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ANALYTICAL TECHNIQUES APPLIED ON SIDDHA MEDICINAL PLANT *ALPINIAOFFICINARUM*. HANCE. (CHITRARATHAI) FOR STANDARDIZATION ASPECTS

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ABSTRACT

The pharmacology of the Siddha system has three chief divisions the plant pharmacology, mineral pharmacology and animal pharmacology. The plant pharmacology has more than 530 herbals explained. The analytical techniques were planned to elucidate bioactive efficacy of *Alpiniaofficinarum* the crude, aqueous and ethanolic fractions isolated from *Alpiniaofficinarum* was evaluated against pathogenic bacteria. The crude samples were further screened using thin layer chromatographic method and confirmed by spectrometric analysis. The atomic absorption spectrometric analysis showed that *Alpiniaofficinarum* rhizomes contain essential elements like Cu, Zn & Mg and the heavy metals were below permissible limits. The ethanolic extraction of *Alpiniaofficinarum* rhizomes has been standardized. Thin layer chromatographic analysis shows the presence of 4 diffenrent active principles in ethanolic extract. The GC MS analysis confirms the presence of active components. The absorbance maxima (λ max) studies of ethanolic extract characteristically was in the terminal UV range substantiates the presence of flavonoids and heptanoids in the extract. The observed antibacterial activity against E coli, Salmonella, Staphylococcus, Klebsiella and Proteus enumerates its bio efficiency and validates its potential use in Siddha system of medicine.

INTRODUCTION

The Siddha system of medicine is one of the ancient systems contemporaneous with those of the submerged lands, Egyptian, Mesopotamian, Chinese and Grecian medicines. The unique nature of this system is its continuous service to humanity for more than five thousand years in combating diseases and in maintaining physical, mental and moral health[1]. The pharmacology of the Siddha system has three chief divisions the plant pharmacology, mineral pharmacology and animal pharmacology. The plant pharmacology has more than 530 herbals explained[2]. *Alpiniaofficinarum.* Hance. Which is called (*Chitrarathai*) as Lesser Galangal of the family Zingiberaceae has been traditionally used in the Siddha system of medicine with much importance[3].

Pharmacognostical Perspective

The rhizome of the herb is used medicinally. The rhizome has an aromatic odour and spicy to taste[4]. *Alpiniaofficinarum.* Hance is similar in appearance to the sword lily. It is a perennial herb[5]. Mother rhizomes used as seed material gave higher yield of both essential oil and oleoresin content. Stage of harvesting does not influence yield[6, 7].

Lesser galangal has antispasmodic, antiphlogistic and antibacterial effects. It is used for loss of appetite, dyspeptic complaints, fevers, cold, cough/bronchitis, tendency for infections, liver and gallbladder complaints, inflammation of the mouth and pharynx,[8]. Galangal is also used for painful upper abdominal syndrome of the Roemheld complex type and for sluggish digestion[9]. Antioxidative compounds were isolated from the methanolic extract of fresh rhizome of smaller galangal (*Alpiniaofficinarum*. Hance)[10]. The rhizome of *Alpiniaofficinarum*. Hance is recommended in the form of its decoction for the treatment of rheumatism. The decoction obtained from it contains eugenol, cineole, pinene, cadinene and a methyl derivative of cinnamic acid[11]. In search of potent anti-inflammatory compounds from dietary components, a diarylheptanoid molecule (7-(4'-hydroxy-3'-methoxyphenyl)-1-phenylhept-4-en-3-one) was isolated from rhizomes of *Alpiniaofficinarum* by bioassay directed fractionations. The effect of this compound was tested on lipopolysaccharide induced activation of various inflammatory responses in vitro using mouse macrophage cell, RAW 264.7 and normal healthy volunteers peripheral blood mononuclear cells (PBMCs)[12]. The analytical techniques were planned to elucidate bioactive efficacy of *Alpiniaofficinarum* the crude, aqueous and ethanolic fractions isolated from *Alpiniaofficinarum* was evaluated

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against pathogenic bacteria. The crude samples were further screened using thin layer chromatographic method and confirmed by spectrometric analysis.

MATERIALS AND METHODS

The raw drug of Siddha Medicinal plant *Alpiniaofficinarum.* Hance. *(Chitrarathai)* was subjected for various analyses and is applied for standardization and anti bacterial activity for the evaluation. Analytical tests of AAS, GC-MS, UV Visible Spect rometer, TLC analysis, Extraction and Anti bacterial activity were done by applying standard protocols.

