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## Phytochemical analysis of siddha polyherbal formulation arathai kudineer churnam

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**Abstract**

Recently, there is a surge among people in choosing alternative systems of medicine, especially Siddha, over modern medicine. Hence, it is very much essential to standardise Siddha drugs as specified by World Health Organisation. In the present study, we carried out phytochemical analysis of Siddha drug formulation Arathai Kudineer Churnam. HPTLC finger printing profile was developed in addition to assessing total phenols and flavonoids. The formulation was also screened for its cytotoxic effects against HepG2 liver carcinoma cells. HPTLC fingerprinting along with quantitative determination suggests the presence of several key phytochemical components including phenols and flavonoid compounds. Arathai kudineer significantly inhibited the growth of HepG2 cells growth as well as depicted significant inhibition of free radicals in a dose dependent manner. Detailed studies will be conducted to characterise the bioactive components as well as elucidate the potential of Arathai kudineer to be used as a viable therapeutic agent.

**Keywords:** Siddha formulation, arathai kudineer, HPTLC, DPPH, HepG2

**1. Introduction**

Siddha Medicine' is an ancient indigenous medical practice that dates back between 10,000BC and 4,000BC which is written in the ancient language 'Tamil'. The alchemy is believed to be given by 18 Siddhars (Ancient Scientists) including the spiritual sage, Agastiyar<sup>[1]</sup>. Siddha medicine is a conglomeration of 96 philosophies, 7 basic tissues and 3 humors present in the human body. According to Siddha, the total number of diseases is 4448 which are mainly categorised based on the vitiation of humor or clinical symptoms<sup>[2]</sup>. It is said to be the most conserved medical practices in the world due to its enigmatic nature and consists of several rejuvenating and life-prolonging treatments<sup>[1]</sup>. However, many Siddha drugs have not been characterised completely using modern scientific methods and the key bioactive components are yet to be explored to a greater extent. Owing to increased usage of Siddha Medicine by the people in the recent times<sup>[3, 4]</sup>, it is essential to standardise Siddha drugs to assess its potentiality in treating acute and chronic disorders at par with the modern system of medicine and for the betterment of the society<sup>[5, 6]</sup>. The present study involves preliminary investigation of the Siddha Polyherbal formulation 'Arathai Kudineer Churnam', and assessment of cytotoxic effect *in vitro*. Both the varieties of Arathai (*Alpinia galanga* & *Alpinia officinarum*) have been used in formulation. The churnam is used in treating immunocompromised disorders such as eczema, arthritis, respiratory infections, Dropsy and fever<sup>[7]</sup>. In addition, the bioactive components reported in the plant possess various therapeutic activities.

**2. Materials and Methods****2.1 Materials****2.1.1 Polyherbal formulation**

The Siddha polyherbal formulation 'Arathai Kudineer Churnam' was prepared by standard methods by the Pharmacy of Siddha Central Research Institute, Chennai, Tamil Nadu, and India based on the Siddha Text (SFI 2014). The Siddha Polyherbal formulation contains five plants namely *Alpinia galanga* (L.) Willd. *Abies webbiana* (Wall. ex D. Don) Lindl, *Alpinia officinarum* Hance, *Glycyrrhiza glabra* L. and *Piper longum* L. in equal parts (details in Table 1). The formulation was identified by Dr. K.N. Sunil kumar Narayanan, department of Pharmacognosy, Siddha Central Research Institute, Chennai.

**Table 1:** Ingredients of the Arathai Kudineer

S. No	Binomial Name	Common Name	Traditional Name	Part Used	Medicinal Uses
1	<i>Alpinia galanga</i> (L.) Willd.	Greater Galangal	Perarathai	Rhizome	Analgesic [8], Respiratory ailments [9], Antimalarial [10], hepatic protective [11]
2	<i>Abies webbiana</i> (Wall. ex D. Don) Lindl.	Talispatra	Thalisapathri	Leaves	Bronchodilation; antiviral, antifungal, antimicrobial [12], anti-inflammatory [13]
3	<i>Alpinia officinarum</i> Hance	Lesser Galangal	Citrarathai	Rhizome	anti-inflammatory, anticancer, antioxidant, antibacterial [14]
4	<i>Glycyrrhiza glabra</i> L.	Liquorice	Adhimadhuram	Rhizome	Anti-inflammatory, spasmolytic, laxative, anti-depressive, anti-ulcer and anti-diabetic [15], neuroprotective [16]
5	<i>Piper longum</i> L.	Long Pepper	Arisi Thippili	Fruit	Immunomodulatory, anti-oxidant, anti-asthmatic, anti-carcinogenic, anti-inflammatory, anti-ulcer, and anti-amoebic properties [17], Hepatoprotective [18]

