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STANDARDIZATION OF AMUKKARA CHOORANUM- SIDDHA DRUG

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Keywords:

Siddha, Amukkara Chooranam, Rheumatoid arthritis, Anti-arthritic, Flavonoids

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ABSTRACT: Siddha medicine originated from Tamil culture in southern India. Worldwide there are 75% of herbal plants are used for AYUSH and Allopathy medicines. Amukkara Choornam is a polyherbal Siddha formulation. Amukkara Choornam has anti-arthritic activity, and it helps to cure rheumatoid arthritis. The demand for Siddha medicines in India is increasing daily; we have to overcome some problems like quality control issues, processing, administrative issues, and clinical trials. From the above, quality control issues are the most important ones. The quality, safety and, side effects, knowledge about herbs can be determined while standardizing each herbal formulation. The knowledge about the herbs and herbal medicines by the villagers and tribes is unknown to scientists. Scientists are well-known about the standardization process unknown by rural peoples and tribes. Hence the present study is carried out to identify the bioactive phytochemical constituents present in Amukkara Choornam by spectroscopic analysis. Standardization is a tool in the quality control process must for rural health care. Siddha medicines are our traditional medicines, which have lesser side effects. Since, it is a plant-based product.

INTRODUCTION: India is one of the mammoths of biodiversity countries; its tradition is related to using herbs as medicine. In the present situation, there is a growing demand for herbal products in the global pharmaceutical market. Hence pharmaceutical companies thrive research on plant-based compounds for their potential [3-4]. Herbal medicines are preferred over allopathy medicines because of their widespread availability, lower cost, effectiveness with chronic conditions, reduced risk of side effects, and may be safer to use over time [5].

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The Siddha system of medicine is the oldest Indian medicine system. Siddha is the mother medicine for all the Indian systems of medicine [1]. It has been practiced mostly in the southern part of this country for treating various ailments and chronic diseases [6]. According to Siddha, the first third of life is predominated by wind, the first third of life, the second third by bile, and the last third by phlegm. The greatest emphasis on examining the pulse is given for diagnosis by Siddha experts [7].

A literature search revealed that three reports on the scientific validation of Amukkara Chooranum deal with the physicochemical and HPTLC assessment of the Chooranum [8, 9, 10]. The authors (D. Sivaraman *et al.*,) demonstrated Amukkara Chooranum for strong antilithogenic effects due to therapeutic phytochemicals interacting with ions that produce urolithiasis crystals and prevent their

formation and aggregation [11]. Amukkara Chooranum Tablet was one of the Siddha Sasthric Medicines in Fixed Regimen (SSM-FiRe) given to the COVID - 19 patients with asymptomatic and mild symptomatic category as an oral dose and the regimen had safe, no modification in biochemical parameters [12]. Amukkara Chooranum inhibited biofilm formation against *Candida albicans* with a concentration of 20 µg/ml and demonstrated a 60% inhibition [13]. Diabetes can be lowered by using Amukkara Chooranum regularly as an aduvant in Rasa Chendruram [14]. Sulekha reported Amukkara remedy for diseases and infections specific to women's health [15]. Amukkara Chooranam Mathirai possesses anti-diarrheal activities and helps to minimize depressive symptoms while receiving treatment for COVID-19 in TPEC COVID care center at Vellore [16]. A commercial sample of AMC was investigated and validated in the present work for its organoleptic characteristics, physico - chemical characteristics, and flavonoid content. GC-MS chromatographic and IR, NMR spectral fingerprint and thermogravimetry analysis have been documented in this paper.

Standardization of Siddha Medicine: Knowledge about the herbs and herbal medicines by the villagers and tribes is unknown to scientists. Standardization is required for herbal medicines to ascertain their quality, safety, and side effects. Inherent characteristics, definitive qualitative and quantitative analysis, and constant parameters of herbal medicines can be done through standardization. Quantitative and qualitative analysis of herbal medicines helps to improve efficacy, safety, and reproducibility [17].

Standardization involves the development of technical standard methods for herbal drugs and is the tool to improve the quality control process [2]. The technical methods for standardization of herbal drugs include chromatographic and spectroscopic analysis, chemical parameters, microbiological parameters, pharmacognostic evaluation, physicochemical parameters *etc.* [11].

