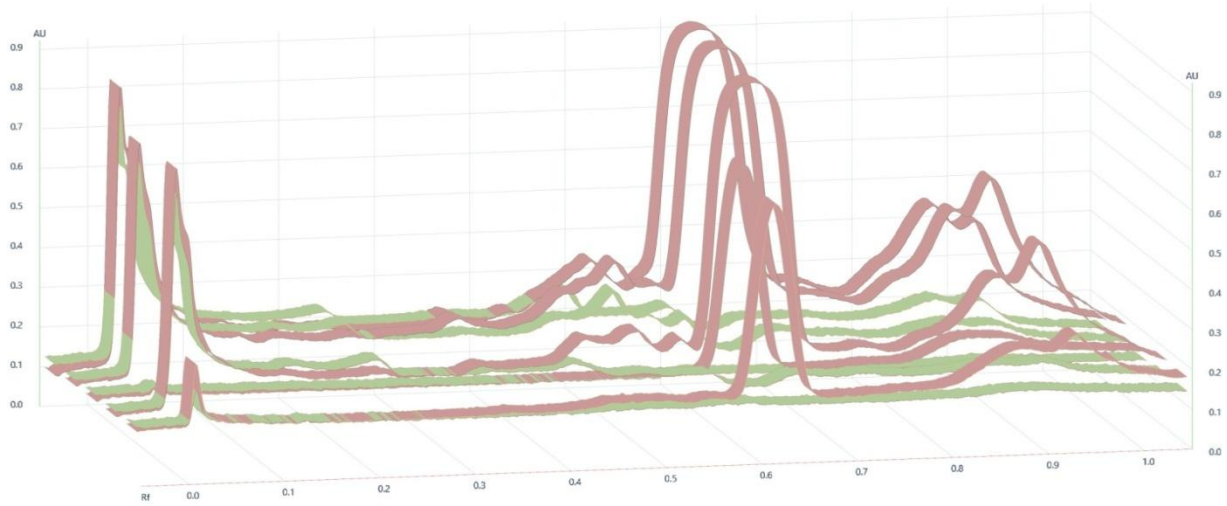


Figure 4: HPTLC isometric chromatograms of *Coffea cruda* inhouse HT and commercial market samples at wavelength λ=254 nm.

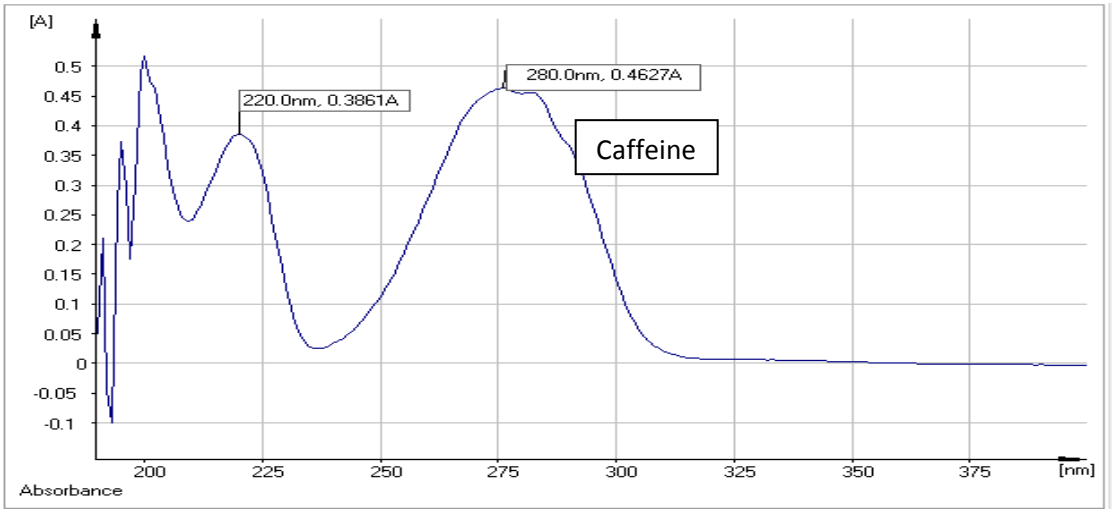


Figure 5: U.V. Absorption spectra of extract from HT of *Coffea cruda.*

DISCUSSION

Raw material used in the preparation of mother tincture **(**In house sample) was cultivated in Survey of Medicinal Plants Unit, Ooty, Tamil Nadu and freshly collected without any adulterations so it contains excess amount of bioactive compounds than that of market samples. More study required to validate the result of other mother tinctures in respect of authentic as well as market sample.

CONCLUSION

It can be concluded on the basis of HPTLC results that the sample collected from SMPCU OOTY, for preparation of mother tincture having higher content of caffeine in comparison from the sample collected from market (Figure 1-4). UV analysis also performed on the inhouse HT *(Coffea cruda)* results maximum absorbance was found at 280 nm shows presence of caffeine in inhouse HT and favour the above said result (Figure 5). Hence we can say chemo profiling of homoeopathic drug/tincture (HT) represents a comprehensive approach for evaluation of quality, purity, safety and efficacy of HT.

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