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Naga Lakshmi and Manoharan

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Original article

Physico-chemical and Phytochemical Evaluation of Siddha Drug Amirdhavalli Chooranam

M Naga Lakshmi*, A Manoharan

Department of Pothu Maruthuvam, Government Siddha Medical College, Palayamkottai, Tirunelveli-627002, Tamilnadu, India.

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Corresponding author *
M Naga Lakshmi, PG Scholar, Department of Pothu Maruthuvam, Government Siddha Medical College, Palayamkottai, Tirunelveli-627002, Tamilnadu, India.
Email:nagalakshmimuthumalai@gmail.com

ABSTRACT:
Background: Standardization of the drug brings the validation to be used as a medicine by subjecting the drug into many analysis and determining its quality and effectiveness. Amirdhavalli Chooranam. The classical siddha drug is indicated for Madhumega noi (Diabetes Mellitus). Aim: The aim of the present study is to investigate the Physico-Chemical parameters like Ash value, Extractive value and Loss on Drying, etc and Preliminary Phytochemical analysis such as High Performed Thin Layered Chromatography, Fourier Transport Infra-red Rays analysis, Powder Microscopy and UV-Visible Spectroscopy of Amirdhavalli Chooranam. Methods: The dried parts of whole plant of Seendhil were ground into fine powder and filtered to get the chooranam. The study was carried out as per Pharmacopeial laboratory standards of Indian Medicine.
Results: Preliminary Phytochemical screening found, the presence of phytochemicals such as saponin, glycosides and alkaloids. The ash value is 8.34% , loss on drying is 8.54% and high performance thin layer chromatography finger printing revealed the presence of many phytochemicals with different Rf values and densitometric scan of the plates showed numerous bands and peaks. FT-IR analysis was used to identify the functional groups of compounds such as alcohol, phenols, carboxylic acid, alkenes etc. The Powder Microscopy reveals the presence of crystal fibers, scalariform vessel, starch grains, stone cells, reticulate vessels. UV-Visible Spectroscopy peaks showed the peak value with appropriate absorption value. Conclusion:A study concluded the bioactive components like saponin, glycosides, alkaloids are effective in the Management of Madhumegam.

Keywords: Amirdhavalli Chooranam, Physico-chemical, High performance thin layer chromatography finger printing, Fourier Transport Infra-Red rays analysis, Powder microscopy, UV-Visible Spectroscopy.

1. INTRODUCTION

The Siddha system of medicine is one of the oldest medical systems known to mankind. It is assumed that when the normal equilibirium of the three humors (vatha, pitha, kapha) is disturbed, disease is caused [1]. Siddha Medicine including mostly plants which plays an important role in preventive and curative. Medicinal plants are richest bio resource of drugs in traditional system of medicine and its physico-chemical and phytochemical properties responsible for different colours, flavours and smells of plant. These medicinal values of plants are in some chemically active substance they produce a definite physiological action on human body [2].

Amirdhavalli Chooranam is prepared from the plant Seendhil (*Tinospora cordifolia*). The plant is a large, glabrous, deciduous climbing shrub belonging to the Menispermaecae family. It is reported the medicinal properties like Anti-Diabetic, Anti-Spasmodic, Anti-Oxidant, Hepatoprotective, Immuno-modulatory and anti-neoplastic activities [3, 4]. The study was designed to screen the Physico-chemical and Phytochemical analysis of Amirdhavalli Chooranam and the work was carried out in Siddha Research Regional Institute, Thiruvanandhapuram, Kerala.

2. MATERIALS AND METHODS

The whole plants of Seendhil were collected from in and around places of Palayamkottai. The raw drug is identified and authenticated by Medicinal Botanist of Govt. Siddha Medical College, Palayamkottai. All the parts of the plant of Seendhil were shade dried ground into fine powder and filtered to get the chooranam. The chooranam is stored in air tight container and labelled as “Amirdhavalli Chooranam” which was used for experimental purposes.

