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# Quality Standards and Antioxidant Activity of Siddha Formulation Cuvacakutori Chooranam

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## ABSTRACT

**Objectives:** To evaluate quality standards and to screen antioxidant activity of Cuvacakutori Chooranam (CC) a poly herbomineral Siddha formulation. **Methods:** The drug was prepared with authenticated ingredients. Powder microscopic, phytochemical, physicochemical, HPTLC fingerprint profiling (*n*-hexane, successive chloroform, successive ethanol), total phenol and flavonoid estimation, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and superoxide scavenging activities of CC were studied. **Results:** Microscopic studies revealed diagnostic features to identify the formulation using microscope. Preliminary phytochemical screening revealed the presence of different secondary metabolites, unique chromatographic fingerprint and antioxidant activities. **Conclusion:** The powder microscopic characters and the evolved physicochemical data arrived could serve as a reference tool for quality control. The presence of different categories of phytochemicals and antioxidant activity of the drug gives broad scope for further efficacy studies of this drug.

**Key words:** Respiratory diseases, Cuvacakutori chooranam, Standardization, HPTLC, Antioxidant.

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## INTRODUCTION

There has been growing interest in usage of traditional medicines in daily life due to impact of plant phytochemicals on maintenance and prevention of several diseases. But there is a major vexation regarding the adulteration, quality, safety and efficacy of the available drugs. In order to maintain all these, standardization of the drug is a primary necessity. Cuvacakutori Chooranam (CC) is a polyherbomineral siddha drug, used for 96 types of respiratory diseases and comprises of 8 powerful herbal ingredients with a mineral ingredient.[1]

*Alpinia officinarum* (L.) Willd. is used traditionally for the treatment of cold related problems.[2-4] It contains mainly diarylheptanoid compounds, which possess inhibitory effect on platelet activity factor (PAF), responsible for several pathological condition including asthma, allergic reaction, inflammation, etc.[5]

*Anacyclus pyrethrum* (L.) Lag. root is widely used in ASU systems of medicine. Its root contains pellitorin, anacyclin, phenylethylamine, inulin, polyacetylenic amides I-IV and sesamin.[6] Pharmacologically it shows antioxidant,[7] antidiabetic,[8] immunostimulating[9] and antidepressant[10] effects.

The essential oil of *Piper cubeba* L.f. berries contain consisting of monoterpenes, sesquiterpenes and oxides. Sequiterpenes Cubebone is the main active principle.[11]

The root, stem and fruit of *P. longum* L. are used in folk medicine to treat chronic bronchitis, asthma, constipation, gonorrhoea, paralysis of the tongue, cholera, diarrhoea, chronic malaria, viral hepatitis, respiratory infection, stomach-ache, bronchitis and spleen diseases. The fruit contains a large number of alkaloids, lignans, esters, volatile oils, organic acids, etc.[12] The most abundant compound of fruit is piperine whereas cepharadione B, cepharadione A, cepharanone B, aristolactam, norcepharadione B, quinolone-4, piperolactam A, piperolactam B were reported as from the root of *P. longum*.[13] Pharmacologically, it possesses anticancer,[14] antioxidant,[15] hepatoprotective,[16] immunomodulatory,[17] antimicrobial,[18] anti-inflammatory,[19] analgesic,[20] coronary vasodilation[21] and antifungal effects.[22]

The king of spices *Piper nigrum* L. fruit is a rich source of numerous biologically active constituents such as alkaloids, monoterpenes, sesquiterpenes, etc. The effect of both *P. nigrum* and *P. longum* are mainly due to the presence of piperine which is a carminative, diuretic, anti-asthmatic, sialagogue and stimulant. Conventionally pepper is used for pain relief, rheumatism, flu, cold, cough, sore throat and even coma. Modern cell line, animal and clinical trial studies have shown that *P. nigrum* has immunomodulatory, bioavailability enhancing, antioxidant, lung metastasis inhibition, hyper sensitivity response, enzyme activity enhancement, gastrointestinal stimulant, anti-asthmatic, anti-carcinogenic, anti-hyperlipidemic, anti-diabetic, anti-inflammatory, anti-metastatic, anti-infertility, anti-ulcer, anti-amoebic, anti-fungal, antibacterial and anti-fibrotic effects.[23]

Leaf juice of *Taxus wallichiana* Zucc. is used for cancer and bronchitis, whereas dried leaves are effective for asthma, bronchitis, hiccough, epilepsy, diarrhoea and headache.[24] It shows analgesic, antipyretic, anticonvulsant, anti-inflammatory, antimicrobial, antifungal and immunomodulatory effects.[25-28] Terpene, alcohol, aldehyde, organic acid, acid esters, alkanes and alkenes are the main phytochemicals of *T. wallichiana* leaves.[29] Taxol is the main active compound is widely used in cancer treatment.[30]

*Zingiber officinale* Roscoe rhizome is a dietary supplement and an important ingredient in several traditional system it is used to treat

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various diseases such as asthma, gingivitis, stroke, toothache, diabetes, rheumatism, headache, gastrointestinal discomforts, nausea, vomiting and common cold. Ginger possesses various pharmacological activities such as anti-apoptotic,[31] anti-cancer effects,[32] antimicrobial, anti-inflammatorymatory, anti-hyperglycaemic and antioxidant.[33] It contains biologically active gingerol, shogaols and zingerone.[34] Borax along with pepper powder and honey is given for the treatment of cough and bronchial asthma. For the prevention of cold fever, borax with *P. betle* can be taken.[35]

In the present study, standardization of CC was performed to assess its quality control parameters along with antioxidant assay.

