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Comparison of *Curcuma caesia* Roxb. with other Commonly Used Curcuma Species by HPTLC

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ABSTRACT

The aim of this study was to evaluate phytochemical constituent of *Curcuma caesia* Roxb. and compare it with other curcuma species like *Curcuma longa* and *Curcuma amada* by using HPTLC. The plant extract is obtained by soxhlet extraction method by using methanol as solvent. Phytochemical analysis was performed on plant extract to detect the presence of phytoconstituents and comparison is done by using HPTLC. Phytochemical screening revealed the presence of phenolics, alkaloids, tannins, terpenoid in *Curcuma caesia*. HPTLC fingerprinting shows presence of camphor in *Curcuma caesia* but absent in *C. amada* and *C. longa* while curcumin is absent in *C. caesia* which is marker of *C. amada* & *C. longa*. The HPTLC is also suitable for rapid and simple authentication and comparison of differences among the samples of identical plant resource but of different species.

Keywords: HPTLC, *Curcuma caesia, Curcuma longa, Curcuma amada.*

1. Introduction

In ethno medicinal practices, the traditional healers use the genus *Curcuma* for the treatment of various ailments but *Curcuma caesia* Roxb. is very less known and untouched drug. The genus *Curcuma* is a member of the ginger (family Zingiberaceae), which comprises over 70 species of rhizomatous herbs [1]. The plants have lot of potential in terms of medicinal properties. Literature reveal its anti-inflammatory, hepatoprotective, blood purifier, antioxidant, antiasthmatic, anti-tumour, stomachic and carminative properties [2]. The plant originates from India and South-East Asia. It grows in rich humid and clayey soils. Among them *Curcuma longa* is commonly known as Haldi in Hindi, is a perennial plant having a short stem with large oblong leaves. It bears ovate pyriform or oblong, ovate or cylindrical rhizomes, which are often branched and brownish yellow in color. It is commonly cultivated in Ceylon, Belgium, Indonesia, France and in South India and Bengal and used in Indian traditional system of medicine and also in several food stuff preparation for its medicinal properties [3, 4]. Rhizomes of another species *Curcuma amada* (Mango ginger) commonly known as Amba Haldi, grown in west Bengal and on the hills of West cost of India. It has Rhizome buff colored with short and smooth fracture. It is used in manufacturing of pickles, culinary preparation and salads as a source of raw mango flavor and high medicinal properties [5]. However the rhizome of species *Curcuma caesia* Roxb. popularly known as Kali haldi is very less known and almost untouched drug. In India it is found in West Bengal, Madhya Pradesh, Orissa, Chhattisgarh and Uttar Pradesh states [6]. It flourishes well in moist deciduous forest areas. Rhizomes of the plant are used for sprains and bruises and also employed in the preparation of cosmetics [7]. According to literature, various other species of *Curcuma* are sold under the name of *C. caesia* and no scientific parameters are available to identify the true plant material and ensure its quality.

Therefore, the present work has been performed to establish the various phytochemical and HPTLC parameters, which could serve as a measure of authentication and differentiation for commercial samples of crude drug among this species.

2. Material and Method
2.1 Plant collection and preparation
Dried rhizomes of *Curcuma* species were collected from local market of Pune. Their botanical

identifications and authentication were confirmed at the Agharkar Research Institute, Pune. Then they were cut into small pieces and ground to fine powder.

2.2. Crude Extraction [8]
Known quantities of the powdered rhizome material were de-fatted with n-hexane and then extracted with methanol using soxhlet apparatus for 24 hrs (for 8-9 cycles). The extract was collected and solvent was evaporated to dryness at constant temperature of 65 °C at reduced pressure. The residues were weighed and stored at room temperature.

2.3. Phytochemical Analysis of Extracts [9-12]
The following procedures were adopted for testing the presence of various chemical constituents in the fractions.