2.1. Physico chemical analysis:

The physico-chemical analysis such as determination of loss on drying, total ash value,acid insoluble ash,water soluble

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ash, sulphated ash, pH value, volatile oil, alcohol soluble extractives, water soluble extractives were carried out by standard methods[5-7].The information collected from these tests are used for standardization.

2.2. Preliminary Phytochemical Analysis

Preliminary phytochemical screening was carried out to find out the presence of various phytoconstituents using standard procedures [8, 9].

2.2.1. High Performance Thin Layer Chromatography condition:

High Performance Thin Layer Chromatography is a popular method for the quality control of herbal products and the analysis of herbal medicines. It is widely used for separation. qualitative and quantitative estimation of marker compounds present in herbal drugs. HPTLC fingerprint profile is suitable for standardization of components followed by determination of specific bio-active phytoconstituents from plant materials [10, 11].

Preparation of the alcoholic extract of the drug for HPTLC analysis

Five gram of the powdered sample is taken and reflux with 200ml of alcohol using a soxhlet apparatus on a water bath for 30 minutes. Filter the extract and concentrate to 5ml then the sample extract obtained is used for further experimental studies [12, 13].

Procedure:

Developing solvent system:

A number of solvent systems were tried and a system which gave the maximum resolution was selected as the solvent system for the extract. The optimum separations of constituents were achieved using the specified solvent system.

Sample application:

The extracts were applied as different tracks of different concentrations of width 8 mm each on silica gel 60 F_{254} pre-coated aluminium sheets through CAMAG micro litre syringe using Automatic TLC Sampler 4 (ATS4). Development of chromatogram . After sample application the plate was introduced vertically in a CAMAG developing chamber (10 cm × 10 cm) pre-saturated with the mobile phase selected.

Documentation :

The developed chromatogram was air dried to evaporate solvents from the plate and the plate was kept in CAMAG Vizualizer and the images were captured under UV light at 254 nm and 366 nm.

Densitometry :

The plate was scanned at 254 nm and 366 nm using TLC Scanner 4 and the finger print profiles were documented. The R_f values and finger print data were recorded with win CATS software associated with the scanner.

Post chromatographic derivatisation:

The plate was derivatized using vanillin-sulphuric acid reagent, heated at 105^0 C by placing on CAMAG TLC plate heater till the colour of the bands appeared. Then the plate was visualized under white light and the chromatograms were documented. The plate was scanned at 575 nm and the R_f values and finger print data were documented.

2.2.2. Fourier Transport Infra red Rays Analysis:

The FT-IR spectra of Amirdhavalli Chooranam in kBr matrix recorded with rate of 20 spectra per second at the resolution 0.25 cm in the wave number region 400-4000 cm. The samples was mixed with kBr and pelletized by applying pressure to prepare the specimen to record the spectra under standard conditions.

2.2.3. Ultra Violet-Visible (UV-Vis) spectroscopy

The alcohol extract of the drug was subjected to Ultra Violet-Visible spectroscopic analysis. The extract was scanned at wave length ranging from 190 to 1100 nm using UV-VIS spectrophotometer (Model: UV3120) and the characteristic peaks were detected and recorded.

2.2.4. Powder Microscopy:

About 0.5gm of the powdered sample was mounted in glycerin at room temperature for 2 h and observed under 10X and 40X objective of bright field microscope (Meswox, India) for powder characteristics. Photomicrographs of diagnostic characters were captured using attached camera.

3. RESULTS AND DISCUSSION

Physico-chemical analysis:

Table1: Physico-Chemical Analysis of Amirdhavalli Chooranam

Sl. No.	Tests	Result%
1	LOD at 105^0C	8.54
2	Total Ash	8.34
3	Acid insoluble ash	0.43
4	Water soluble ash	5.02
5	Sulphated ash	11.27
6	pH (4% water extract)	5.4
7	Volatile oil	Nil
8	Alcohol soluble extractives	7.27
9	Water soluble extractives	14.82

Phytochemical analysis:

Table 2: Phytochemical analysis of Amirdhavalli Chooranam

Tests	Result
Saponins	+
Tannins	-
Terpenoids	-
Phenols	-
Steroids	-
Quinones	-
Antraquinones	-
Glycosides	+
Carbohydrates	-
Alkaloids	+
Lignans	-
Flavanoids	-
Proteins	-

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Interpretation:

Saponin:

Saponins have been implicated in regulation of energy metabolism through activities of AMPK. In addition most of the signaling pathways (JAK2 /STAT3 /MAPK /PBK / AMPK) being modulated by leptin and saponins.[14]. Saponin present in the extracts suggests that this drug may become one of the possible source in the treatment of cancer. Because saponins are anti-carcinogenic agents as they possess surface active characteristics due to their amphiphillic nature of their chemical structure. It includes direct cytotoxicity, immune-modulatory, bile acid binding and normalization of carcinogen induced cell proliferation [15].

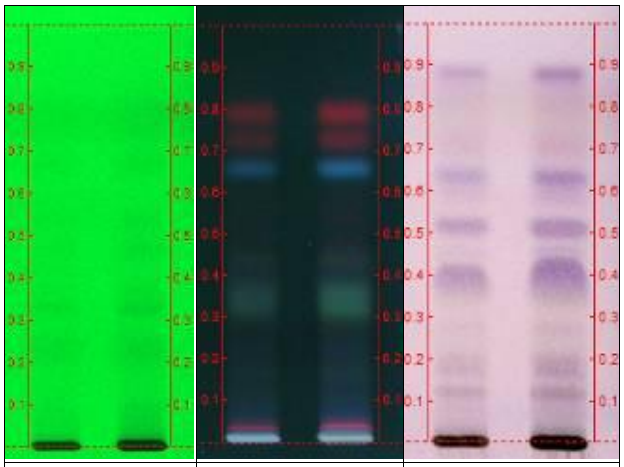
Glycosides:

Glycosides consist of a glucose moiety attached to an aglycan. The aglycan is a molecule that is bio-active in its free form but inert until the glycoside bond is broken by water or by enzyme. This mechanism allows the plants to differ the availability of the molecule to an appropriate time. 18-norclerodendrone, Furanoid diterpene glycoside, Tinocordiside, Tinocordifolioside are some glycosides present in the drug [16]

Alkaloids:

The drug is admired for the valuable bio-active phytoconstituents particularly alkaloids. They are pharmacologically active organic compound present in plant kingdom synthesized from aminoacid having basic or cationic property due to the presence of positively charged α-nitrogen in their heterocyclic ring [17]. It reveals the broad spectrum antibacterial property and analgesic property [18, 19]. Also they possess anti-spasmodic, anti-fungal, anti-fibrogenic effects. Berberine, Choline, Tinosporine, Magnoflorine, Palmetine are some alkaloids present in the drug.

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY:



Under UV short	Under UV long	Under white light after derivatisation

Fig 1: HPTLC profile of alcohol extract of Amirdhavalli Chooranam viewed in UV short; UV long; White light after derivatisation using vanillin-sulphuric acid;Solvent system-Toluene: Ethyl acetate-(5:2);Volume applied; Track 1- 5 µl: Track 2 – 10 µl