Polyherbal Siddha medicine, named Amukka-rachoornam is composed of herbs and spices. The named components are *Withania somnifera, Piper longum, Syzygium aromaticum, Cinnamomum wightii, Elettaria cardamomum, Piper nigrum, Zingiber officinale,* and cane sugar [18]. It is an anti-arthritic active compound prescribed for diseases like rheumatoid arthritis, kapharoga, splenomegaly, vataroga, hiccup, tuberculosis, anemia, leucorrhoea. It aids in secreting bile for better digestion and kapha balance [19].

MATERIALS AND METHODS: In the present study, the evaluation of Amukkara Chooranum was carried out by standard methods. The drug was collected from Siddha drug suppliers, Coimbatore district. The test sample is designed here in after as AMC (Amukkara Chooranum).

Organoleptic Properties: Organoleptic property of the purchased drug was examined according to the conventional method given by Kokate [20]. Organoleptic characteristics like colour, odour, appearance and taste were evaluated.

Physico- Chemical Properties: The solubility of the sample was tested using solvents like water, dichloro methane, ethyl acetate, ethyl alcohol, DMSO, acetone, chloroform, pet ether, and concentrated acids, and the characteristic changes were noted.

For the solubility test, a small amount of the samples were treated individually with water, concentrated hydrochloric acid, nitric acid, concentrated sulphuric acid, 5% aqueous sodium hydroxide, iodine solution, 5% aqueous potassium hydroxide solution and the glacial acetic acid solution respectively.

The ash value, pH value, and loss on drying were determined as per the method described in Indian Pharmacopoeia [21]. The sample's melting point was determined using melting point apparatus (Saffire). The pH value of the sample was determined by a pH meter (QC/Micro/pH – 101, Sr No. 1311605).

Loss on Drying: Accurately, 2 gram of the sample was taken in a tared crucible, and initial weight was taken. The sample was heated in a Muffle furnace maintained at 105-110°C, for 3 h, after which the sample was allowed to cool to room temperature for 30 minutes in desiccators and subsequently weighed. This procedure was repeated until a constant weight was obtained.

Loss on drying (%) = W / Loss in weight x 100

Where W = weight of the sample powder in g.

Total Ash Value: Accurately 2 to 3 g of air–dried samples of the AMC was weighed in a silica dish and incinerated at a temperature not exceeding 700°C until ash free from carbon was obtained. Then it was cooled and weighed. The process was repeated until at least two consecutive constant weights were obtained. The results were expressed as range or mean value ± standard deviation. The percentage of ash was calculated with reference to the air–dried drug.

$$\text{Ash \%} = W / \text{Loss in weight} \times 100$$

W = weight of the air-dried drug.

Phytochemical Screening: To identify the phytochemicals present in the sample phytochemical colour test was carried out according to the standard methods [22].

Quantitative Estimation of Total Flavonoids: Total flavonoid content was determined by standard method [22]. The ethanol extract of the sample (0.5ml) was mixed with a freshly prepared 2% ethanolic solution of aluminium chloride. This mixture was incubated at room temperature for 60 minutes. A yellow colour developed, indicating the presence of flavonoids in the sample. The absorbance was measured by UV-VIS spectro-photometer at 420nm.

Generally, total flavonoids in the plant samples will be expressed as quercetin equivalent (mg/g). Based on the calibration curve, the total flavonoid content was calculated using the formula,

$$Y = (0.217) \times (X)$$

Where X is the absorbance and Y is the quercetin equivalent.

Thermo Gravimetry Analysis (TGA): Thermo gravimetric analysis was conducted using a TA instrument 951 thermo gravimetric analyser (TGA). The 951 model is a horizontal design TGA. Each test was conducted with a flow rate of 50cc/ min of nitrogen through the furnace and balance sides of the TGA. Approximately 6 – 12 mg of the sample was weighed onto a platinum pan for each sulphate decomposition test. The sample was then held at ambient conditions for 40 min before it was heated at a rate of 5°C/ min to 200°C where the temperature was held for 1 hour. The sample was then heated at the same heating rate to 300°C and held for 15 min this step was then repeated, raising the temperature in 100^0C increments and holding for 15 min until a temperature of 700°C was reached (that is 100°C, 200°C, 300°C, 400°C, 500°C, 600°C and 700°C). The final step in the decomposition program was to increase the temperature to 700°C at a rate of 5°C/ min where the temperature was held for 15 min.