2.3.1. Test for Steroids
A. Salkowski test
Chloroform (2 ml) and 2 ml of concentrated sulphuric acid were added to 2 ml of test solution, shaken and allowed to stand. Change in the colour of lower chloroform layer to red and acid layer to greenish yellow fluorescence indicates the presence of steroids.

B. Liebermann-Burchard reaction
T.S 2 ml was mixed with chloroform (2 ml). To the solution, 2 ml of acetic anhydride and 2 drops of conc. Sulphuric acid from the side of test tube were added. Change in colour as, first red, then blue and finally green indicates the presence of steroids.

2.3.2. Test for Triterpenoids
(A) Salkowski test
Concentrated sulphuric acid (2 ml) was added to 2 ml of test solution. The solution was shaken and allowed to stand. The colour of lower layer changes to yellow indicating the presence of triterpenoids.

(B) Liebermann-Burchard Test
The 3 ml of test solution was treated with 3 ml of acetic anhydride, mixed well and then 2 ml of concentrated sulfuric acid was added from the sides of the test-tube. The development of deep red colour indicates the presence of triterpenoids.

2.3.3. Test for Glycosides:
(A) Baljet's test
2 ml of the test solution was treated with 2 ml of sodium picrate solution. The development of yellow to orange colour indicates the presence of cardiac glycosides

(B) Keller-Kiliani test
Glacial acetic acid (3-5 drops), one drop of 5% $FeCl_3$ and conc. Sulphuric acid were added to the test tube containing 2 ml of T.S. Appearance of reddish-brown color at the junction of two layers and bluish green in the upper layer indicates the presence of glycosides

(C) Legal's test
To 2 ml of test solution, 1 ml of pyridine and 1 ml of sodium nitroprusside was added. Change in color to pink or red indicates the presence of cardiac glycosides.

(D) Borntrager's test
Dilute Sulphuric acid was added to 2 ml of solution of extract, boiled for a few min and filtered. To the filtrate 2 ml of benzene or chloroform was added and shaken well. The organic layer was separated and ammonia was added. The change in colour of ammonical layer to pink-red indicates the presence of anthraquinone glycosides.

2.3.4. Tests for Saponin
(A) Foam Test
Powdered extract (10-20 mg) was shaken vigorously with water (1 ml). Development of persistent foam which is stable at least for 15 minutes indicates the presence of saponin.

2.3.5. Tests for Carbohydrates:
(A) Molisch's test
3 ml of Molisch's reagent was added to the 3 ml of test solution, shaken for few minutes. Then 2 ml of concentrated sulphuric acid was added slowly from the sides of the test tube. The development of a purple ring at the junction of two liquids indicates the presence of carbohydrates.

(B) Barfoed's test
Barfoed's reagent (1 ml) and test solution (1 ml) were mixed in a test tube, heated in boiling water bath for 1-2 min. and then cooled. The appearance of red precipitate indicates the presence of monosaccharides.

(C) Fehling's test
Fehling's A and B solutions (1 ml each) were added to the test tube and boiled for 1 min. To this 2 ml of test solution was added and heated in boiling water bath for 5-10 min. Appearance of yellow and then brick red precipitate indicates the presence of reducing sugars.

(D) Benedict's test
Benedict's reagent (1 ml) and T.S (1 ml) were mixed in a test tube and heated in boiling water bath for 5-10 min. Change in colour to yellow; green or red indicates the presence of reducing sugar.

2.3.6. Tests for Alkaloids
To the dry extract (20 mg) dilute hydrochloric acid (1-2 ml) was added, shaken well and filtered. With filtrate the following tests were performed.

(A) Mayer's test
To the 3 ml of test solution 3 drops of Mayer's reagent (potassium mercuric iodide) was added. Appearance of reddish brown or cream precipitate indicates the presence of alkaloids.

(B) Hager's test
To 3 ml of filtrate 4-5 drops of Hager's reagent (saturated picric acid solution) was added. Appearance of yellow precipitate indicates the presence of alkaloids

(C) Dragendorff's test
3 ml of the test solution was mixed with Dragendorff's reagent (potassium bismuth iodide). Appearance of reddish brown precipitate indicates the presence of alkaloids.