Collection and authentication of plant material

The dried rhizomes of *Alpiniaofficinarum.* Hance were procured from authorized suppliers from Chennai. The identity and authenticity of the *Alpiniaofficinarum.* Hance rhizomes was confirmed at Centre for Advanced Studies in Botany, University of Madras.

AAS Study on contents of trace elements

Apparatus

Perkin Elmer, USA model AAS (Sophisticated Instrument Analysis, IIT Madras)

Sampling

100 gms of dried rhizomes were powdered using a pulverizer. The coarse powder of the dried rhizomes of *Alpiniaofficinarum.*Hance.*n* was taken to analyse the trace elements and heavy metals by AAS analysis.

Reagents

All reagents were of analytical grade. Double distilled deionized water was used for all dilutions. Conc. Nitric acid and Conc. Sulphuric acid were of suprapur quality. All glassware was cleaned by soaking in dilute nitric acid and was rinsed with distilled water prior to use. The element standard solutions used for calibration were prepared by diluting a stock solution of 1000 mg/l. Pb, Cd, Hg & Co was supplied by Sigma and Cu & Zn by Aldrich[13].

Digestion procedure

5 grams of the dried *Alpiniaofficinarum.* Hance. rhizome was powdered in a pulveriser and is cast into high form porcelain crucibles. The crucibles are then placed in the Muffle furnace and a temperature of 650º C is maintained for 12 hours in order to dry, ash, and atomize the sample[14]. The ash formed was collected and dissolved in 5 ml of Conc. Nitric acid (25% v/v) and 10 ml of Conc. Sulphuric acid, the mixture when necessary was heated slowly to dissolve the residue. The solution was transferred to 50 ml volumetric flask and made up to volume[15]. The sample solution was clear and ready for analysis.

Analytical procedure

Detection limit is defined as the concentration corresponding to three times the standard deviation of ten blanks. Detection limit values of elements as mg/ml in AAS were 0.005 for Cd, 0.03 for Hg, 0.1 for Li, etc. Sample volume, ramp and hold times for the drying, ashing, atomization and cleaning temperatures were optimized before analysis to obtain maximum absorbance and minimum background.

Aqueous and Ethanolic extraction of Alpiniaofficinarum. Hance

100 grams of the dried *Alpiniaofficinarum.*Hance. rhizomes were powdered in a pulveriser and divided equally for aqueous and ethanolic extracts. The aqueous extract was obtained with the help of Soxhlet's apparatus. The extract was lyophlised and stored in a glass vial. The ethanolic extract was taken by cold storage process where the cycles were repeated by adding fresh ethanol to the extract. The extract was concentrated and stored[16].

TLC analysis of crude extracts of Alpiniaofficinarum. Hance

TLC was used to identify compounds and its purity. As a stationary phase a special fine silica gel plates (Merck precoated TLC plates) were used to analyse the extracts. 5 microlitre of the crude extract was spotted and developed with various solvents as follows,

1. Benzene : Methanol 1 : 2
2. Methanol : Benzene 1 : 2
3. Acetone : Benzene 1 : 1
4. Acetone : Methanol 1 : 1
5. Toluene : Methanol 1 : 2
6. Methanol : Toluene 1 : 2
7. Ethyl acetate : Methanol 2 : 1
8. Benzene : Methanol : Chloroform 1 : 1 : 1
9. Acetone : Methanol : Chloroform 1 : 1 : 1
10. Toluene : Methanol : Chloroform 1 : 1 : 1
11. Benzene : Methanol : Toluene 1 : 2 : 1
12. Benzene: Methanol: Toluene: Chloroform 0.5: 2: 0.5: 1

Toluene: Methanol: Chloroform, 1 : 1 : 1 was optimized for the TLC separation of *Alpiniaofficinarum.*Hance. extract and the spots were visualized in Iodine chamber. R_f values were calculated.

Gas chromatography-mass spectrometry (GC/MS) studies on Alpiniaofficinarum. Hance. extracts

Apparatus

JEOLGC mate

Sampling

100mg of Al. extract was dissolved in 1 ml of ethanol and 0.50 ml of the supernatant transferred to an auto sampler vial.

Procedure

The diluted sample is transferred to an auto sampler vial of the GC instrument. The GC instrument is operated in the ionization mode EI+ and the sample was analysed. In full scan mode a standard chromatogram of the mass details is taken. Then the mass spectra of the peaks were analysed separately and the respective chromatograms where taken[17].

UV-Visible Spectrometer studies on Alpiniaofficinarum. Hance. extracts to detect absorbance maxima (λ max)

Apparatus

UV-1601, SHIMADZU, UV-Visible Spectrometer

Samples

100mg of Al. extract was dissolved in 1 ml of ethanol and made up to 3ml.