## MATERIALS AND METHODS

### Drug Preparation

The ingredients (Figure 1) were procured from local market of Chennai, Tamil Nadu and they were authenticated with reference to floras and chemical test. The borax was purified by frying in low flame. One part of each of *A. officinarum, A. pyrethrum, P. cubeba, P. nigrum, Z. officinale*, Borax and two parts of *T. wallichiana* were taken to prepare CC as per classical text.[1] It was stored in airtight container for further studies.

### Powder Microscopy

The CC was mounted in glycerin on a clean microscopic slide after treating it with 10% NaOH solution. Slides were observed under Nikon ECLIPSE E200 trinocular microscope and diagnostic characters were identified. Individual characters of powder were magnified to 400X and photographed. The specific identifying characters of the individual component herbal drugs were identified.[36]

### Physico-chemical Analysis

All the physico-chemical parameters were performed according to the methods standard.[37]

### Phytochemical Screening

All the phytochemical tests were done by using the standard methods.[38]

### Extract Preparation

CC (4 g) was extracted successively with 100 ml of each of *n*-hexane, chloroform and ethanol using Soxhlet apparatus. Extracts were concentrated to dryness and corresponding weights were taken for determining successive extract values. The total residues were redissolved with the corresponding solvents and made up to 10 ml and transferred into sample vials for TLC application. For TLC of CC with its ingredients and standard piperine, 0.5 each of CC, ingredients and 5mg of piperine were sonicated with 5 ml ethanol for 5 min, filtered and taken into sample vials.

### Chemicals, Solvents and Instruments

Analytical grade solvents *n*-hexane, toluene, chloroform, ethyl acetate, ethanol and formic acid were purchased from Merck. Vanillin (1 g) sulphuric acid in methanol (5%) solution (VSA) was used for visualization. For HPTLC, Autosampler ATS4, twin trough chambers, visualizer, scanner 4 (Scanner_210441) linked with WINCATS software, plate heater (all from CAMAG, Switzerland) were used. For antioxidant studies, Folin-Ciocalteu reagent, quercetin, ascorbic acid, gallic acid, DPPH, nitro blue tetrazolium (NBT) and DMSO (AR grade, Sigma Aldrich), sodium carbonate (LR grade, MERCK), aluminium chloride, potassium acetate, sodium hydroxide (LR grade, Sigma Aldrich) were used. The absorbance was measured by the Perkin Elmer's Lambda 25 UV spectrophotometer.

### TLC/HPTLC Procedure

Extract 20 µl was applied on silica gel $60F_{254}$ coated aluminium plate (3×10 cm) as from 8 mm band and 10 mm. from bottom Plate was developed using *n*-hexane: ethyl acetate (7:3, *v/v*) for *n*-hexane extract, toluene: ethyl acetate: formic acid (6:2:0.5, *v/v/v*) for successive chloroform extract and toluene: ethyl acetate: methanol: formic acid (2:5.5:2:0.5, *v/v/v/v*) for successive ethanol extract separately in presaturated twin trough chamber (10×10 cm). The developed plates was dried and photograph was taken followed by scanning under λ 254/366 in absorbance Fluorescence using $D_2$/Hg lamp respectively with a slit dimension 6×0.45 mm and scanning speed 20 mm/s. The scanned plate was dipped in VSR and heated at 105°C till the appearance of colored bands. Photograph was taken immediately at white light followed by scanning at λ 520 at absorption mode (W lamp).

For TLC of CC with ingredients and standard piperine 10 µl of each of the ingredients along with 2 µl of standard piperine solution were applied on TLC plate (16× 10 cm) keeping other conditions as above. The plate was developed the mobile phase *n*-hexane: ethyl acetate: formic acid (15:5:0.5, *v/v/v*) in the twin trough chamber (20×10 cm), saturated with the mobile phase, Photo documentation, scanning under UV and at 520 nm after derivatization was done as above.

### Antioxidant Activity

The total phenol content was determined by Folin-Ciocalteau method using gallic acid as standard[39] and expressed as Gallic Acid Equivalents (GAE).

The total flavonoid content was estimated[40] using quercetin as standard and expressed in terms of Quercetin Equivalents (QE). The ability of the plant extract to scavenge the stable free radical was assayed by the DPPH method using quercetin as reference standard.[41] The superoxide scavenging effect was studied by the alkaline DMSO method using NBT.[42] Ascorbic acid was used as standard reference The scavenging activities were expressed as $IC_{50}$.

## RESULTS

### Powder Microscopy

Powder microscopy showed the presence of various cellular and sub cellular characters like epicarp cells, perisperm cells, cells with brown content trichomes, fibres, mesophyll cells, different types of vessel, starch grains, tannin cells, etc. (Figure 1).