254 nm

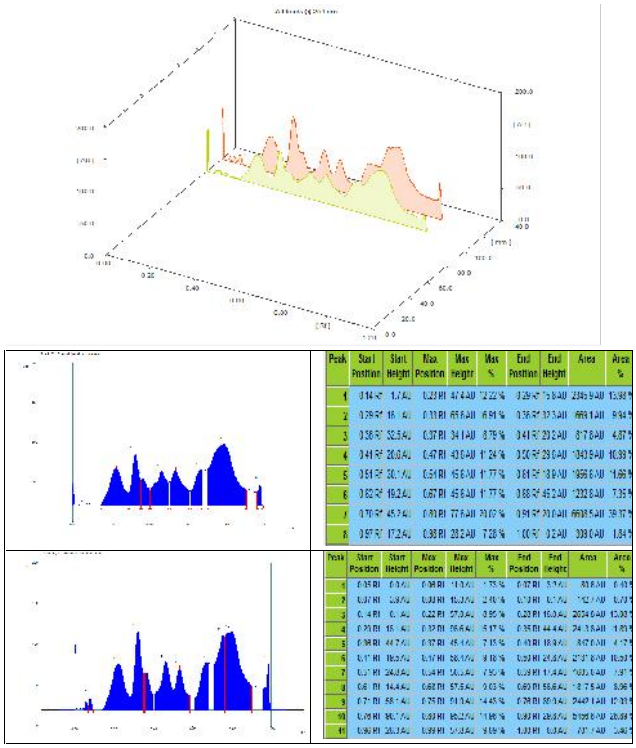


Fig 2: HPTLC fingerprint profile of 5µl and 10µl of alcohol extract of Amirdhavalli chooranam at 254nm after derivatisation.

366 nm

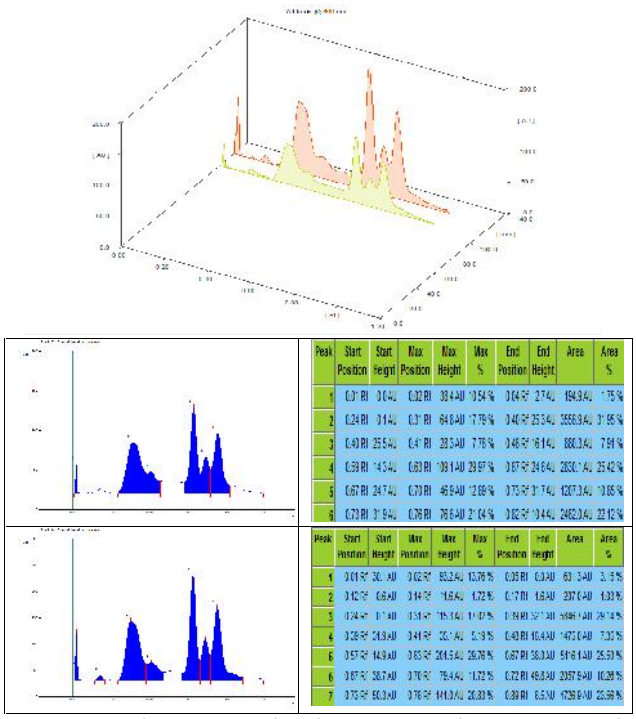


Fig 3: HPTLC fingerprint profile of 5µl and 10µl of alcohol extract of Amirdhavalli chooranam at 366nm after derivatisation.

575 nm

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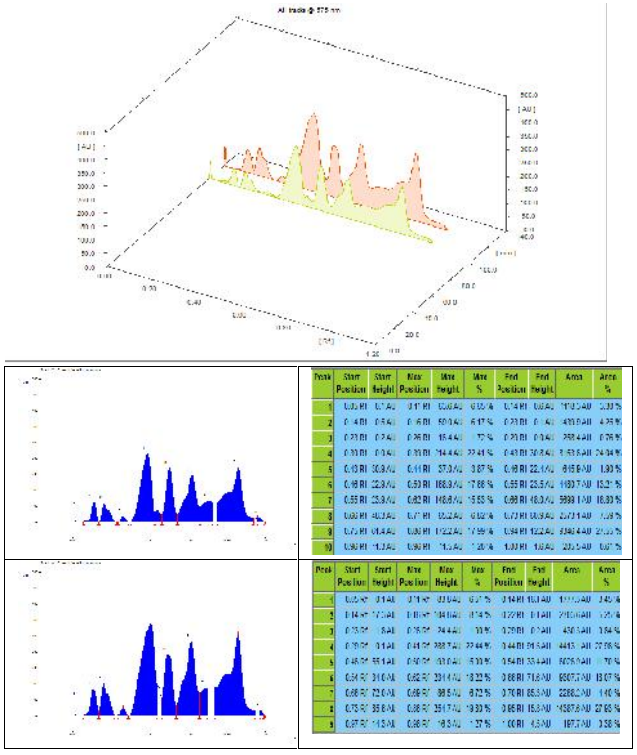