Procedure

The diluted aqueous extract was taken in silica cuvettes and analysed for absorbance maxima in two spectrum i.e., 300-1100 nm and 190-300 nm against the control.

Similarly, the diluted ethanolic extract was taken in silica cuvettes and analysed for absorbance maxima in two spectrum i.e., 300-1100 nm and 190-300 nm against the control[18].

Antibacterial activity of Al. extracts Apiniaofficinarum

Sampling

100mg of Al. extract was dissolved in 1 ml of ethanol and made upto 100 ppm.

Materials

- Disposable sterilized Petri dishes were used for the antibacterial studies.
- Bacterial strains were procured from Centre for Advanced Studies in Botany, University of Madras.
- Mueller-Hinton Agar media was used for the media for bacterial culture on the Petri dishes.
- Standard sterilized paper disks were used for loading samples.

Procedure

Samples were loaded to the sterile disks, under a laminar flow at the concentration of 30µl, 60µl & 90µl and were allowed to dry[19]. Mueller-Hinton Agar media was prepared and sterilized in an autoclave. The media was poured into the sterilized Petri dishes and was allowed to solidify. The bacterial strains were cultured and spread on the media using a cotton swab[20]. Then the loaded disks were placed on the spread bacteria. The Petri dishes were sealed using standard microfilm to prevent contamination[21].

The culture was incubated for twenty four hours and the inhibition was captured using a Nikon digital camera.

RESULTS AND DISCUSSION

Heavy metal analysis

The crude powder of the rhizomes of *Alpiniaofficinarum.* Hance. was analysed by atomic absorption spectrometry which showed the presence of the contents as shown in table 1.

Table.1 Heavy metal and trace elements analysis of crude rhizome powder of *Alpiniaofficinarum.* Hance.

S.NO	Trace Elements & Heavy Metals	Normal Values*	Sample (ppm)
1	Lead (Pb)	0.45-0.75	0.372
2	Mercury (Hg)	0.01-0.045	-0.485
3	Cadmium (Cd)	0.07-0.85	0.714
4	Copper (Cu)	2-5	0.435
5	Zinc (Zn)	15-20	0.945
6	Magnesium (Mg)	300-350	41.02
7	Cobalt (Co)	0.25-0.5	0.009
8	Arsenic (As)	0.015-0.05	0.021

*Normal dietary allowance prescribed by World Health Organization (WHO) in mg/day.

It is evident from table 1 and Fig - 1, that all the heavy metals and trace elements are under the permissible limits for human intake.

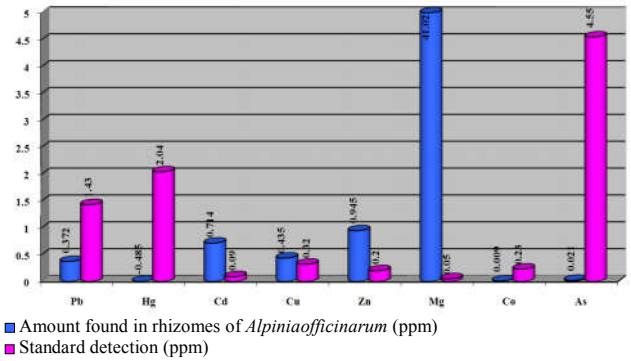

Fig.1 Heavy metals and trace elements analysis of *Alpiniaofficinarum*

Chromatographic separation of Alpiniaofficinarum.Hance. Hance compounds

TLC analysis of Alpiniaofficinarum. Hance. Hance extracts

The optimum solvent concentration was optimized and Toluene: Methanol: Chloroform, 1:1:1 was taken to fractionate the crude *Alpiniaofficinarum.* Hance. ethanolic extracts. The TLC showed four fractioned spots were visualized with the R_f values 0.042, 0.286, 0.336, 0.385. (Table – 2, Fig – 2)

Table – 2 TLC analysis of *Alpiniaofficinarum.*Hance. ethanolic extracts

Solvent	Ratio	R_f
Toluene:Mehanol:Chloroform	1:1:1	0.042 0.286 0.336 0.385
Toluene:Methanol	2:1	0.275 0.385 0.452
Benzene:Methanol	2:1	0.425 0.450