### Physico-chemical Analysis

The physico chemical parameters were carried out in duplicates and the mean values are presented (Table 1).

### Phytochemical Screening

Ethanolic extract of the drug was subjected to phytochemical screening and their presence, absence were documented (Table 1).

### TLC Photo documentation

TLC of *n*-hexane extract of CC showed 10 bands with R*f* 0.03, 0.07, 0.10, 0.15, 0.21, 0.27, 0.33, 0.38, 0.67 and 0.72 (all green) under short UV; it revealed 7 bands with R*f* 0.03, 0.10 (both blue), 0.22, 0.29, 0.35, 0.42 and 0.45 (all pink) under long UV; post derivatized plate showed 10 bands at R*f* 0.06 (blue), 0.11 (black), 0.14 (violet), 0.16 (dark blue), 0.24 (violet), 0.29 (grey), 0.34, 0.45, 0.67 (all violet) and 0.72 (violet) [Figure 2].

TLC of successive chloroform extract revealed 6 bands at R*f* 0.26, 0.34, 0.43, 0.50, 0.58, 0.79 (all green) under short UV. Under long UV, 6 bands came out with *Rf* 0.24 (yellow), 0.29 (pink), 0.38 (pink), 0.49 (blue), 0.65 (pink) and 0.72 (fluorescent blue); 9 bands at 0.07 (violet), 0.12 (blue),

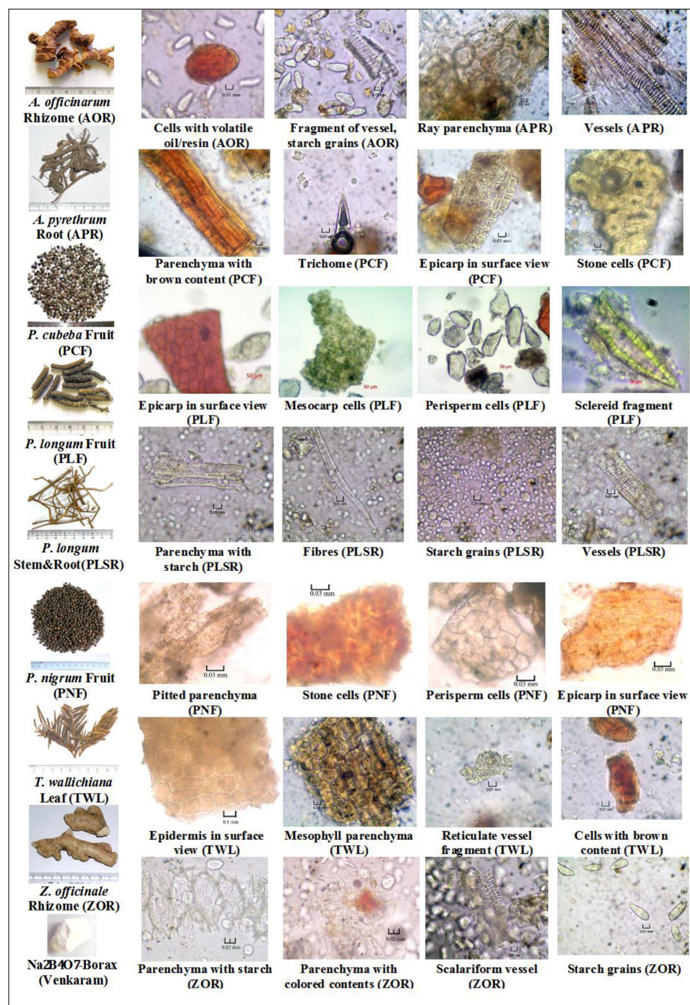



**Figure 1:** Ingredients and powder microscopy of CC.

**Table 1: Physico-chemical Constants and Phytochemicals.**

| Parameter | Result | Phytochemical | Inference |
|---|---|---|---|
| Loss on drying at 105°C | 9.95% | Alkaloid | + |
| Total ash | 12.48% | Phenol | + |
| Water soluble ash | 2.35% | Flavonoid | + |
| Acid insoluble ash | 1.33% | Steroid | + |
| Water soluble extractive | 26.10% | Tannin | + |
| Ethanol soluble extractive | 12.25% | Coumarin | + |
| pH | 8.48 | Glycoside | + |
| *Successive Extraction* | | Reducing sugar | + |
| *n*-Hexane | 5.80% | Quinone | - |
| Chloroform | 1.50% | | |
| Ethanol | 6.30% | | |

0.25 (violet), 0.32 (violet), 0.34 (yellow), 0.37(blue), 0.45 (violet), 0.53 (violet), 0.61 (yellow) in post derivatized plate [Figure 3].