Spectroscopic Analysis: The UV-Visible, Infra-Red and NMR spectral fingerprints were recorded for the sample. UV – Visible spectrum was recorded using Science World AU - 2701. The IR of AMC was recorded using Affinity – I Shimatzo FT – IR instrument. GC-MS analysis was performed using the Clarus 680 GC. The H^1NMR of AMC was recorded in 500 MHz Bruker Avance NMR instrument, using DMSO solvent.

RESULT AND DISCUSSION:

Organoleptic Properties: Organoleptic property includes the study of morphology and other sensory characteristics like the drug's shape, size, and fracture. The results were summarized in **Table 1.**

TABLE 1: RESULTS OF ORGANOLEPTIC CHARACTERISTICS OF AMC

Parameters	Observation
Colour	Light Brown
Odour	Dried Ginger Smell
Taste	Sweet
State of Drug	Powder
Consistency	Soft

Physico Chemical Properties: The solubility of the drug in aqueous media in the pH range of 1-7.5 is important for the efficient release of the drug when it is administered orally [23].

Hence, the behavior of the drug in acidic, basic, and neutral reagents was observed. The sample was soluble in concentrated hydrochloric acid and Partially Soluble in nitric and sulphuric acid. The sample was insoluble in an iodine solution and glacial acetic.

The solubility of a drug is an important biopharmaceutical parameter as it affects the rate of dissolution and thus affects the rate of absorption.

The AMC was found to be partially soluble in ethanol and chloroform and soluble in DMSO.

TABLE 2: SOLUBILITY OF AMC

Chemical treatment	Observation
Concentrated Hydrochloric acid	Soluble
Concentrated Nitric acid	Partially soluble
Concentrated Sulphuric acid	Partially Soluble
5% Aqueous Sodium Hydroxide	Insoluble
Iodine Solution	Insoluble
5% Aqueous Potassium Hydroxide	Insoluble
Glacial Acetic Acid	Insoluble
Chloroform	Partially soluble
Ethanol	Partially Soluble
DMSO	Soluble

The melting point of the drug was 260°C. pH of the formulation plays a significant role in the living biological system with respect to aiding in absorption and distribution through systemic circulation. The pH of the sample was found to be 7.0-7.1. Hence, the sample is neutral. Ash value was found to be 0.0438%. At higher temperature the sample charred, which indicated the presence of organic matter and the absence of inorganic salts. Loss on drying of the sample was carried out using muffle furnace maintained at 105-110°C and the loss on drying was found to be 0.3618%**,** indicating AMC's low moisture content.

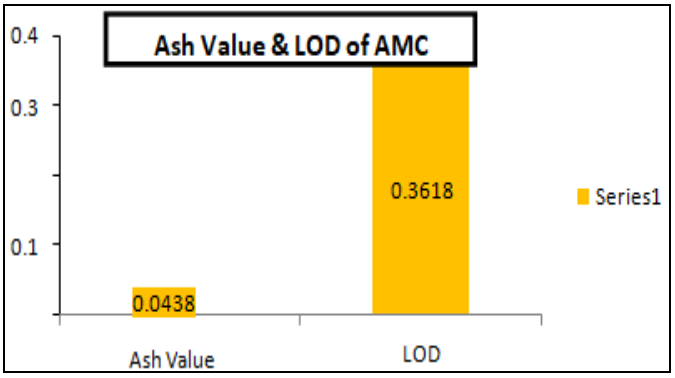


FIG. 1: ASH VALUE AND LOSS ON DRYING OF AMC

Phytochemical Screening: The colour tests of the sample indicated the presence of alkaloids, flavonoids, and carbohydrates in the polyherbal formulation AMC.

