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RESEARCH COMMUNICATION

Powder microscopic, physicochemical and chromatographic approach for the quality control of anti-hypertensive drug *Rattha Piththathirku Kudinir Chooranam*

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ABSTRACT

The present work aims to study powder microscopy, physicochemical and high-performance thin-layer chromatography photo documentation and fingerprint profiles of a Siddha drug, *Rattha Piththathirku Kudinir Chooranam* (RPK). The raw drugs were collected, authenticated and the RPK was prepared. Then the drug was investigated for powder microscopic characters, physicochemical parameters, Thin Layer Chromatographic photo documentation (TLC), High-Performance Thin-Layer Chromatographic (HPTLC) fingerprint profiles of successive *n*-hexane, successive chloroform, successive ethanol and hydro alcohol (1:1) extracts. The successive and hydro alcohol extracts of the drug displayed distinct TLC spots and HPTLC peaks which are distinct to this drug.

Introduction

Hypertension is a major health issue that leads to an increase in the risks of heart, brain, kidney and other organ related diseases. It is one of the major reasons for early death of public (1). According to the World Health Organization, it is estimated that 1.13 billion people are suffering from hypertension worldwide, and two-thirds of them belong to low and middle income countries (2). Traditional and complementary medicine plays an important role in maintaining good health due to its easy acceptance by the human body (3) and lesser side effects. Among 170 Member States of WHO, 88% recognised their use of traditional and complementary medicine (4). *Rattha Pitthathirku Kudinir* (RPK) is a Siddha drug consisting of *cukku* [*Zingiber officinale* Linn. (rhizome)] and *caranai* [*Zaleya decandra* (L.) Burm. (root)]. *Ratthapitham* refers to *Purpura* i.e. hypertension. According to Siddha literature, 30 to 60 ml of medicine has to be taken twice daily for treating hypertension (5).

The plant *Zaleya decandra* is commonly called Purslane (English) and belongs to Aizoaceae (6). It is native to India, Sri Lanka and Australia and introduced to tropical regions of Africa and South America (7, 8). Traditionally the root is used for the treatment of hepatitis, asthma and orchitis (9). Decoction of root bark is well known as an aperient (10). The preliminary qualitative phytochemical screening has been reported (6) to show alkaloids, carbohydrates, phenols, terpenoids, cardiac glycosides, fatty acids, saponins, tannins and proteins. GC-MS analysis of *Z. decandra* revealed that momeinositol (26.57%), methyl ester of 2-hydroxy-valeric acid (18.9%), 2-methyl-hexanoic acid, (7.74%), 2-amino-5-(2-carboxy)vinyl) imidazole, (7.71%), decanoic acid (7.45%), allantoic acid (6.90%) are present in major concentrations; whereas dl-citrulline (3.8%), benz(e)azulene-3,8-dione, octanoic acid (2.16%), 1,3-dioxolan-4-one, strogogenin (1.04%), 12,15-octadecatrienoic acid, *n*-hexadecanoic acid (0.89%), 1H-pyrazole, 4,5-dihydro-3-methyl- (0.66%), E-11-tetradecenol, trimethylsilyl ether (0.59%) and malonic acid were reported to be present in minimal concentrations (7). Oleic acid has been isolated from the root of *Z. decandra* (11). Pharmacologically it shows antidiabetic (12), hepatoprotective, antioxidant (13), cytotoxic activity (7) and anthelmintic activity (6).

Zingiber oficinale is used in several ancient systems of medicines for the treatment of catarrh, rheumatism, nervous diseases, gingivitis, toothache, asthma, stroke, diabetes, headache, gastrointestinal discomforts, nausea, vomiting and the common cold (14-16). Traditionally ginger is being administered for hypertensive patients since ancient days (17). It consists of volatile oils, gingerol, diarylheptanoids, proteins, amino acids, sugar,