TLC analysis of *Alpiniaofficinarum* crude ethanolic extract

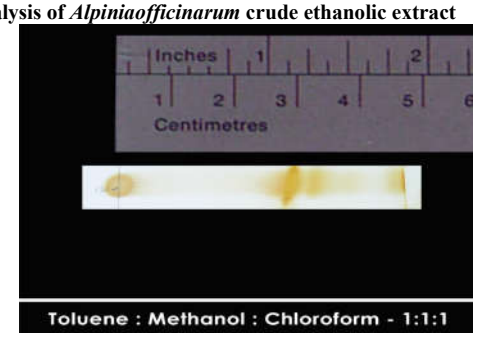

Fig 2

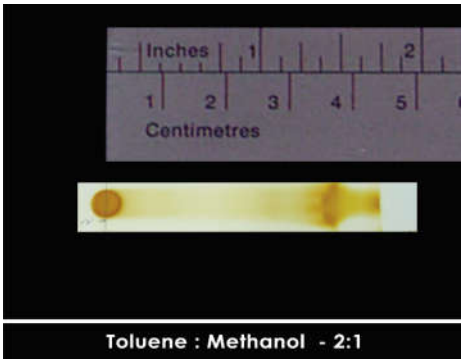

Fig 3

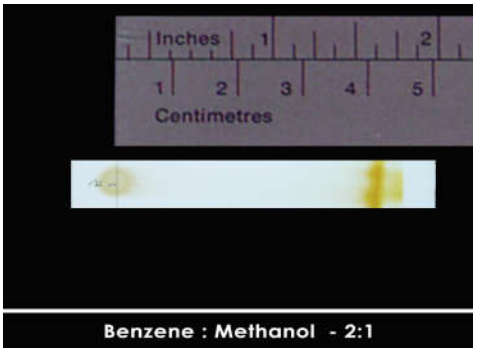

Fig.4

GC Ms Analysis of extracts of Alpiniaofficinarum. Hance.

Fig- 5 gives the GC MS details of crude Alpinia officinarun ethanolic extracts. A total of four peaks with the following retention time and the respective mass details (8.6, 418) (9.37, 456) (12.7, 623) (12.95, 634) were elucidated.

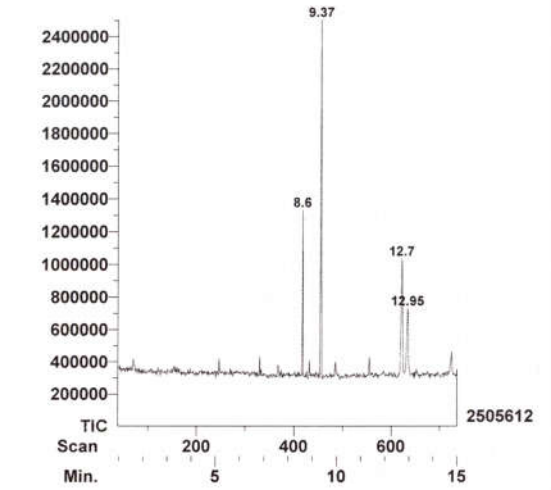

Fig5 GC-MS Spectral Analysis of Ethanolic Extract of Alpiniaofficinarum

Spectral analysis of extracts of Alpiniaofficinarum. Hance.

Crude ethanolic extract analysed spectrometrically shows the absorbance maxima in the Ultra Violet spectrum of light. The ethanolic extract had the λ max with their corresponding absorbance as follows, (361, 0.55) (268.76, 0.92) (211, 2.34). (Fig – 6)

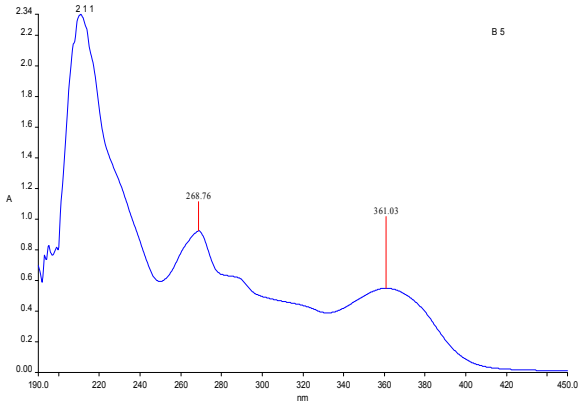

Fig. 6 UV Spectral Analysis of Ethanolic Extracts of Alpiniaofficinarum

Antibacterial activity of ethanolic extract of Alpiniaofficinarum. Hance.

The ethanolic extract of *Alpiniaofficinarum*.Hance. rhizomes were found to have antibacterial activity against Proteus (Fig – 7), E coli (Fig – 8), Klebsiella (Fig – 9), Salmonella (Fig – 10) and Staphylococcus (Fig – 11). The radial inhibitory zone was identified for all the concentration of crude ethanolic extract (30µl, 60µl and 90µl). As the concentration increases there is a gradual increase in the radial inhibitory zone size for all the pathogenic organisms tested.