In the TLC of successive ethanol extract, 6 bands appeared under short UV with R*f* 0.03, 0.19, 0.28, 0.38, 0.47 and 0.79 (all green); 6 bands with R*f* 0.19, 0.41 (both blue), 0.46 (fluorescent blue), 0.57 (blue), 0.74 (yellowish green) and 0.80 (yellowish green) under long UV; 9 bands with

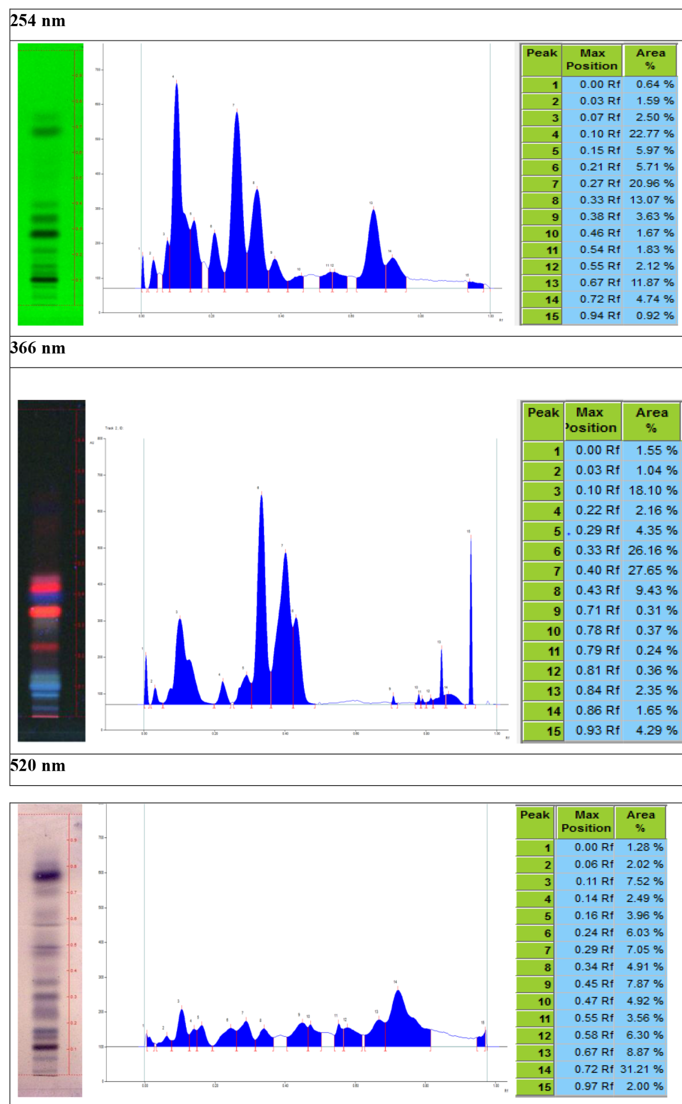



**Figure 2:** TLC/HPTLC of n-hexane extract of CC.

R*f* 0.02 (light pink), 0.06 (pink), 0.12 (grey), 0.20 (grey), 0.28 (black), 0.37 (violet), 0.47 (yellow), 0.54 (violet), 0.79 (yellowish green) under white light (post derivatization) [Figure 4].

TLC photo documentation of CC with ingredients and piperine revealed the presence of 6 bands in AOR at *Rf* 0.18, 0.30, 0.51, 0.60, 0.64, 0.72 (all green); 2 bands in APR at R*f* 0.18, 0.60 (both green); 4 bands in PCF at R*f* 0.15, 0.24, 0.39, 0.60 (all green); 7 bands in PLF at R*f* 0.17, 0.29, 0.33, 0.40, 0.44, 0.56, 0.79 (all green); standard piperine at *Rf* 0.33; 11 bands in drug CC with R*f* 0.14, 0.17, 0.21, 0.26, 0.28, 0.33, 0.39, 0.44, 0.51, 0.59, 0.78 (all green); 3 bands in PLSR at *Rf* 0.33, 0.46, 0.60 (all green); 11 bands in PNF at *Rf* 0.06, 0.11, 0.14, 0.25, 0.27, 0.30, 0.33, 0.44, 0.51, 0.60, 0.78 (all green); 9 bands in TWL at *Rf* 0.10, 0.15, 0.18, 0.21, 0.29, 0.44, 0.59, 0.69, 0.78 (all green) and 3 bands in ZOR at *Rf* 0.29, 0.55, 0.66 (all green) under short UV; Under long UV, AOR showed 5 bands at *Rf* 0.23 (blue), 0.33 (green), 0.56 (green), 0.64 (blue), 0.69 (blue); 4 bands in APR at *Rf* 0.18 (Fluorescent blue), 0.29 (blue), 0.56 (F. green), 0.65(blue); 5 bands in PCF at R*f* 0.18 (pink), 0.25 (pink), 0.56 (F. green), 0.64 (blue), 0.69 (pink); 7 bands in PLF with R*f* 0.16 (pink), 0.19 (pink), 0.24 (pink), 0.33 (F. blue), 0.48 (blue), 0.65 (blue), 0.78 (blue); standard piperine at *Rf* 0.33 (F. blue); 11 bands in CC with *Rf* 0.09, 0.16, 0.19 (all