2.3.7. Tests for Flavonoids
(A) Ferric-chloride test
Test solution with few drops of ferric chloride solution shows intense green colour indicating the presence of flavonoids.

(B) Shinoda test
To the powdered extract (10 mg), 5 ml of ethanol (95%), 3 drops of hydrochloric acid and 0.5 gm magnesium turnings were added. Change of colour of solution to pink indicates the presence of flavonoids.

2.3.8. Tests for Tannins
(A) Ferric-chloride test
3 ml of test solution treated with few drops of ferric chloride solution. Development of dark colour indicates the presence of tannins.

(B) Gelatin test
03 ml of test solution when treated with gelatin solution (3 ml) gives white precipitate indicating the presence of tannins.

2.3.9. Test for Proteins
(A) Millon's test
T.S (3 ml) and Million's reagent (5 ml) were mixed in a test tube. The appearance of white precipitate changing to brick red or dissolved and gave red color to solution on heating indicates the presence of proteins.

(B) Xanthoproteic test
To the test tube containing T.S (3 ml), 1 ml of conc. Sulphuric acid was added. Appearance of white precipitate which turns yellow on boiling and orange on addition of NH_4OH indicates the presence of tyrosine and/or tryptophan containing proteins.

(C) Biuret test
3 ml of the test solution was treated with 4% sodium hydroxide (3-5 drops) and 1% copper sulphate solution (3-5 drops). The appearance of blue colour indicates the presence of proteins.

(D) Ninhydrin test
Test solution (3 ml) and 3 drops of 5% lead acetate solution were boiled on water bath for 10 min. Change in the colour of solution to purple or blue indicates the presence of amino acids.

2.3.10. Test for Gums
(A) Hydrolyze test solution using dilute HCl. Perform Fehling's or Benedict's test. The appearance of red color indicates the presence of gums.
(B) To 2 ml of extract, add 1 ml of 0.2 N iodine solution. Mixture does not acquire olive green color indicates the presence of gums.

2.4 Chromatography
2.4.1 Sample Preparation
Accurate quantity of extracts was dissolved in methanol to make concentration of 1 mg/ml. All the three extracts of these three species were prepared by same manner and the standard solution of curcumin and camphor (1 mg/ml) was prepared in methanol.

2.4.2 Chromatographic Conditions
Chromatography was performed on Merck TLC plates precoated with silica gel 60 F_{254} (10 cm X 10 cm with 250 μm layer thickness) and developed using mobile phase toluene: ethyl acetate: methanol (18:1:1) up to 80 mm distance. Anisaldehyde sulfuric acid reagent is used as derivatizing agent for visualization. All three extracts and reference standards camphor and curcumin is applied on the plate as a band (5 mm long and 8 mm apart) by using CAMAG linomat 5 sample applicator equipped with 100 μl syringe using wincats software.

2.4.3 Detection
After removal of plates from chamber, plates were completely dried in air at room temperature and derivatised by using anisaldehyde sulfuric acid reagent and the R_f value were recorded by CAMAG TLC scanner 3.

2.4.4 Validation parameters
Repeatability of method was calculated by performing linearity study 5 times of standard.

3. Result and Discussion
Table 1 shows the result of Phytochemical screening for three different curcuma species considered in this study. The result indicates preliminary chemical analysis which can be useful for phytochemical characterization and confirmation of *curcuma* species as well as estimation of bioactive compound. The extract from *Curcuma caesia* revealed the presence of carbohydrate, flavonoid, steroid, phenol, alkaloid, tannin, amino acid, terpenoid and glycoside compounds which are known to have curative activity against diseases producing pathogen. Therefore it can be used pharmacologically to develop new compounds for health benefit.