organic acids and inorganic elements. It comprises more than 300 types of compounds, mainly categorised into 3 different types: 194 types of volatile oil compounds, 85 types of gingerols and 28 types of diarylheptanoids (18). The major phytoconstituents of *Z. officinale* are 6-shogaol, 6-gingerol, 8-gingerol, 10-gingerol, zingiberol, *β*-phellandrene, *α*-zingiberene, *ar*-curcumene and *β*-bisabolene along with the compounds gingerdiols, *α*-santalol, *β*-eudesmol, nerolidol, farnesol, elemol, neral, geranial, *α* and *β*-pinene, camphene, sabinene, myrecene and limonene (18-24). Pharmacological actions of ginger are anti-diabetic, anti-inflammatory, analgesic, antimicrobial, tissue and radio-protective, anti-emetic, anti-tumorigenic and antioxidant effects (25). The hypertensive effect and antiplatelet effect of *Z. officinale* have been well registered. The study of fresh ginger in anaesthetised rats exhibited a blood pressure lowering effect, possibly through a specific blockade of the voltage-dependent Ca^{2+} channels (26). The study of *Z. officinale* on the pharmacodynamic and pharmacokinetic of losartan in hypertensive rats divulged the hypertension management effect of it (27).

As hypertension is a common illness, and there are medications available in several brand names; thus quality control parameters are required for the analysis of commercial samples. Hence, it is decided to evaluate the powder microscopic, physicochemical values, document thin layer photography and record the HPTLC fingerprint profiles.

Materials and Methods

Plant material

Zaleya decandra root and *Zingiber oficinale* rhizome were collected from the Survey of Medicinal Plants Garden, Mettur, Tamil Nadu and were authenticated by referring floras and chemical test.

Formulation of RPK

The rhizome of *Zingiber officinale* and the roots of *Zaleya decandra* were washed well in running water and then shade dried. The dried samples were powdered and passed through a sieve no. 60. The powdered drugs were mixed thoroughly in the ratio of 1:1 (5).

Powder Microscopy

A pinch of the mixed powdered sample was mounted on a microscopic slide with a drop of 50% glycerol after treating it with 0.1 % chloral hydrate solution. Characters were observed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss ERc5s digital camera under bright field light. Photomicrographs of diagnostic characters were captured and documented (28).

Physico-chemical Analysis

All the physicochemical parameters were evaluated according to the standard methods (28).

Chemicals, Solvents and Instruments

Analytical grade solvents *n*-hexane, toluene, chloroform, ethyl acetate, ethanol, methanol and formic acid were purchased from Merck. Vanillin (1 gm) sulphuric acid in methanol (5%) solution (VSR) was used for visualisation. For HPTLC, Autosampler ATS4, twin trough chambers, visualiser, scanner 4 (Scanner_210441) linked with WINCATS software, plate heater (all from CAMAG, Switzerland) were used.

Extract Preparation

RPK (4 gm) was subjected to successive extraction with 100 ml of each of *n*-hexane, chloroform and ethanol using Soxhlet apparatus. Extracts were concentrated to dryness. The total residues were re-solvated with the corresponding solvents and made up to 10 ml, and transferred into sample vials for TLC application. For hydro alcohol extract, 1 gm of the drug was refluxed with 25 ml of aqueous ethanol (1:1 ratio) for 2 hrs, cooled, filtered and used for TLC in the sample vial.

HPTLC analysis

Successive *n*-hexane, chloroform and ethanol extract, 10 µl each, were applied on three different silica gel $60F_{254}$ pre-coated aluminium plate (6 × 10 cm) as 8 mm band 10 mm from the bottom. The plate containing *n*-hexane extract was developed using toluene: ethyl acetate: formic acid (8: 2: 0.5, *v/v/v*), chloroform extract plate using toluene: ethyl acetate: formic acid (6.0:4.0:1.0, *v/v/v*) and ethanol extract by using toluene: ethyl acetate : methanol: formic acid (3.0:4.0:3.0: 0.5, *v/v/v/v*) separately in pre-saturated twin trough chamber (10 × 10 cm). The developed plates were dried, and photographs were taken under UV, followed by scanning under λ 254 (absorbance mode, D_2 lamp) and λ 366 (fluorescence mode, Hg lamp) respectively with a slit dimension 6 × 0.45 mm and scanning speed of 20 mm/s. The scanned plates were dipped in VSR and heated at 105°C till the appearance of coloured bands. Photographs were taken immediately at white light, followed by scanning at λ 520 (absorption mode, W lamp).