Antibacterial activity of Alpinia officinarum crude ethanolic extract

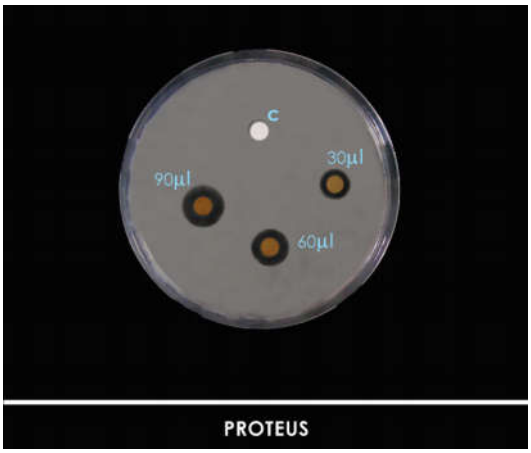

Fig. 7

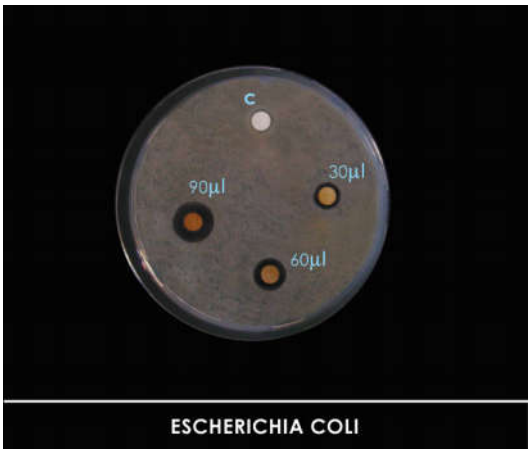

Fig.8

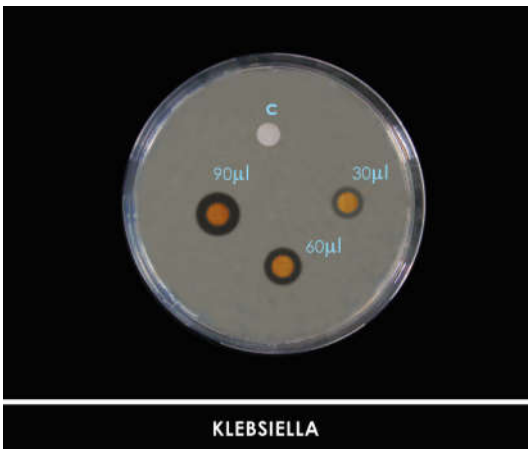

Fig. 9

Fig. 10

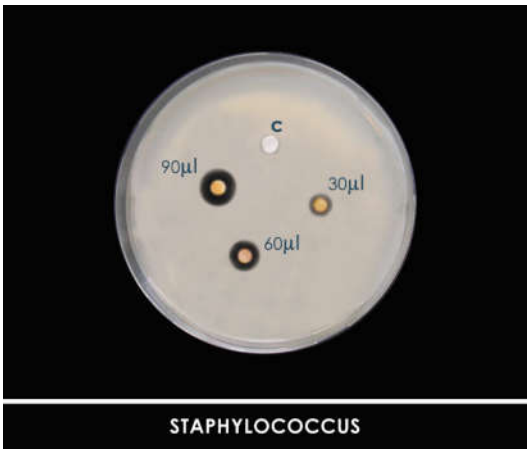

Fig 11

CONCLUSION

The present study is an attempt to explore the efficacy of *Alpiniaofficinarum* rhizomes and scientifically validate the ancient use of the drug. Various techniques like chromatographic analysis, UV spectrophotometeric analysis, Atomic absorption spectrometric determination of heavy metals and GC Ms analysis was carried out. The various assays carried out were compared with the previous literatures and the authenticity of the drug was confirmed. From the observation the following interferences could be drawn, the atomic absorption spectrometric analysis showed that *Alpiniaofficinarum* rhizomes contain essential elements like Cu, Zn & Mg and the heavy metals were below permissible limits. The ethanolic extraction of *Alpiniaofficinarum* rhizomes has been standardized. Thin layer chromatographic analysis shows the presence of 4 diffenrent active principles in ethanolic extract. The GC MS analysis confirms the presence of active components. The absorbance maxima (λ max) studies of ethanolic extract characteristically was in the terminal UV range substantiates the presence of flavonoids and heptanoids in the extract. The observed antibacterial activity against E coli, Salmonella, Staphylococcus, Klebsiella and Proteus enumerates its bio efficiency and validates its potential use in Siddha system of medicine.

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