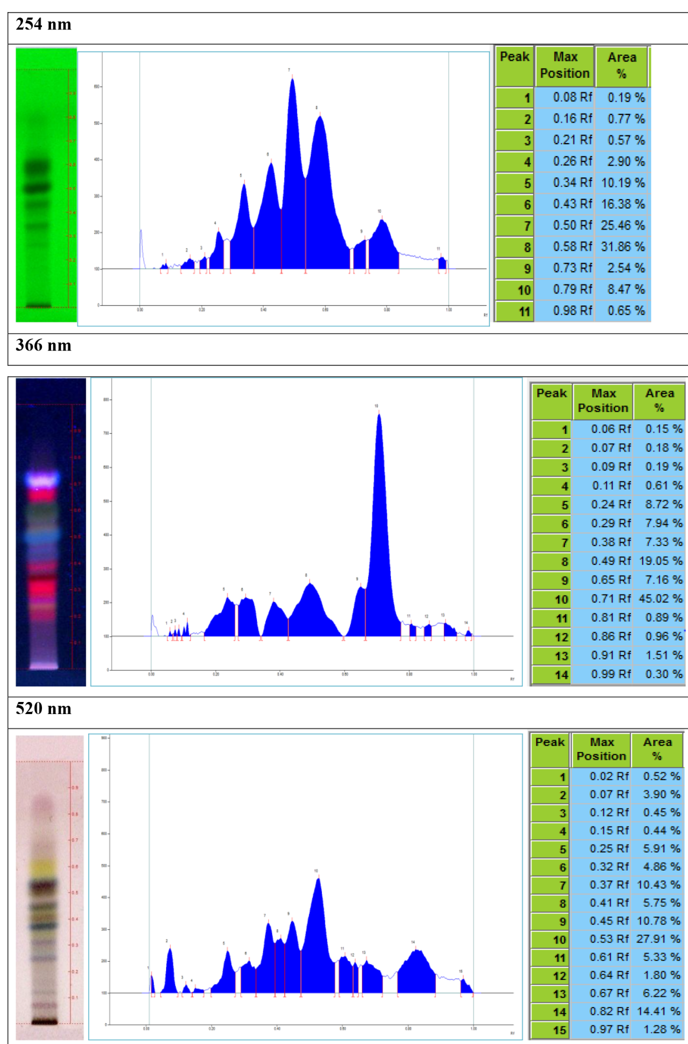



**Figure 3:** TLC/HPTLC of successive chloroform extract of CC.

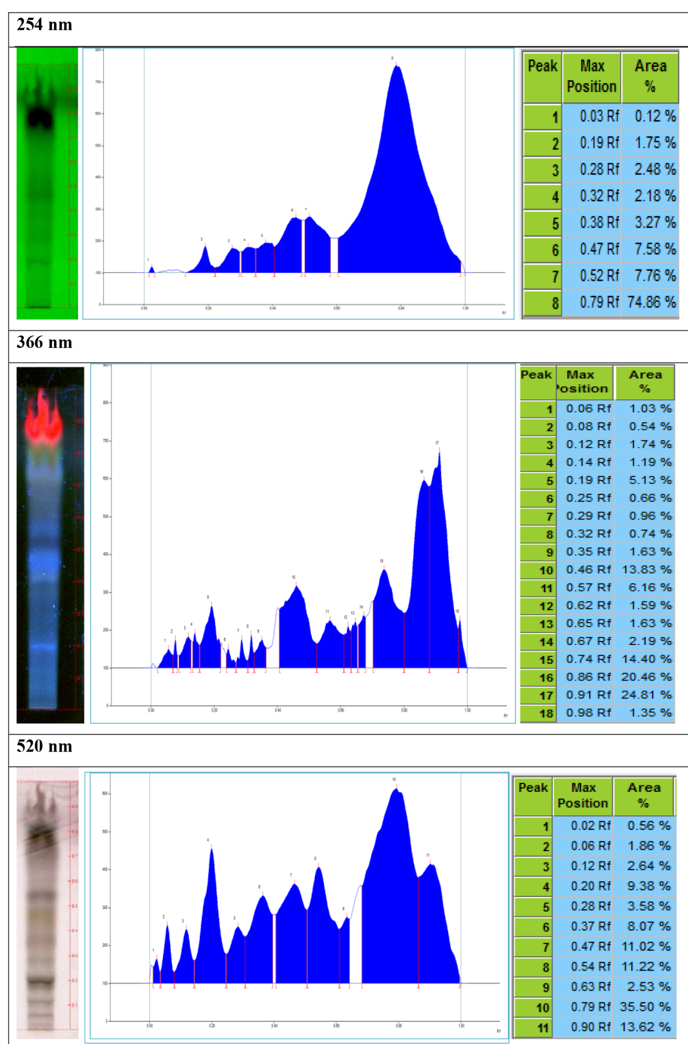



**Figure 4:** TLC/HPTLC of successive ethanol extract of CC.

pink), 0.24 (F. blue), 0.33 (F. Blue), 0.41 (yellowish green), 0.56 (green), 0.60 (pink), 0.65 (blue), 0.72 (pink), 0.78 (blue); 5 bands in PLSR with R*f* 0.18 (blue), 0.29 (blue), 0.33 (F. blue), 0.56 (F. green), 0.64 (blue); 7 bands in PNF with R*f* 0.15 (blue), 0.19 (blue), 0.23 (pink), 0.33 (F. blue), 0.41 (green), 0.56 (green), 0.64 (blue); 8 bands in TWL with *Rf* 0.15 (pink), 0.18 (pink), 0.23 (pink), 0.37 (pink), 0.48 (pink), 0.56 (F. green), 0.60 (pink), 0.69 (pink) and 5 bands in ZOR with *Rf* 0.10 (green), 0.25 (blue), 0.29 (green),0.37 (green), 0.56 (F. green) [Figure 5].