Results and Discussion

Powder Microscopy

The powder microscopic observation showed the following characters, *Zingiber officinale*: suberised cork; scalariform and pitted vessels; septate non lignified fibre; oleoresin and sac shaped starch grains; *Zaleya decandra*: thick-walled cork cells, fibrous layers, crystal fibres, pitted vessels, spiral vessels and heterogenous prismatic crystals (Fig. 1). The powder is pale yellow with a characteristic odour and taste.

Microscopic examination of the powdered compounded formulation revealed suberised cork, oleoresin and sac shaped starch grains which form the diagnostic characteristics of *Zingiber officinale* rhizome and thick-walled cork cells, crystal fibres together with heterogenous prismatic crystals could be attributed to *Zaleya decandra* root. Each species has its own diagnostic character, which can be used to confirm its identity. In the present study, the powder microscopic characterisation of the compound

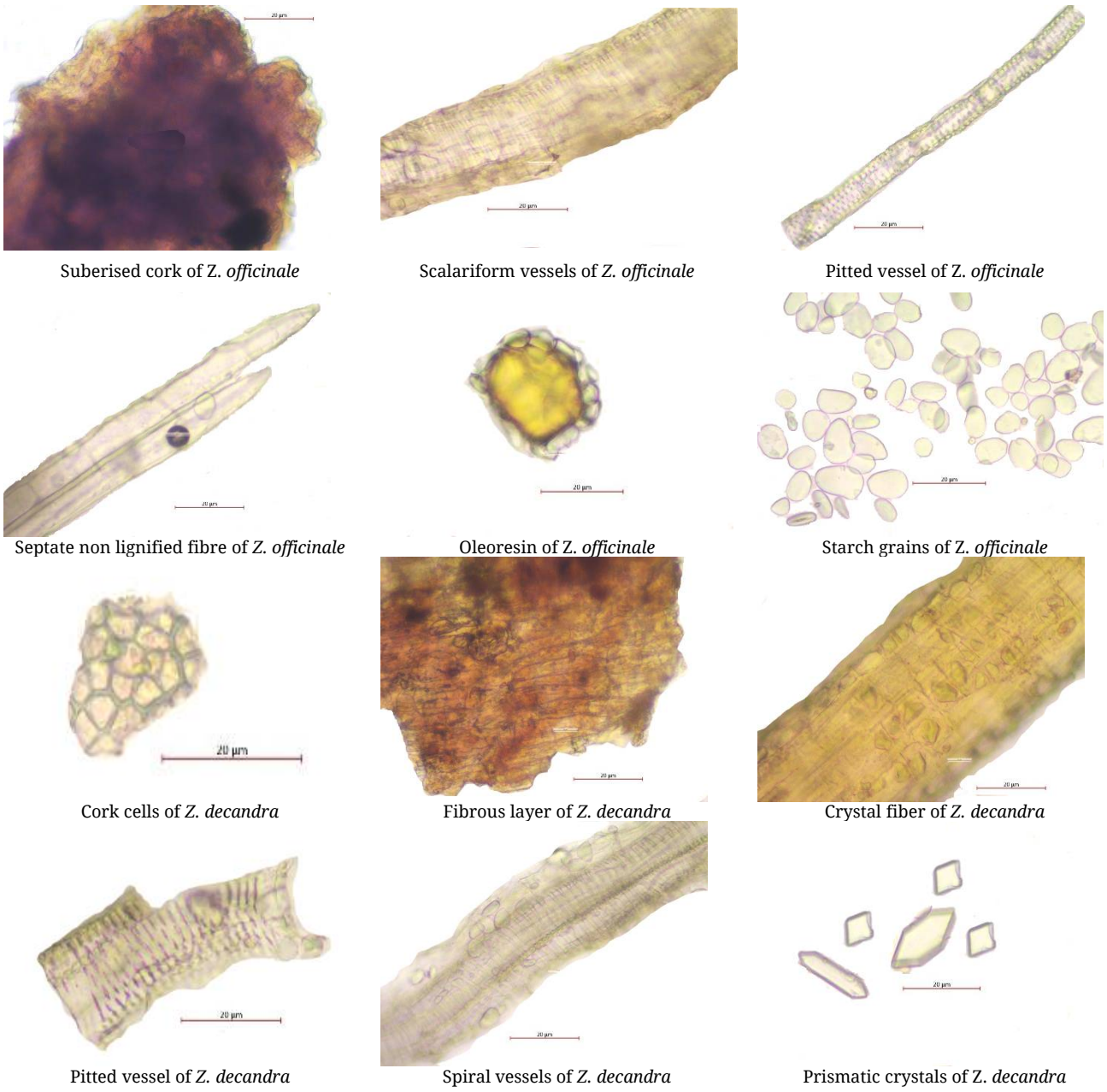

Fig. 1. Powder Microscopy of Rattha Piththathirku Kudinir Chooranam.

formulation has led to the effective diagnosis of the individual herbal drugs used in it.

Physicochemical results

The physicochemical values of the tested drug are presented in Table 1. Drug quality is markedly affected by moisture content denoted by loss on drying (LOD), which alters the shelf life of herbal drug. LOD value was found to be 10.38 ± 0.17 %. Total ash value was determined as 6.87 ± 0.09 %. Total ash contains physiological and non-physiological inorganic salts of phosphates, silicates and carbonates. Acid insoluble ash (AIA) value represents the amount of earthing matters present in roots and rhizomes. AIA was found to be 1.43 ± 0.09%. The alcoholic soluble extractive value was estimated as 17 ± 0.15%, whereas the water-soluble extractive value was 12.26 ± 0.16 %. The pH of the drug was determined as 4.62, which revealed the acidic nature of the drug. The physicochemical values of any herbal drug would vary and the same could be considered for the quality control of drugs (29-31).

