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Phytochemical screening and characterization of bioactive compounds in *Kalarchi chooranam* using GC-MS technique

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Abstract
The aim of this study was to carry out for identification of bioactive compounds from the *Kalarchi chooranam* by qualitatively and Gas chromatography-Mass Spectroscopy (GC-MS) technique. The qualitative analysis of *Kalarchi chooranam* showed the presence of alkaloids, phenols, saponins and phytosterols while protein, aminoacids and flavonoids were absent. GCMS analysis of *Kalarchi chooranam* was done by standard protocol using the equipment Perkin-Elmer Gas Chromatography–Mass Spectrometry while the mass spectra of the compounds found in the *Kalarchi chooranam* was matched with the National Institute of Standards and Technology (NIST) library. The GC-MS analysis revealed the presence of fifteen compounds. The prevailing compounds are 2,5-Furandione, dihydro-3-methylene, Phthalic anhydride and L-Glutamic acid 5-ethyl ester. These findings support the traditional use of *Kalarchi chooranam* in various disorders.

Keywords: *Kalarchi choornam*, gas chromatography and mass spectroscopy, bioactive compounds, phytochemistry

1. Introduction
Natural products are used extensively throughout the world, at least in 70% of drugs available in the global market. However, there is a great threat to biodiversity [1] due to over harvesting raw materials for herbal medicines and health care products. The knowledge of herbo mineral origin and animal origin that have curative and palliative effects were transmitted from one generation to another and it is the outcome of bold experimentation through trial and error method over hundreds of years. Ethno medicine is the mother of all other systems of medicine such as Siddha, Ayurveda, Unani, Nature cure and even modern medicine. The traditional herbalists are part and parcel of the community and are often familiar with the details of each family and its environs, so that they are in a better position to deal with their day-to-day problems. In fact the native healers take care of the common ailments of the folk in their home setting [2].

In spite of incredible advances in modern scientific technology and allopathic medicine till we are unable to provide quality healthcare to all. Traditional medicine particularly herbal medicine considered as a major healthcare provider around the globe particularly in rural and remote areas. A large section of people depends on such medicine for their primary healthcare mainly in underdeveloped or developing countries. Indian traditional medicinal system like Siddha, Ayurveda and Unani has a very rich history of their effectiveness; modern research also acknowledged the importance of such medicine. Indian traditional medicine or medicinal plants are also considered as a vital source of new drug [3].

Within a decade, there were a number of dramatic advances in analytical techniques including TLC, UV, NMR and GC-MS that were powerful tools for separation, identification and structural determination of phytochemicals [4].Gas Chromatography Mass Spectroscopy (GC-MS) a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification of biochemical components of medicinal plants [5]. So, the present study was aimed to investigate the possible chemical components of the *Kalarchi chooranam* by subjecting it to GC-MS analysis.

2. Materials and methods
Preparation of *Kalarchi choornam*
Ingredients
Kalarchi paruppu (*Caesalpinia bonduc*) - 1 part
Milagu (*Piper nigram*) - 1/4 part
The above-mentioned drugs are finely powdered and tored in air tight container.
Indications: *Andavayu, soothakavayu, Yanaikal* [6].

2.1 Phytochemical Analysis
The *Kalarchi chooranam* was qualitatively tested for the presence of phytochemicals such as alkaloids, flavonoids, saponins, terpenoids, steroids and tannins using the standard procedures as previously described [7].

2.2 Test for alkaloids
10 mg of the *kalarchi chooranam* in 5 mL of 1% ethanolic HCl and 5 drops of Drangendorff 's reagent were added. The formation of orange precipitate indicates the presence of alkaloids.

2.3 Test for saponins
The persistent frothing test for saponin was used. To 50 mg of the extract was added 5 ml of distilled water. The mixture was vigorously shaken and heated to boil for a stable persistent froth. The frothing was mixed with 4 drops of olive oil and shaken vigorously for the formation of emulsion thus indicating the presence of saponins.

2.4 Test for tannins and phenols
Small quantity of the extract was dissolved in water and to that ferric chloride solution (5%) or gelatin solution (1%) or lead acetate solution (10%) or 10% Sodium Chloride were added separately. Appearance of blue colour with ferric chloride (or) precipitation with other reagent indicates the presence of tannins and phenols

2.5 Test for phytosterol
The *Kalarchi chooranam* was qualitatively tested for the presence of phytochemicals such as alkaloids, flavonoids, saponins, terpenoids, steroids and tannins using the standard procedures as previously described [7]. To the test sample few drops of acetic anhydride was added and mixed well. 1 ml of concentrated sulphuric acid was added along the side of the test tube and set aside for awhile. Brown ring was formed at the junction of the two layers indicating presence of sterols.