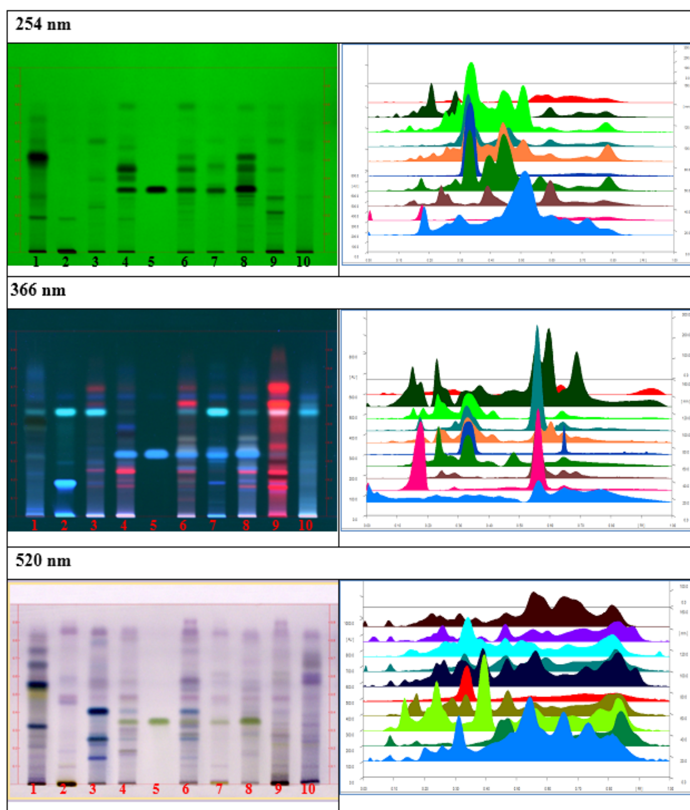
In the post derivatized TLC plate, 10 bands appeared for AOR with *Rf* 0.02, 0.14, 0.20, 0.26 (all violet), 0.31 (dark blue), 0.49 (yellow), 0.54 (dark blue), 0.65, 0.73, 0.81 (all violet); 6 bands for APR with *Rf* 0.09, 0.17, 0.45 (all violet), 0.47 (pink), 0.58 (violet), 0.84 (violet); 8 bands for PCF with *Rf* 0.14 (violet), 0.24 (dark blue), 0.39 (dark blue), 0.47, 0.56, 0.68, 0.77, 0.83 (all violet); 9 bands for PLF with *Rf* 0.17 (violet), 0.24 (blue), 0.29 (violet), 0.33 (green), 0.39 (blue), 0.47 (pink), 0.56, 0.80, 0.83 (all violet); standard piperine at *Rf* 0.33 (green); 12 bands for CC with R*f* 0.09, 0.14 (both violet), 0.24 (blue), 0.26 (dark), 0.33 (green), 0.41 (dark blue), 0.47 (pink), 0.52, 0.56, 0.73, 0.83, 0.89 (all violet); 7 bands for PLSR with R*f* 0.01, 0.09 (both pink), 0.33 (green), 0.47 (pink), 0.56, 0.59, 0.82 (all violet); 7 bands for PNF with R*f* 0.15 (violet), 0.27 (blue), 0.33 (green), 0.47 (pink), 0.52, 0.67, 0.81 (all violet); 9 bands for TWL with R*f* 0.03, 0.09, 0.26, 0.32, 0.38 (all violet), 0.46 (pink) 0.60 (green), 0.83, 0.88 (both violet) and 9 bands for ZOR with *Rf* 0.08 (violet), 0.21 (yellowish green), 0.25, 0.31, 0.37 (all violet), 0.47 (pink), 0.56, 0.66 and 0.81 (all violet) [Figure 5].

## HPTLC densitometric scan

Densitometric scan of *n*-hexane extract showed 15 peaks under short UV, 15 peaks under long UV and also 15 peaks under white light of post derivatized plate (Figure 2). Fingerprint profile of the successive chloroform extract possessed 11 peaks under short UV, 14 peaks under long UV and 15 peaks under white light scanning (Figure 3). Scanning of successive ethanol extract revealed 8 peaks under short UV, 18 peaks under long UV and 11 peaks under white light scanning (Figure 4). In the HPTLC scan of CC with the ingredients and piperine, AOR showed 8, 9 and 15 peaks; APR showed 6, 4 and 9 peaks; PCF exhibited 8,6 and 10 peaks; PLF separated with 10, 9 and 11 peaks, PLSR gave 6, 5 and 9 peaks, PNF represented 14, 7 and 8 peaks, TWL turned with 13, 10 and 11 peaks and ZOR appeared with 8, 7 and 10 peaks at UV 254/366 nm and after derivatization at white light respectively. The 3D chromatogram of all tracks are shown in Figure 5.