The HPTLC images (after derivatization) shown in Fig.1 indicate that all the reference substances and sample constituents were clearly separated. The R_f values of the bands for reference standards are: Camphor at R_f 0.6, curcumin at R_f 0.38, demethoxycurcumin at R_f 0.3 and bis-demethoxy curcumin at R_f 0.24. Constituent of the sample extracts were identified by comparison of bands in sample with reference substances on the same plate.

Table 1: Phytochemical Evaluation

Test	*Curcuma caesia*	*Curcuma longa*	*Curcuma amada*
Carbohydrate	+	-	+
Protein	+	+	+
Steroids	+	+	+
Glycoside	+	-	+
Flavonoid	+	+	-
Alkaloid	+	-	+
Tannin	+	-	-
Saponin	-	-	-
Triterpenoids	+	-	-

+ present; - absent

As seen in Fig. 2 and Fig. 3 camphor is present in *C. caesia* which is absent in *C. longa* and *C. amada* while curcumin is absent in *C. caesia* which is a marker for *C. longa* and *C. amada*. To confirm these results UV spectra of respected bands was taken. Fig. 4 shows the overlain spectra of camphor and band in *C. caesia* track at R_f 0.6 which reveled the presence of camphor in *C. caesia*.

Overlain spectra of standard curcumin and all the three extracts *C. amada, C. longa & C. caesia* at R_f 0.385 (Fig: 5) shows absence of curcumin in *C. caesia* which is present in the other two species. Also Fig. 6 shows overlain spectra for demethoxycurcumin at R_f 0.3 which shows presence of demethoxycurcumin in *C. longa* and *C. amada*.
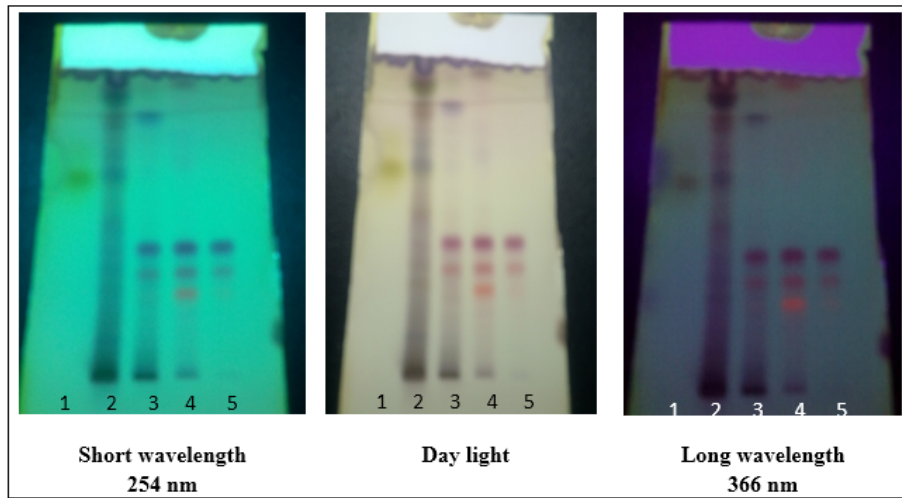
Track 1: Camphor RS; Track 2: *C. caesia*; Track 3: *C. amada*; Track 4: *C. longa*; Track 5: Curcumin RS
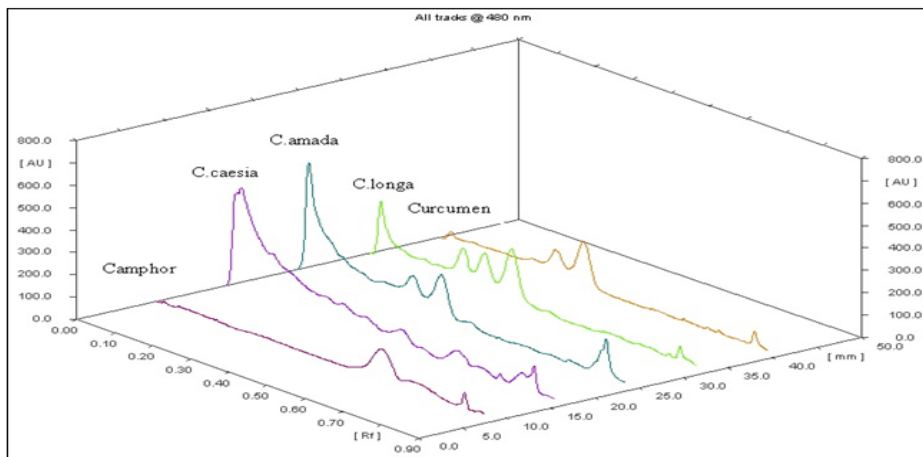**Fig 1:** Developed TLC plate of *Curcuma* species with standards (after derivatization)