Quantitative Estimation of Total Flavonoids: Many herbs and plant products have been shown to have hypoglycemic action. Flavonoids are known to be bioactive antidiabetic principles. Flavonoid compounds such as Boswellic acid, Ellagic acid, Quercetin, and Rutin revealed a maximum reduction in blood glucose levels [24].

The quantitative estimation of flavonoids was done by the method described by Ordonez *et al*., [25] based on the formation of a flavonoid-aluminum complex. The results showed that 0.328mg/1g of flavonoid was in the sample by Ordonez method.

TGA Analysis: Thermal analysis is a technique used to study the properties of a material with temperature change. **Fig. 2** and **3** represent the TG curve of the sample. The weight loss commenced at 203°C and completely decreased at 575°C above that; the curve observed no change in weight. The Derivative thermo-gravimetric (DTG) curve showed the exothermic nature of AMC, as reported in **Fig. 4** and **5,** indicating two decomposition peaks at 190°C and 512°C.

The DTG of organic matter usually shows three exothermic peaks due to oxidation i.e., recalcitrant organic matter (380°C – 475°C), refractory organic matter including black carbon and labile organic matter (200°C – 380°C) [26]. In the DTG curve of AMC **Fig. 6,** the regions corresponding to the peak at 300°C and 450°C are due to aliphatic, carboxylic acids, and aromatic groups. The aliphatic content was 139%, and the aromatic content was 75%.