## Total Phenol/Flavonoids

The total phenol content in aqueous and methanol extracts (CCAE and CCME) was found to be 28.56 ± 0.36 and 27.93 ± 0.67 mg of GAE/g of the extract respectively. Whereas, the total flavonoid content for the




1.AO R; 2.AP R; 3.PC F; 4.PL F; 5.PN F; 6.CC; 7.PL SR; 8.PN F; 9.TW L; 10.ZO R

**Figure 5:** TLC of ethanol extract of of CC and ingredients.

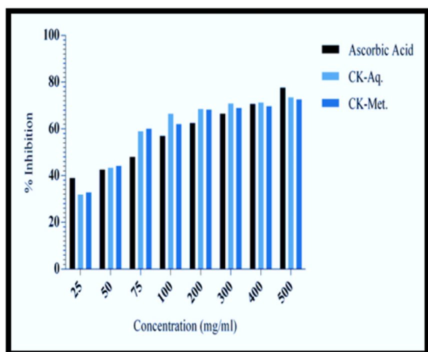



**Figure 6B:** DPPH Radical Scavenging Activity.

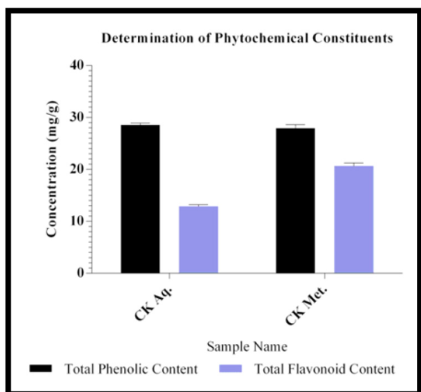



**Figure 6A:** Total phenol/flavonoid content.

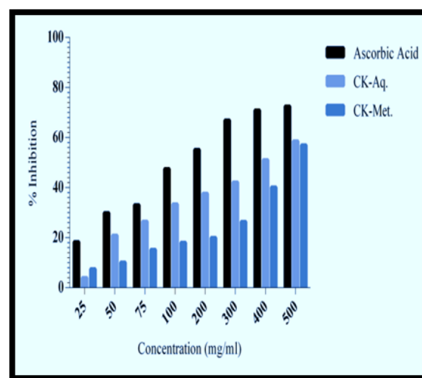



**Figure 6C:** Superoxide Anion Radical Scavenging Activity.

**Table 2: Free radical scavenging activity by CC.**

| Concentration (µg/ml) | Ascorbic Acid | CCAE | CCME |
|---|---|---|---|
| DPPH free radical Inhibition (%) | | | |
| 25 | 38.98 | 31.88 | 32.81 |
| 50 | 42.58 | 43.36 | 44.14 |
| 75 | 47.97 | 58.98 | 60.00 |
| 100 | 56.95 | 66.48 | 62.03 |
| 200 | 62.42 | 68.44 | 68.20 |
| 300 | 66.48 | 70.86 | 68.98 |
| 400 | 70.63 | 71.25 | 69.69 |
| 500 | 77.66 | 73.44 | 72.66 |
| Superoxide anion radical inhibition (%) | | | |
| 25 | 18.26 | 3.95 | 7.44 |
| 50 | 29.88 | 20.86 | 10.15 |
| 75 | 33.03 | 26.49 | 15.22 |
| 100 | 47.46 | 33.37 | 18.04 |
| 200 | 55.13 | 37.66 | 19.95 |
| 300 | 66.85 | 42.16 | 26.38 |
| 400 | 70.91 | 51.07 | 40.14 |
| 500 | 72.49 | 58.51 | 56.93 |

CCAE and CCME were estimated to be 12.84 ± 0.366 and 20.624 ± 0.621 mg of QE/g of extract respectively. The results are represented graphically in Figure 6A.

## DPPH radical scavenging activity

The absorbance was measured at 517 nm using UV-spectrophotometer. The percentage of inhibition at various concentration of CC as well as standard ascorbic acid (25 to 500 µg) were calculated (Table 2, Figure 6B). In higher concentration, ie., 500 µg/ml, CCAE.

Inhibited 73.44%, CCME inhibited 72.66% and the standard ascorbic acid inhibited 77.66%. In lower concentration, ie., 25 µg/ml, CCAE inhibited 31.88%, CCME inhibited 32.81% and the standard ascorbic acid inhibited 38.98%.

## Superoxide radical scavenging

The absorbance was measured at 560 nm in UV spectrophotometer. The % of inhibition at various concentrations (25 to 500 µg/ml) of drug and standard ascorbic acid were calculated (Table 2, Figure 6C). The result showed 58.51% for CCAE, 56.93% for CCME and 72.49% of inhibition

for standard ascorbic acid at 500 μg/ml concentration. In lower concentration 25μg/ml, showed 3.95% for CCAE, 7.44% for CCME and 18.26% inhibition for standard.