### 2.1.2 Experimental chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, ascorbic acid, gallic acid, quercetin, Nitro Blue Tetrazolium (NBT), 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), Antibiotics and antimycotics were purchased from SRL Pvt. Ltd. (Mumbai, India). DMEM was purchased from Lonza Biotech. Fetal Bovine Serum (FBS) was purchased from GIBCO. All other reagents, solvents and chemicals were of analytical grade and procured locally in Chennai, India.

### 2.1.3 Preparation of the Extract

75g of the crude drug was immersed in a mixture containing water and ethanol (1:1) for 48 hours. The extract was filtered and then concentrated using a rotary evaporator at 55°C with a pressure of 100mbar. The extract was stored in separate air tight container.

### 2.1.4 HPTLC Finger Printing

HPTLC for creating a phytochemical profile of Arathai Kudineer Churnam was performed by standard procedure using CAMAG HPTLC apparatus [19, 20].

### 2.1.5 Estimation of Total Phenolic Content

The total phenolic content in the formulation was determined by Folin-Ciocalteu method using Gallic acid as a reference standard [21]. The amount of phenolic content was expressed in terms of Gallic acid equivalents.

### 2.1.6 Determination of Flavonoid Content

The flavonoid content was estimated using quercetin as a reference standard [22]. The flavonoid content was expressed in terms of Quercetin equivalents.

### 2.1.7 Antioxidant Analysis

#### 2.1.7.1 DPPH scavenging activity

DPPH scavenging activity was carried out using quercetin as reference standard [23]. DPPH alone served as control while methanol alone served as blank. The activity was expressed in terms of IC<sub>50</sub>.

#### 2.1.7.2 Superoxide radical scavenging activity

The assay was performed using NBT [24]. NBT and DMSO together served as control while DMSO alone served as blank.

### 2.1.8 Cell Culture and Cytotoxicity Assessment

HepG2 cells were purchased from National Center for Cell Sciences (Pune, India). The cells were cultured in DMEM supplemented with 10% FBS, 100 U/mL penicillin and 100 µg/mL streptomycin in 5% CO<sub>2</sub> at 37°C. Upon achieving more than 90% confluency, approximately 5X10<sup>4</sup> cells were seeded in 96-well plate and maintained in the aforementioned

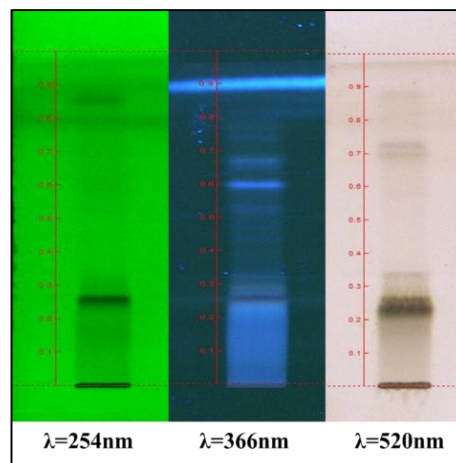
conditions. After 24 hours, the cells were treated with arathai kudineer churnam extract (concentration 25-250 µg/mL). Cytotoxicity was assessed using 3-(4, 5-Dimethylthiazol-2-Yl)-2, 5-Diphenyltetrazolium Bromide (MTT) [25]. DMSO served as control.

### 2.2. Statistical Analysis

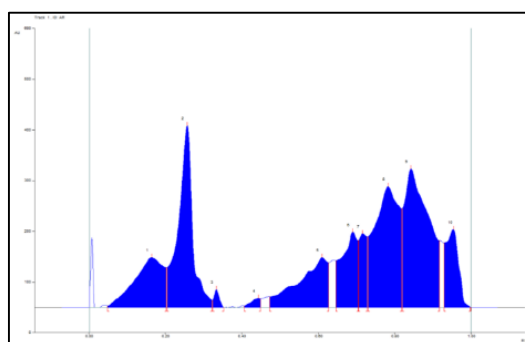
The experiments were performed in two different sets with each set triplicated. The data was analysed using Graph Pad Prism version 6.01 and the data is expressed in terms of mean ± standard error mean (SEM).