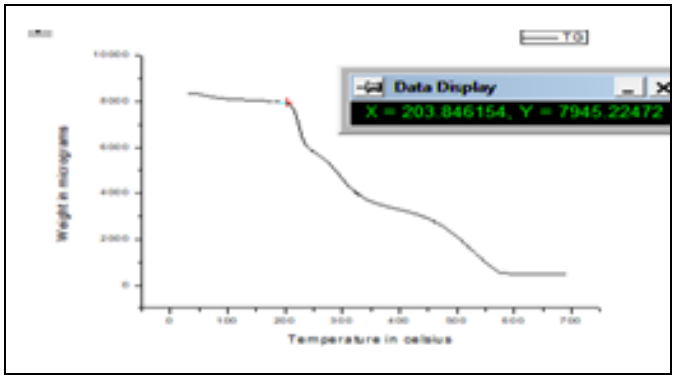


FIG. 2: TG CURVE OF AMC

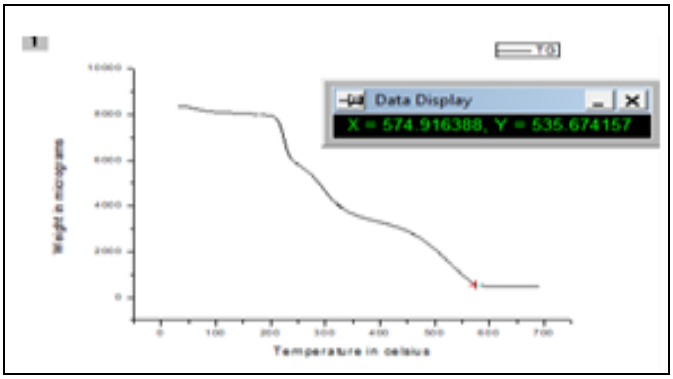


FIG. 3: TG CURVE OF AMC

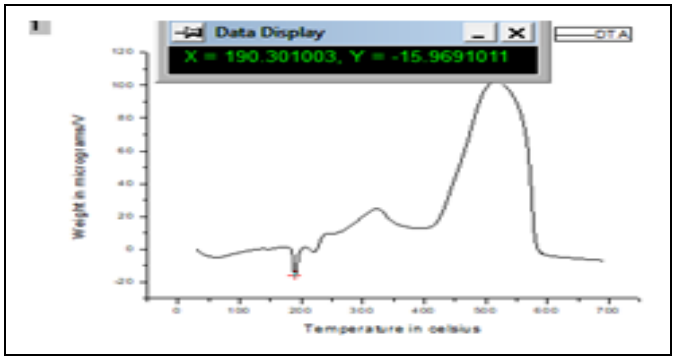


FIG. 4: DTA CURVE OF AMC

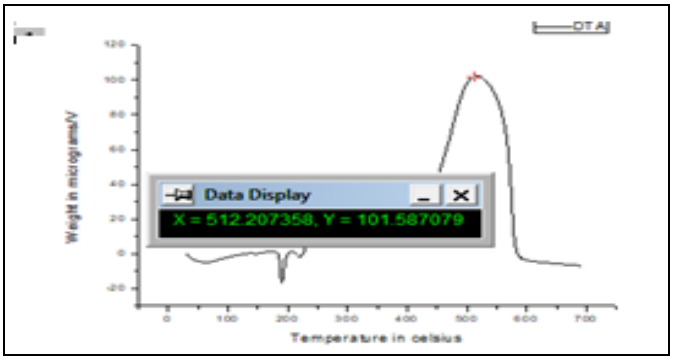


FIG. 5: DTA CURVE OF AMC

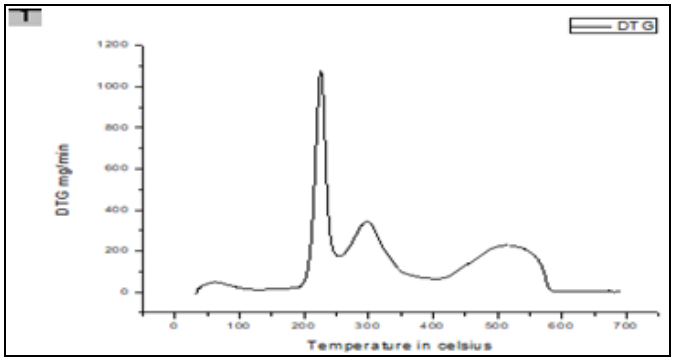


FIG. 6: DTG CURVE OF AMC

FT – IR: IR spectrum **Fig. 7** indicated the presence of amides, alcohols/phenols, amines, and alkyl halides in the AMC sample **Table 3**, which may be due to the presence of plant ingredients used in the AMC sample.

TABLE 3: IR RANGE OF AMC

Frequency	Peak Assignment	Possibility
3329.14, 1612.49	N-H Stretching (strong), N-H Bending for 1^0&2^oamides	Amides
3329.14, 1411.87, 1029.99	O-H Bond is broad, C-O-H Bending appears broad (weak), C-O Stretching	Alcohols & Phenols
3329.14, 1334.74	N-H Stretching, C-N Stretching	Amines
586.36	C-Br stretching (strong)	Alkyl halide

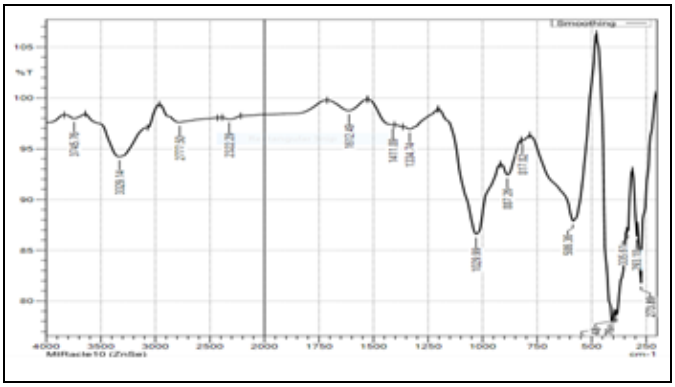


FIG. 7: IR FINGERPRINTING OF AMC

GC-MS: The GC–MS chromatogram of the formulation is shown in **Fig. 8, 9, 10, 11, 12, and 13.** The spectrum showed nine major peaks. The compounds pertaining to the peaks were identified by comparing with NIST library data of the peaks and mass spectra of the peaks with those reported in the literature. The following compounds were identified in AMC *viz*, Chrysoeriol, Apigenin, Adipic acid, Piperine, and Squalene **Fig. 18, 19, 20, 21, 22. Table 4** indicates the identified compounds and their uses**.**