2.6 Test for Carbohydrates
300 mg of the extract was dissolved in distilled water and filtered. The filtrate was boiled with Fehling's and with Benedict's solution. Formation of brick red precipitate in Fehling's and Benedict's solution is the positive result for reducing sugars and non-reducing sugars respectively.

2.7 Proteins and free amino acids
A small quantity of extract was dissolved in a few ml of water and subject the solution to Millon's or Biuret tests, red or pink-purple colour indicates the presence of proteins.

2.8 GC –MS analysis
GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25mm ID x 1µMdf, composed of 100% Dimethyl polydiloxane), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 250 ºC; ion-source temperature 280 ºC. The oven temperature was programmed from 110 ºC (isothermal for 2 min), with an increase of 10 ºC/min, to 200ºC, then 5ºC/min to 280ºC, ending with a 9min isothermal at 280ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36min. min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0. [8].

3. Results & Discussion (Times New Roman, 12, Bold)
In the present preliminary phytochemical analysis on the *Kalarchi chooranam* revealed the presence of medicinally active compounds and summarized in Table-1. The phytochemical analysis of *Kalarchi chooranam* showed the presence of carbohydrate, alkaloids, phenols, saponins, phytosterol and tannin while flavonoids protein and amino acids were absent.

Fifteen (15) compounds were identified in *Kalarchi chooranam* extract by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and area of percentage (%) are presented in (Table 2 and Fig. 1). The prevailing compounds were Ethyl citrate (36.29%, 16.69%, 6.05%, 1.06%), 2,6-Dihydroxyisonicotinic acid(12.28%), 2,5-Furandione, dihydro-3-methylene(12.01%), 2,6-Dihydroxyisonicotinic acid(3.63%), Phthalic anhydride(2.65%), 4,6(1H)-Pyrimidinedione,3,4,5,6-tetrahydro-2-imino-(2.06%),L-Glutamicacid,diethyl ester(1.89%),Pyrimidine-4(3H)-one,3-amino-2,6dimethyl-(1.74%), L-Glutamic acid 5-ethyl ester (1.30%), Dimethyl ethylidenemalonate(0.82%), Adipic acid,ethyl 3-methylbutyl ester(0.80%) and Citric acid trimethyl ester(0.72%).

Phytochemicals are known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect and anticancer property. It is well-known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases [9].

Alkaloids have established broad spectrum antibacterial activity and are also used as analgesics and narcotics for pain relief. Alkaloids are very important in medicine and constitute most of the valuable drug. They have marked physicological effect in animals [10]. The presence of saponin and tannin is also indicative of its antioxidant, antimicrobial and anti-carcinogenic properties [11]. Tannins inhibit the pathogenic fungi and antimicrobial activity of extracts showed better activity by the presence of tannins [12]. Phenols in plants are reported to show multiple activities like antioxidant, anti-carcinogenic, anti-inflammatory etc. [13]. The presence of the phytochemicals such as alkaloids, phenols, saponins, tannins, phytosterol and carbohydrates in this *Kalarchi chooranam* might be responsible for their therapeutic effects.

Gas chromatography – mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample [14]. In the last few years, GC-MS has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species [15]. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives.

The biological active compound, 2,5-Furandione, dihydro-3-methylene present in the *kalarchi chooranam* possess anticancer effect. Phthalic anhydride is reported to be antiulcer, antimicrobial, anxiolytic, antiviral, antitumor and cytotoxic activities [16]. L-Glutamic acid 5-ethyl ester act as antiepileptic, antiprostatitic, antiretardation, anxiolytic and neurotoxic [17].

3.1 Tables and Figures

Table 1: Preliminary phytochemical evaluation of *Kalarchi chooranam* extract

Sl. No.	Test	Result
1	Alkaloids	Present
2	Phenols	Present
3	Flavonoids	Absent
4	Saponins	Present
5	Tannins	Present
6	Phytosterol	Present
7	Carbohydrates	Present
8	Proteins	Absent
9	Aminoacids	Absent

Table 2: GC-MS analysis of *Kalarchi chooranam* extract

Peak number	RT	Area of %	Identified compound
1	5.331	12.01	2,5-Furandione, dihydro-3-methylene-
2	8.664	2.06	4,6(1H)-Pyrimidinedione, 3,4,5,6 tetrahydro-2-imino-
3	9.064	0.82	Dimethyl ethylidenemalonate
4	10.586	1.74	Pyrimidin-4(3H)-one, 3-amino-2,6 dimethyl-
5	10.897	2.65	Phthalic anhydride
6	11.408	3.63	2,6-Dihydroxyisonicotinic acid
7	11.586	12.28	2,6-Dihydroxyisonicotinic acid
8	12.530	1.30	L-Glutamic acid 5-ethyl ester
9	12.608	1.89	L-Glutamic acid, diethyl ester
10	14.308	0.72	Citric acid, trimethyl ester
11	14.474	1.06	Ethyl citrate
12	14.985	0.80	Adipic acid, ethyl 3-methylbutylester
13	15.152	16.69	Ethyl citrate
14	15.652	36.29	Ethyl citrate
15	15.741	6.05	Ethyl citrate