### 3. Results and Discussion

HPTLC fingerprinting analysis of *Arathai Kudineer Churnam* revealed the presence of several phytochemical constituents (Figure 1a). When viewed in UV range, the compound separation was dense at peaks 9, 8 and 2 when viewed at 254nm (Figure 1b) with 22.91%, 19.87% and 17.67% density. At 366nm (Figure 1c), peaks 13, 3 and 2 showed maximum density of 31.83%, 20.72% and 15.86% respectively. In the visible range, at 520nm (Figure 1d), the compound separation was more than 50% that is 57.71%.



**Fig 1a:** HPTLC Finger Printing



**Fig 1b:** HPTLC Profile at 254nm

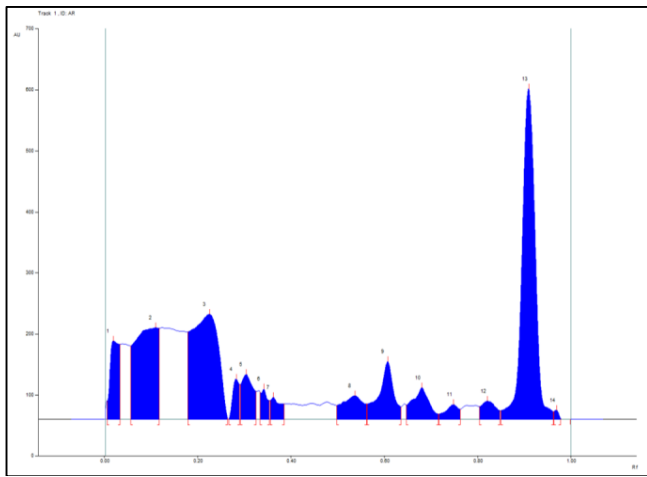


Fig 1c: HPTLC Profile at 366nm

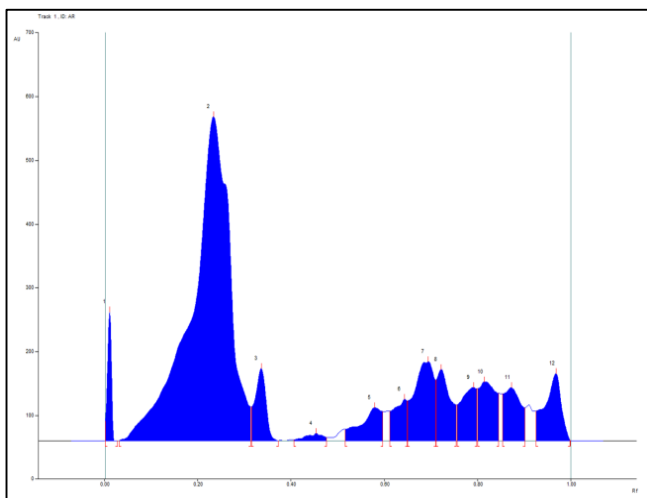


Fig 1d: HPTLC Profile at 520nm

The amount of total phenolic content (Figure 2) was found to be  $404.8 \pm 18.194$  mg/g of gallic acid equivalents. The flavonoid content (Figure 2) was found to be  $297.8 \pm 10.933$  mg/g of quercetin equivalents. It is evident that Arathai Kudineer Churnam may contain many key phytochemical constituents.