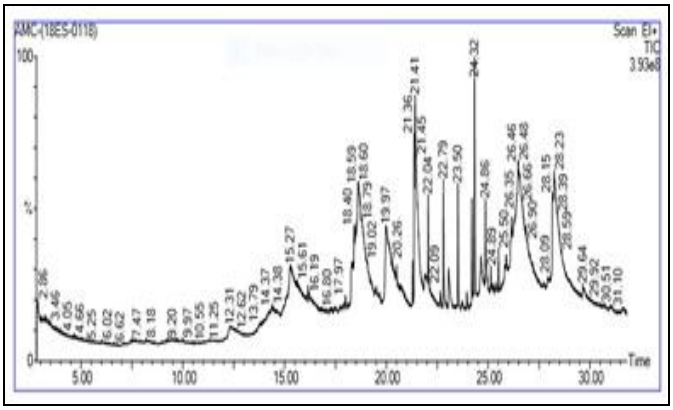


FIG. 8: GC SPECTRUM OF AMC

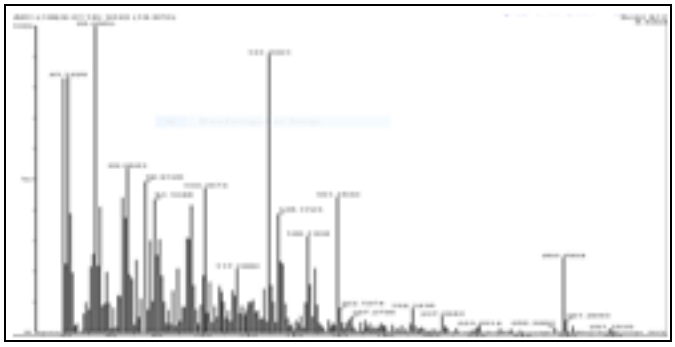


FIG. 9:

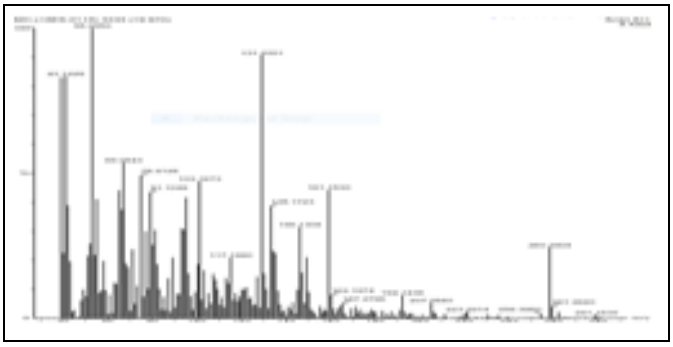


FIG. 10:

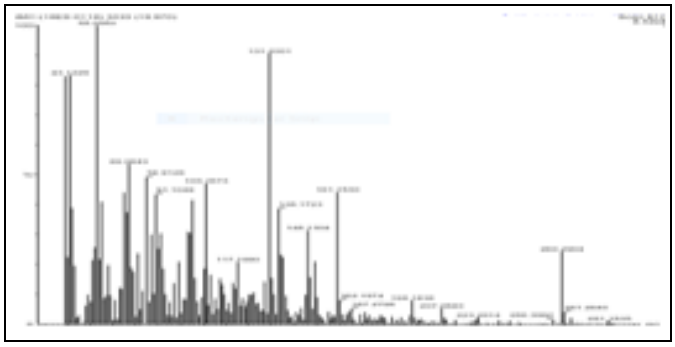


FIG. 11:

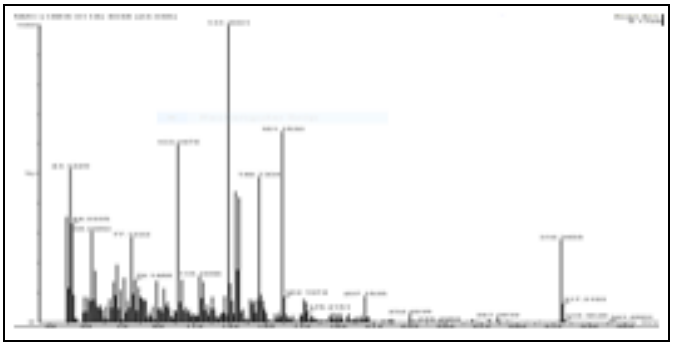


FIG. 12:

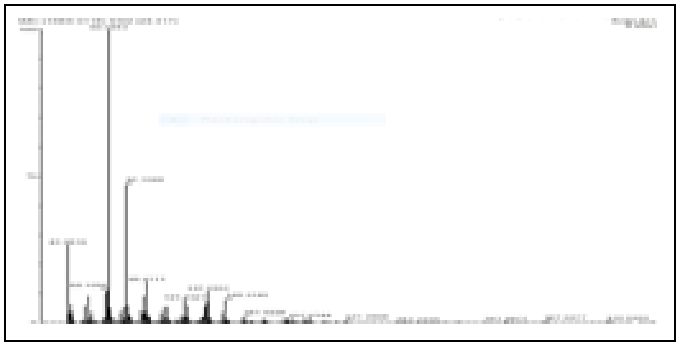
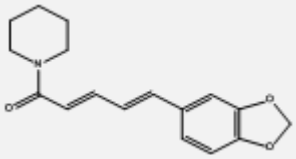
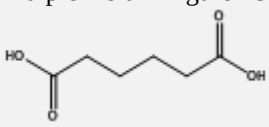
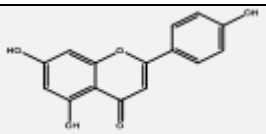
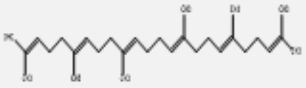


FIG. 13:

FIG. 9, 10, 11, 12, and 13: MASS-SPECTRUM OF AMC

TABLE 4: STRUCTURE OF IDENTIFIED CONSTITUENTS IN THE AMC BY GC – MS ANALYSIS

RT	Area%	Molecular Weight	GC–ESI–MS	Identified Compound	Nature & Uses
26.478	19.575	285.343g/mol	MS: 285.18,207.01, 173.12,137.12, 115.16,71.11.	Piperine Figure-18 	Pungent, increase the bioavailability of nutritional compounds
21.406	10.537	146.142g/mol	MS: 103.1,83.1, 77.05,54.9,43.05.	Adipic Acid Figure-19 	Acidic, used in the production of pharmaceuticals, bactericides
19.970	7.826	270.24g/mol	MS: 260.29,256.3, 207.08,194.14, 161.15,148.13, 117.18,103.16.	Apigenin Figure – 20	Natural flavones, anti-oxidant, anti-inflammatory & anti – tumor properties

23.046	1.474	300.27g/mol	MS: 316.34,232.26, 207.15,161.15, 148.13,131.2,103.09.	Chrysoeriol Figure – 21 	Flavonoid, anti-oxidant, anti – inflammatory properties
24.317	2.812	410.718g/mol	MS: 149.31,137.19, 121.23,95.211, 81.104,69.0843,68.105	Squalene Figure – 22 	emollient, anti-oxidant

NMR: NMR gives the largest amount of information about the structure of a compound. The comparison of the spectrum showed the presence of chemical constituents, namely, Piperine, Apigenin, Zingiberene and Squalene. Hence, H^1 NMR analysis was done for the AMC sample to identify the components present in the sample. In the spectrum the peaks were seen, in the regions 5.9 – 7.0 ppm, 3.54ppm and 0.8 – 1.55ppm, indicating the presence of both aromatic and aliphatic regions. The NMR values were compared with the standard spectrum present in the SDBS database. Piperine– 5.9–7.0 ppm, Apigenin – 3.54 ppm, Zingiberene– 0.8– 1.55 ppm, Squalene–0.91–5.04 ppm **Fig. 14, 15, 16, 17.**