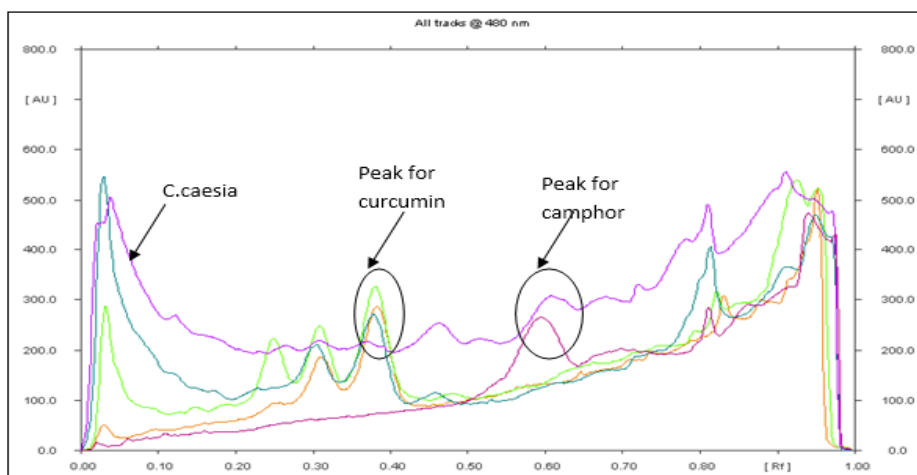**Fig 2: 3D** Chromatogram of *Curcuma* species along with reference standards

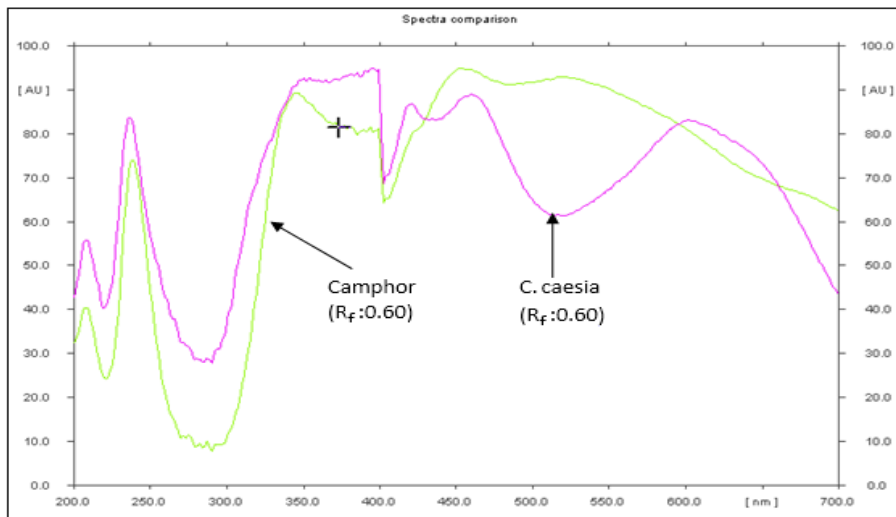**Fig 3: 2D** Chromatogram of *Curcuma* species along with reference standards