TLC Photo documentation

In the TLC photo documentation of *n*-hexane extract of RPK (Supplementary Fig. 1), 8 bands were visualized with R_f 0.11, 0.25, 0.28, 0.33, 0.41, 0.52, 0.67 and 0.73 (all green) under short UV; 8 bands

Table 1. Physicochemical values of *Rattha Piththathirku Kudinir Chooranam*

Parameters	Mean ± SD (n=2)
Loss on drying (105°C) (%)	10.38 ± 0.17
Total ash (%)	6.87 ± 0.09
Acid insoluble ash (%)	1.43 ± 0.09
Water-soluble extract (%)	12.26 ± 0.16
Alcohol soluble extract (%)	5.17 ± 0.15
pH (10 % solution)	4.62 ± 0.01

were visualized with R_f 0.08 (blue), 0.12 (green), 0.18 (blue), 0.25 (green), 0.34 (red), 0.50 (violet), 0.58 (blue) and 0.77 (blue) under long UV and 10 bands were visualized with R_f 0.04 (violet), 0.10 (violet), 0.24 (violet), 0.28 (violet), 0.34 (red), 0.45, 0.69 and 0.77 (all violet).

The photo documentation of successive chloroform extract (Supplementary Fig. 2) revealed 6 bands with R_f 0.03, 0.42, 0.48, 0.54, 0.60 and 0.76 (all green) under short UV; 6 bands with R_f 0.05, 0.23, 0.40, 0.44, 0.54 (all blue) and 0.72 (fluorescent blue) under long UV and 7 bands with 0.03 (brown), 0.23, 0.25, 0.42, 0.52, 0.63 and 0.83 (all violet) under white light post derivatization.

In the successive ethanol extract of RPK (Supplementary Fig. 3), 7 bands with R_f 0.04, 0.09, 0.20, 0.28, 0.47, 0.67 and 0.77 (all green) under short UV; 9 bands with R_f 0.04,0.09, 0.21,0.24, 0.31, 0.45, 0.53, 0.66 and 0.82 (all blue) under long UV and 6 bands for post derivatized plate under white light with 0.05, 0.11, 0.47 (brown), 0.66, 0.72, 0.79 (violet) were observed.