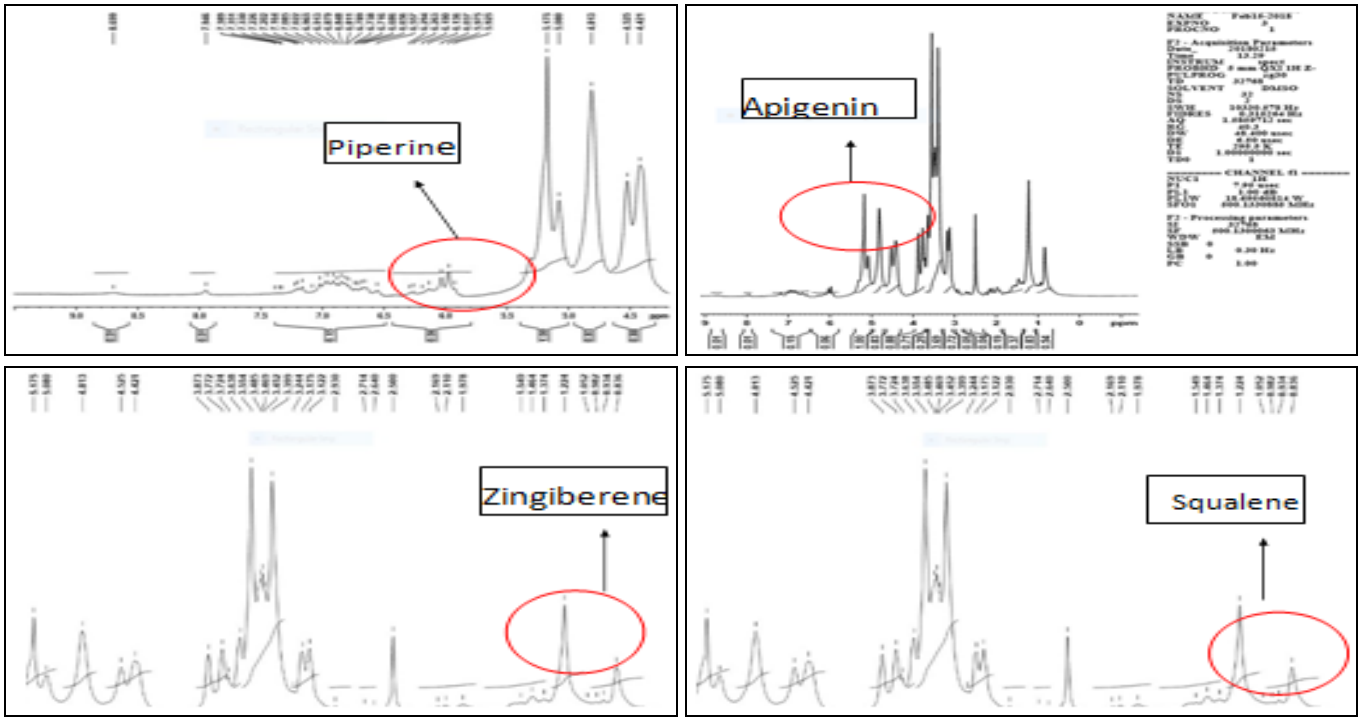


FIG. 14, 15, 16, and 17: NMR OF AMC

HPTLC of AMC showed Withaferine A and Piperine [8]. Flavonoids are used as ingredients in cosmetic products and drugs. Flavonoids also possesses anti - bacterial, anti - cancer and anti-oxidant properties [27]. The present study of GC-MS and NMR analysis of the choornam also revealed the presence of the flavonoids piperine, apigenin and Zingiberene respectively. The total flavonoid content of AMC was found to be 0.328mg/1g. Many herbal formulations were found to exhibit hypoglycemic action. Those formulations mainly contain flavonoids. Flavonoids are known to be bioactive anti-diabetic principles. Flavonoid compounds such as Boswellic acid, Ellagic acid, Quercetin, and Rutin revealed a maximum reduction in blood glucose levels [28]. Evaluation of

AMC against chickengunya proved the anti-inflammatory action of the choornam [29].

CONCLUSION: Siddha medicines are our traditional medicines with lesser side effects. Since, they are plant-based products. The compound Piperine identified in the AMC increases the bioavailability of nutritional compounds, thus enhancing a person's briskness. Apigenin and Chrysoeriol have anti-inflammatory activity, thus validating the anti–rheumatoid arthritis activity of the choornam; the scientific validation of the Siddha system of medicine may give an approach for enhancing wealth.

Footnote:

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Ethics Statement: This article does not contain any studies with human participants or animals.

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CONFLICTS OF INTEREST: The authors declare no conflicts of interest.

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