Fig 4: Overlain spectra of Camphor RS and Camphor in *C. caesia* at R_f 0.60
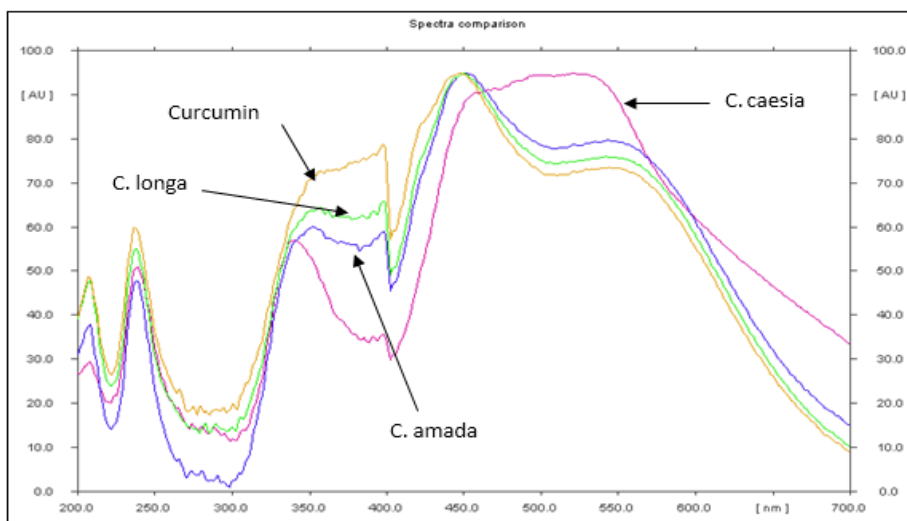

Fig 5: Overlain spectra of Curcumin RS and Curcumin in extracts at R_f 0.385
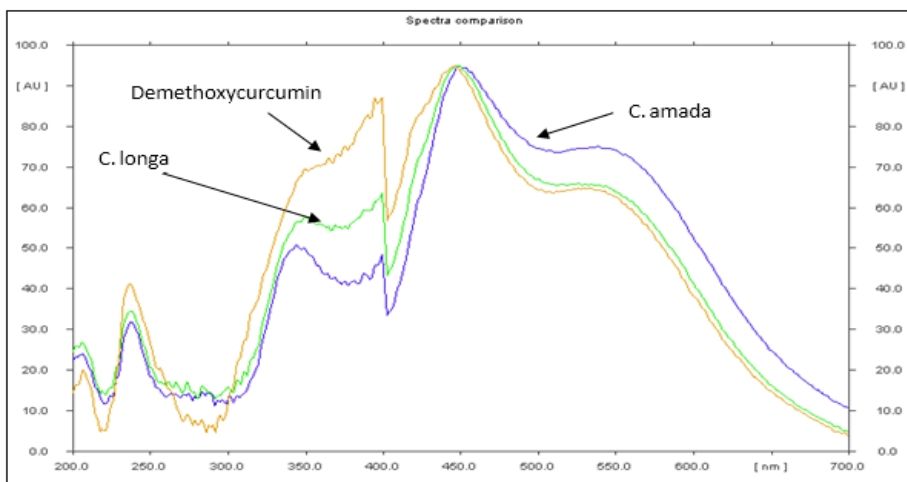

Fig 6: Overlain spectra of Demethoxycurcumin RS and Demethoxycurcumin in extracts at R_f 0.3

4. Conclusion

Although TLC is a conventional method used generally for standardization, the HPTLC method is more practical. HPTLC is feasible to precisely determine major active constituents of medicinal plants. The separation and resolution are better and the results are much more reliable and reproducible than TLC. HPTLC method can be used for differentiation, qualitative and quantitative determination of compound.

The present work has been performed to establish the various phytochemical and HPTLC parameters, which could serve as a measure of authentication and differentiation for commercial samples of crude drug among the curcuma species. The methanolic

extract of studied plants shows the presence of camphor in *C. caesia* while curcumin in *C. longa* and *C. amada* species, which can be used as markers for respective species.

5. Acknowledgement

6. References

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