## DISCUSSION

Botanical identity of medicinal plants is essential to ensure the potentiality and safety of herbal drugs. The pharmacognostic study helps the correct authentication of herbal material used for the preparation of herbal medicines. The morphological and anatomical features are very important and can be used as a diagnostic key to differentiate one plant from another.[43] The specific characters observed in the powder of CC formulation gave correct authentication of its plant ingredient from which it is prepared. The diagnostic features will also give information on presence of adulterants or substitutes to the official sources. Presence of elongated rectangular epidermal cells, parenchyma cells with brown contents and pappillate lower epidermal cells confirms the presence of *T. wallichiana* leaf in the formulation.[44] The fruit characters observed in the powder exactly matches with that of *P. cubeba*, *P. longum* and *P. nigrum*. The presence of scalariform vessels and oleoresin cells in *A. officinarum* are reported[45] gives key character to discriminate it from other *Alpinia* species.

Moisture content indicated by loss on drying was found to be 9.95%. Total ash reveals the inorganic content including physiological and non-physiological salts of the drug which was evaluated as 12.48%. Water soluble ash which predicts the inorganic content was found to be 2.35 %. The acid insoluble ash was found to be 1.33 % which composed of siliceous matter mostly from the rhizomic ingredient drugs. The water-soluble extractive value and alcohol soluble extractive value were estimated as 26.10 % and 12.25 % respectively. Water is better extracting solvent than ethanol for this drug. The pH of the drug was determined as 8.48 which indicates the basic nature of the drug due to the presence of alkaloids from PCF, PNF, PLF and PLSR.

Densitometric scan of *n*-hexane extract of CC showed three major peaks at $R_f$ 0.10 (22.77 %), 0.27 (20.96 %) and 0.33 (13.07 %); three major peaks at $R_f$ 0.40 (27.65 %), 0.33 (26.16 %), 0.10 (18.10 %); two major peaks at $R_f$ 0.72 (31.21%) and 0.67 (8.87%) at 254,366 and 520 nm respectively.

Finger print of successive chloroform extract possessed, three major peaks with *Rf* 0.58 (31.86%), 0.50 (25.46%) and 0.43 (16.38%); two major peaks with R*f* 0.71 (45.02%) and 0.49 (19.05%); two major peaks with $R_f$ 0.53 (27.91%) and R*f* 0.82 (14.41%) at 254,366 and 520 nm respectively.

Densitometric scan of successive ethanol extract of CC revealed one major peak at *Rf* 0.79 (74.86%); three at $R_f$ 0.91 (24.81%), 0.86 (20.46%) and 0.74 (14.40%); three major peaks at $R_f$ 0.79 (35.50%), 0.90 (13.62%) and 0.54 (11.22%) under 254, 366 and 520 nm respectively.

TLC of CC along with its ingredients and piperine confirmed the presence of piperine in drug CC, PLF, PLSR and PNF by the green colored band with $R_f$ 0.33 under short UV, blue colored band under long UV and green colored band under white light of post derivatized plate. Densitometric scan revealed the appearance of piperine with an area 21.42 % in PLF track, 27.02 % in CC track, 51.24 % in PLSR track and 37.12 % area in PNF under UV 254 nm; 27.48 % in PLF track, 33.36 % in CC track, 21.02 % track in PLSR and 36.46 % area in PNF track under UV 366 nm and 8.40 % in PLF track, 10.57% in CC track, 14.84 % track in PLSR and 30.04 % area in PNF track under white light of post derivatized plate. CC showed a band with R*f* 0.46 which resembled with the band present in TWL; CC, PLF, PNF revealed a common band at R*f* 0.80; one pink colored band with R*f* 0.24 appeared in CC, PCF, PLF, PNF, TWL; the band with $R_f$ 0.42 of CC resembled with the band in PNF under long UV.

The total phenol content were 28.56±0.36 and 27.93±0.67 mg of GAE/g; the total flavonoid content were 12.84±0.366 and 20.624±0.621 mg of QE/g of aqueous and methanol extracts of CC respectively.

The $IC_{50}$ for DPPH free radical inhibition of ascorbic acid was 81.2.99±0.243 μg/ml while that of CCAE was 62.3±0.874 μg/ml and CCME was 61.3±0.797 μg/ml. This indicates that both extracts of CC are inhibiting the DPPH free radicals better than ascorbic acid. The $IC_{50}$ for superoxide radical scavenging activity of ascorbic acid was 112.78±0.974 μg/ml, the CCAE as 385.43±0.543 μg/ml and CCME as 467.24±0.967 μg/ml. It is inferred that the superoxide radical scavenging activity of CCAE is three times less while that of CCME is four times less than ascorbic acid. The drug is effective scavenger of DPPH free radicals rather than superoxide radicals.

## CONCLUSION

The results from powder microscopic study would be supportive for finding the specific characters of the ingredient drugs and rule out the adulterant or substituent. The detailed literature on chemical constituents and phytochemical study confirmed that the drug CC consists of wide variety of phytochemicals which could be responsible for the expected therapeutic activities of the drug. The TLC and HPTLC data would serve as reference assist for the quality control of the drug in chemical point of testing. The presence of different categories of phytochemicals and antioxidant activity of the drug gives scope for further pharmacological studies.

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## CONFLICT OF INTEREST

Authors declare no conflict of interest.

## ABBREVIATIONS

**NaOH:** Sodium hydroxide; **DMSO:** Dimethyl sulphoxide.

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