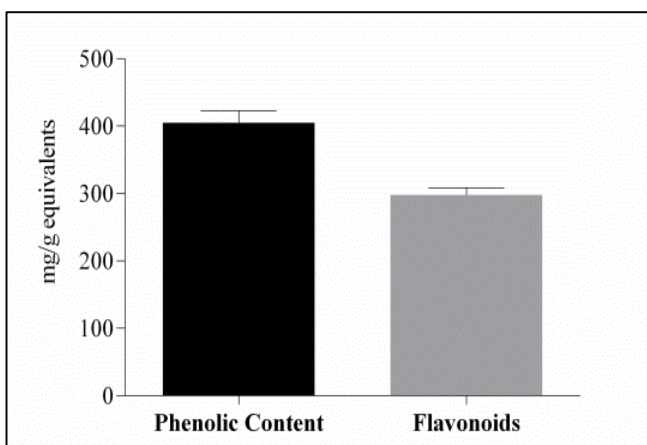


Fig 2: Quantitative Analysis of Phytochemical Constituents

The inhibitory activity of DPPH radical (Figure 3a) by quercetin ( $IC_{50} = 36.99 \pm 2.206$   $\mu$ g/ml) was better when compared to the scavenging activity of Arathai Kudineer Churnam ( $IC_{50} = 53.65 \pm 3.439$   $\mu$ g/ml). The superoxide radical scavenging activity (Figure 3b) of ascorbic acid

( $IC_{50} = 17.88 \pm 0.620$   $\mu$ g/ml) was better when compared to the Arathai Kudineer Churnam ( $IC_{50} = 32.85 \pm 0.851$   $\mu$ g/ml).

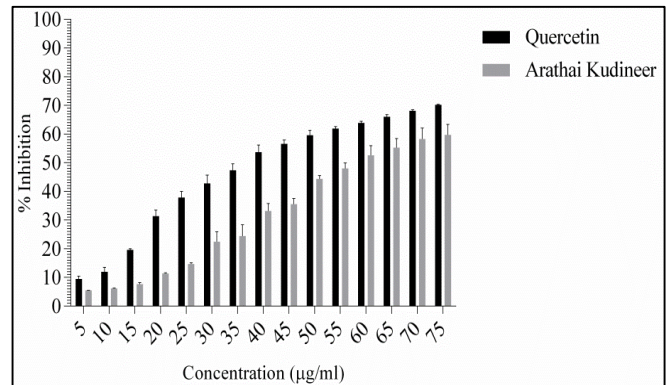


Fig 3a: DPPH Radical Scavenging Activity

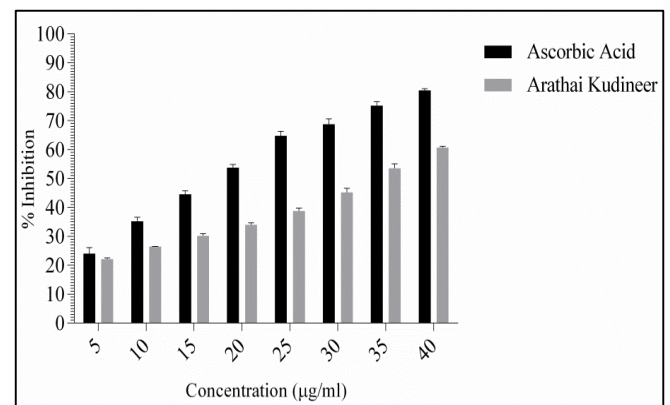


Fig 3b: Superoxide Radical Scavenging Activity

Arathai Kudineer Churnam is generally used for treating digestive complaints of Kapha imbalance, cough with sputum, headache, fever and painful joints and swelling (*Siddha Vaidya Thirattu*). This is the first time its cytotoxic potential has been assessed using HepG2 hepatocarcinoma cells. Cytotoxicity increased with increase in the concentration with an  $IC_{50}$  of  $331.883 \pm 10.931$  mg/ml.

Generally, HPTLC is chiefly used for phytochemical profiling of herbal drugs, validating its composition and also to overcome adulteration, etc., [26]. This is the first time phytochemical profile is being reported for Arathai Kudineer Churnam for identifying the richness of phytochemical constituents present. The quantification of phytochemical components, antioxidant and the cytotoxicity assays elucidate the potency of Arathai Kudineer Churnam. The individual components of the formulation have the tendency to detoxify and are also used in treating several disorders. Further studies will be carried out to explore the potential of Arathai Kudineer Churnam in treating hepatocellular carcinoma using *in vivo* models and characterise specific compounds which we will enable to develop new therapeutic compounds.

#### 4. Conclusion

The present study provides us an overview of the Siddha Polyherbal formulation arathai kudineer possessing several phytochemical constituents and its activity against hepatocarcinoma cells. A detailed study will be carried out to characterise the formulation and develop it as a viable therapeutic alternative.

#### 5. Funding

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## 6. Acknowledgement

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## 7. Conflict of Interest

The authors declare no conflict of interest.

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