Fig 4: HPTLC fingerprint profile of 5µl and 10µl of alcohol extract of Amirdhavalli chooranam at 575nm after derivatisation

The HPTLC fingerprinting patterns of alcohol extract of Amirdhavalli chooranam was developed at 254nm, 366nm and after derivatisation with vanillin sulphuric acid at 575nm.The solvent system, Toluene:Ethyl acetate-(5:2) efficiently resolved the components. HPTLC photo documentation profile of the Amirdhavalli chooranam at 254nm, 366nm and after derivatisation is given in Figure.1,2,3. The fingerprint profile and the R_f value and percentage area of the peaks are shown in corresponding figures. High Performance Thin Layer Chromatography shows the presence of many phytochemicals with different Rf values and densitometric scan of the plates showed numerous bands and peaks. On observation 10 bands were appeared under UV short with R_f 0.06,0.08,0.22,0.32,0.37,0.47,0.54,0.68,0.75,0.80,0.99. Out of which R_f value at 0.80 has the maximum area 26.89% indicating the presence of highest concentration of phytoconstituents. HPTLC pattern at 366nm showed the peak at R_f 0.31 having the maximum area of 29.14%. HPTLC pattern after derivatisation showed 9 bands with R_f 0.11, 0.16, 0.26, 0.41, 0.50, 0.62, 0.69, 0.86, 0.98. Out of which R_f value at 0.41 has the maximum area 27.98%. Each band indicates the presence of phytoconstituent present in the extract.

FT-IR ANALYSIS:

FT-IR spectroscopy is used primarily for qualitative and quantitative analysis of organic compounds and also for determining the chemical structure of inorganic materials. The region between 400-4000 wave number is referred to as the finger print region. Absorption band in this region are generally due to intramolecular phenomena and are highly specific for each material.

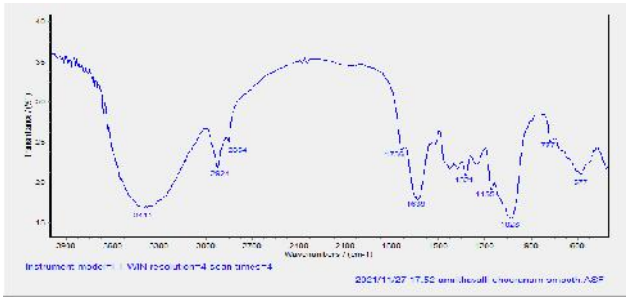

Fig 5: FTIR analysis

Interpretation:

In this study, Amirdhavalli chooranam has various components like alcohols, phenols, carbonyl acid, carboxylic acid, alkenes, alkyl halides, aliphatic amines, nitro compounds identified by reference values [20].

UV- VISIBLE SPECTROSCOPY:

UV absorption spectroscopy was used to recognize quantitative determination of different analytes by using wavelength and absorbance value. It can be used for the quantitative determination of compounds that absorb UV radiation [21].

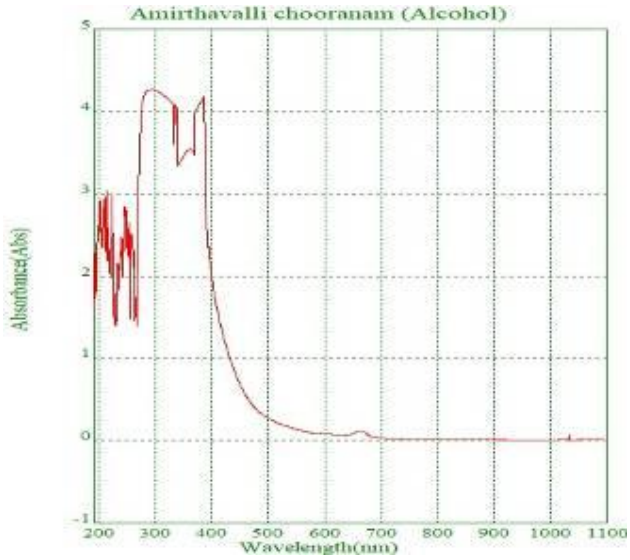

Fig 6: UV-Visible spectroscopy

Interpretation:

UV-Visible Spectroscopy interpretation of Amirdhavalli chooranam showed double high peak waves. The UV spectrum peaks showed the peak value at290 nm with absorption value of 4.2 and 390 nm with absorption value of 4.1 mentioned in fig.6

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POWDER MICROSCOPY:

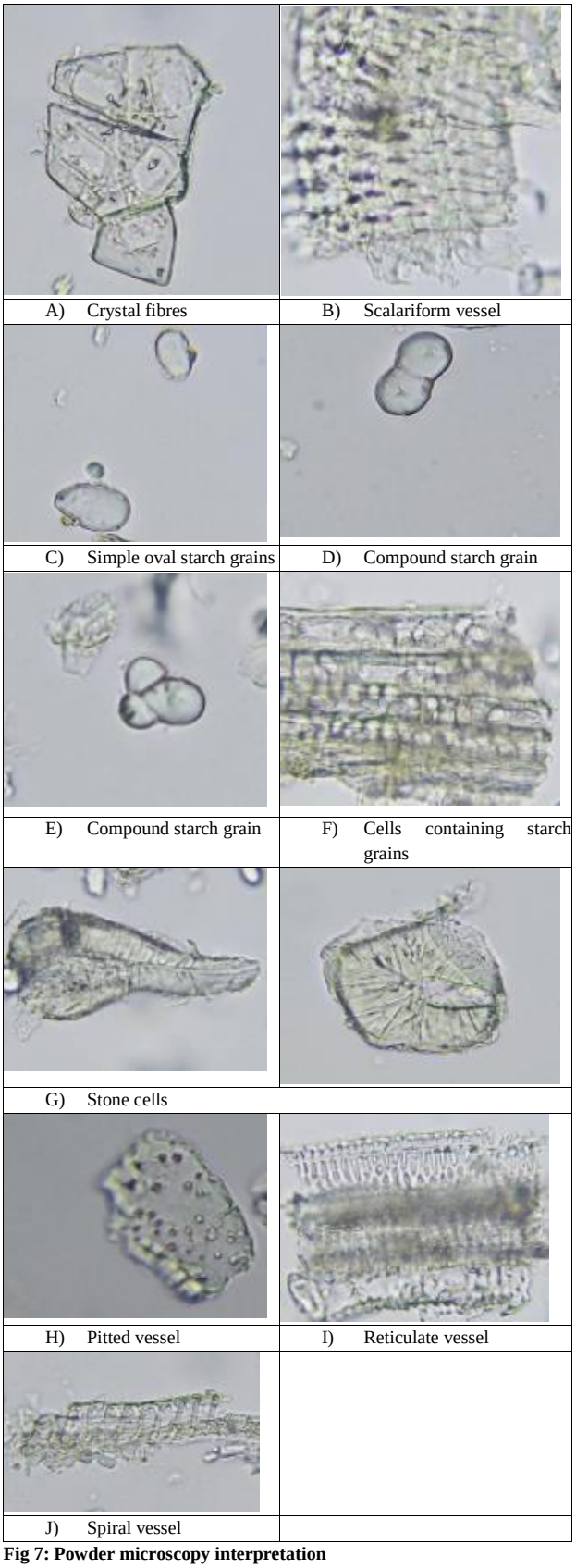

Fig 7: Powder microscopy interpretation

Under microscope showed numerous crystal fibers, scalar form vessels, simple oval starch grains, compound oval starch grains, stone cells, pitted vessels, reticulate vessels, spiral vessels.

4. CONCLUSION

The above results obtained to helps in the correct identification and authentication may help to prevent adulteration. The bio-active constituents like saponin, glycosides, and alkaloids are responsible for its therapeutic activity. FT-IR, UV-Visible spectroscopy, Powder microscopy showed the structural compounds and its active compounds.

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