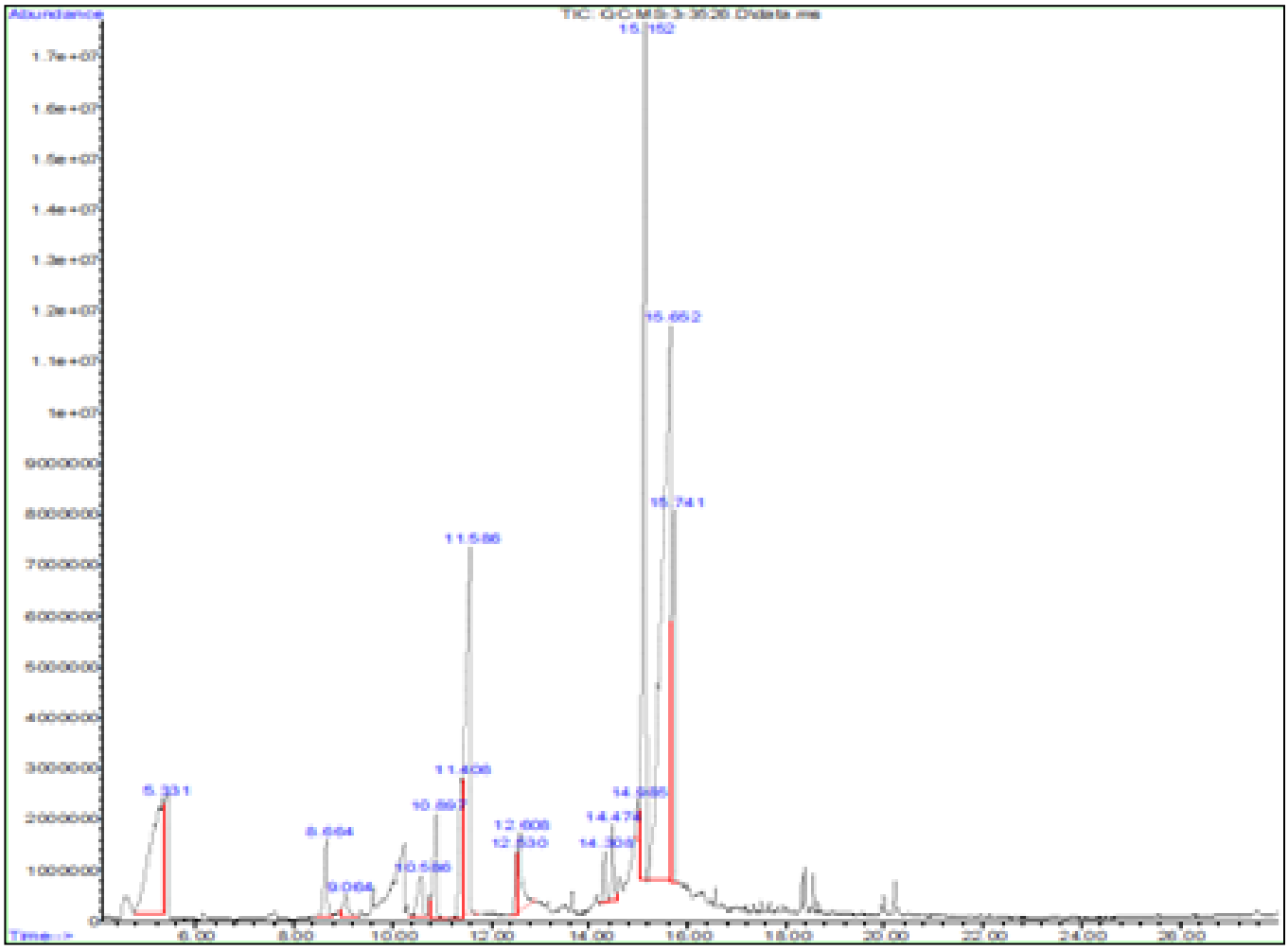

Fig 1: GC-MS chromatogram of *Kalarchi chooranam* extract.

4. Conclusions

In conclusion, the phytochemical analyses have revealed the presence of alkaloids, phenols, saponins, phytosterols and carbohydrate compounds. In GCMS analysis fifteen compounds identified in this study scientifically validated that the extract of *Kalarchi chooranam* has potent anticancer, antiulcer, antimicrobial, anxiolytic, antiviral, antitumor and cytotoxic activities. So that those might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the *kalarchi chooranam* which may be created a new way to treat many incurable diseases.

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