TLC photo documentation of hydro-alcohol extract of RPK (Supplementary Fig. 4) showed 5 bands with R_f 0.09, 0.14, 0.52, 0.66 and 0.70 (all green) under short UV; 5 bands under long UV with R_f 0.16 (blue), 0.59 (blue), 0.66 (blue), 0.67 (light green) and 0.82 (blue) and 6 bands with R_f 0.06 (black), 0.12 (black), 0.29 (black), 0.52 (green), 0.67 (violet) and 0.70 (violet) under white light post derivatization.

HPTLC Densitometry

Scanning of plate developed using *n*-hexane extract of RPK (Supplementary Fig. 1) divulged 13 peaks with R_f 0.11 (area 10.88%), 0.19 (1.96%), 0.25 (13.64%), 0.28 (8.00%), 0.33 (6.45%), 0.41 (5.06%), 0.52 (11.32%), 0.59 (6.64%), 0.67 (11.84%), 0.73 (19.92%), 0.87 (1.67%), 0.89 (2.16%) and 0.97 (0.46%) under short UV; 12 peaks under long UV with R_f 0.08 (3.84%), 10 (1.89%), 0.12 (2.28%), 0.14 (1.85%), 0.18 (4.00%), 0.25 (22.78%), 0.34 (48.84%), 0.50 (1.56%), 0.58 (9.86%), 0.77 (0.56%), 0.83 (0.16%) and 0.93 (2.39%) and 12 peaks with R_f 0.04 (1.29%), 0.10 (12.62%), 0.24 (8.59%), 0.28 (4.99%), 0.34 (10.13%), 0.45 (13.00%), 0.53 (7.33%), 0.69 (16.47%), 0.77 (24.88%), 0.91 (0.21%), 0.96 (0.25%) and 0.98 (0.23%) under white light post derivatization.

For successive chloroform extract (Supplementary Fig. 2) 9 peaks were detected with R_f 0.01 (1.08%), 0.03 (1.51%), 0.20 (0.26%), 0.42 (27.34%), 0.48 (10.30%), 0.53 (13.29%), 0.60 (26.21%), 0.76 (19.40%) and 0.94 (0.62%) under short UV; 10 peaks with R_f 0.06 (1.18%), 0.23 (1.30%), 0.28 (1.06%), 0.40 (7.03%), 0.44 (4.56%), 0.49 (2.86%), 0.54 (5.74%), 0.71 (74.39%), 0.80 (1.42%), 0.86 (0.45%) under long UV and 13 bands with R_f 0.01 (0.76%), 0.03 (0.80%), 0.06 (0.13%), 0.15 (0.24%), 0.23 (3.77%), 0.25 (1.92%), 0.35 (10.91%), 0.42 (16.23%), 0.46 (6.09%), 0.52 (13.84%), 0.63 (23.29%), 0.83 (20.68%) and 0.93 (1.36%) under white light post derivatization.

Successive ethanol extract (Supplementary Fig. 3) showed 8 peaks with R_f 0.04 (0.14%), 0.09 (1.02%), 0.17 (1.01%), 0.20 (2.66%), 0.28 (7.95%), 0.47 (26.62%), 0.67 (41.41%) and 0.76 (19.19%) under short UV; 13 peaks with R_f 0.01 (1.70%), 0.04 (2.08%), 0.09 (0.39%), 0.13 (0.59%), 0.21 (3.61%), 0.24 (1.98%), 0.31 (12.25%), 0.45 (2.74%), 0.53 (5.07%), 0.66 (53.74%), 0.82 (14.37%), 0.95 (0.95%) and 0.97 (0.55%) under long UV and 9 peaks with R_f 0.01 (3.83%), 0.05 (9.72%), 0.11 (20.84%), 0.26 (0.46%), 0.47 (9.43%), 0.66 (22.30%), 0.72 (6.39%), 0.79 (17.15%) and 0.89 (9.88%) in white light post derivatization.

Scanning of hydro-alcohol extract (Supplementary Fig. 4) of revealed 11 peaks with R_f 0.01 (area 13.59%), 0.09 (2.54%), 0.14 (0.48%), 0.17 (0.95%), 0.24 (3.96%), 0.26 (6.23%), 0.34 (0.71%), 0.52 (22.87%), 0.66 (31.70%),0.70 (13.75%) and 0.79 (3.22%) under short UV; 11 peaks with R_f 0.02 (area 48.05%), 0.08 (3.30%), 0.11 (1.18%), 0.16 (11.53%), 0.20 (1.26%), 0.59 (8.85%), 0.66 (9.52%), 0.67 (9.80%), 0.82 (5.63%), 0.96 (0.35%) and 0.97 (0.51%) under long UV and 12 peaks with R_f 0.01 (area 6.28%), 0.06 (10.88%), 0.12 (22.52%), 0.29 (6.39%), 0.34 (3.18%), 0.52 (13.57%), 0.67 (20.84%), 0.70 (11.70%), 0.79 (2.89), 0.87 (0.45), 0.88 (0.93) and 0.95 (0.38%) under white light post derivatization.

Scanning of *n*-hexane extract of RPK revealed major peaks at R_f 0.73 (area 19.92%), 0.25 (13.64%) and 0.67 (11.84%) under short UV; at R_f 0.34 (area 48.84%), 0.25(22.78%) and 0.58 (9.76%) under long UV; and peaks at R_f 0.77 (area 24.88%), 0.69 (16.47%) and 0.45 (13.00%) under white light scanning of post derivatized plate. Scanning of successive chloroform extract revealed prominent peaks R_f 0.42 (area 27.34%), R_f 0.60 (26.21%) and R_f 0.76 (19.40%) under short UV; peaks R_f 0.71 (area 74.39%), R_f 0.40 (7.03%) and 0.44 (4.56%) under long UV and peaks R_f 0.63 (area 23.29%), R_f 0.83 (20.68%) and R_f 0.42 (16.23%) under white light of post derivatized plate. Finger print profile of successive ethanol extract exposed major peaks R_f 0.67 (area 41.41%), R_f 0.47 (26.62%) and R_f 0.76 (19.19%) under short UV, peaks R_f 0.66 (area 53.74%), R_f 0.82(14.37%) and R_f 0.31 (12.25%) under long UV and peaks R_f 0.66 (22.30%), R_f 0.11(20.84%) and R_f 0.79 (17.15%) under white light of post derivatized plate.

Densitometric scan of hydro-alcohol extract of RPK divulged paramount peaks R_f 0.66 (area 31.70%), R_f 0.52(22.87%) and R_f 0.70 (13.75%) under short UV, peaks R_f 0.02 (48.05%), R_f 0.16 (11.53%) and R_f 0.66(9.52%) under long UV and peaks R_f 0.12 (area 22.52%), R_f 0.67 (20.84%) and R_f 0.52 (13.57%) under white light of post derivatized plate. The TLC/HPTLC of successive hexane, chloroform, ethanol would reveal the presence of a number of phytoconstituents which are low polar, medium polar and high polar respectively (32-34).

Conclusion

The powder microscopic characters, TLC/HPTLC photos and fingerprint profiles are distinct to *Rattha Piththathirku Kudinir Chooranam* and the same could be applied for the quality control of the drug as pharmacopoeial reference standards.

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Authors' contributions

AKM, ST and RA carried out the physicochemical parameters and TLC/HPTLC studies. KGD and KNSK contributed to microscopic studies and RS designed the study, compilation, coordination and final proofing.

Conflict of interests

The authors declare no conflict of interest.

Supplementary files

Fig. 1. TLC/HPTLC of n-hexane extract of Rattha Piththathirku Kudinir Chooranam.

Fig. 2. TLC/HPTLC of chloroform extract of Rattha Piththathirku Kudinir Chooranam.

Fig. 3. TLC/HPTLC of ethanol extract of Rattha Piththathirku Kudinir Chooranam.

Fig. 4. TLC/HPTLC of hydro-alcohol extract of Rattha Piththathirku Kudinir Chooranam.

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