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Research Article

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Standardization of the Siddha herbal formulation Inji dravagam for the treatment of peptic ulcer disease

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Abstract

Inji dravagam is a siddha herbal medication which can be prescribed for peptic ulcer disease as per *siddha* literature. A detailed research of organoleptic characters, physico-chemical characterization, phytochemical analysis by TLC and HPTLC, elemental analysis by ICP-OES and Gas Chromatography mass spectrometry study was done on *Inji dravagam* to determine the qualitative, quantitative analysis and the presence of any toxic compounds or heavy metals. This study proved that there were no significant heavy metallic compounds identified in the drug which may fear to cause any toxicity in humans and was found to be within normal limits. Further, the evaluation of phytoconstituents present in the *Inji dravagam* was identified by using ICP-OES and GC-MS study which revealed its pharmacological and therapeutic value. Thus the drug has medicinal property and can be prescribed for peptic ulcer disease without any hesitancy.

Keywords: Siddha medicine, peptic ulcer disease, ICP-OES, gas chromatography

1. Introduction

Centuries back, when the people of India got affected by any illness or disease they approached the ancient saints called siddhars to getrelieved from their issues permanently. That is how Siddha system of medicine originated thousands of years ago by siddhars to treat the illness of the people with the medicines madeof plants, animals and minerals. In Siddha system of medicine, there are about 32 types of internal medicines such as surasam, saaram (juice extract), kudineer (decoction), chooranam (medicated powder), legiyam, mathirai (tablet), etc. Among them dravagam is one of the medications, which is in the liquid state prepared by the distillation process using an instrument called valuka yenthiram[2].

Dravagam or Theeneer is the distilled essence, which contains the volatile constituents of the drugs used in the preparation, in a medium of water and is equivalent to “aqua” or water of the western pharmacopoeia. The term Dravagam or Theeneer denotes the acceptable aromatic nature of the drug and indicates that it is in the aqueous state. Inji dravagam is an herbal formulation taken from Siddha literature **Yaakopu Vaithiya Chinthamani-700** which has been indicated for the treatment of gunmam (peptic ulcer disease) [1].

An analytical study was made on *Inji dravagam* which deals with the qualitative and quantitative analysis, phytochemical analysis and elemental analysis carried out in National Institute of Siddha and Captain Srinivasamurthi Research center for Ayurveda and Siddha drug Development in Arumbakkam, chennai.

2. Materials and Methods

2.1. Standard operative procedure of *inji dravagam:*

2.1.1. Ingredients:

1. Purified fresh Ginger juice - *(Zingiber officinale)*
2. Purified Omam - *(Carum copticum)*

1.1.2. Source of Collection:

Fresh gingers were collected from Tambaram sanatorium, Chennai, Tamilnadu. Omam was procured from a well reputed country shop in Parrys, Chennai.

2.1.3. Identification and Authentication of the drug:

The Herbal drugs were Identified and authenticated by competent authority department of Gunapadam, National Institute of Siddha, Tambaram sanatorium, Chennai.

2.2 Purification of Ingredient:

2.2.1. Purification of *Omam*

Omam was purified by soaking it in limestone water for three hours and then it was fried[3].

2.2.2. Purification of Inji

Inji was purified by removing the outer layer of skin[3].

2.3. Preparation and storage of *Inji dravagam*:

The distillation process was done at Gunapadam practical lab of National Institute of Siddha. The theeneer collected was stored in air tight container made of glass. The container was kept closed. If it was kept open, then the volatile active principles present in the drug will be lost by evaporation[2].

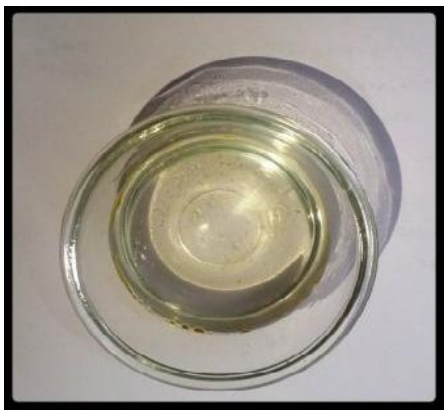


Figure 1: Inji dravagam

Labeling:

Name of the preparation:		***Inji dravagam***
Date of preparation	:	12/06/15 and 25/02/16
Dose	:	5ml BD
Adjuvant/Vehicle	:	Water
Indications	:	Gunmam (Peptic ulcer disease)
Date of expiry	:	1 year from the date of manufacture

Therapeutic Administration of drug:

Form of medicine - Liquid (Pale yellow)
Route of administration - Oral
Dose-5ml
Vehicle-Water
Time of administration-Twice a day

2.4. Analytical study of *Inji dravagam*:

2.4.1. Organoleptic character

Colour: The medicine was taken into watch glasses and placed against white back ground in white tube light. It was observed for its colour by naked eye.

Odour: The medicine was smelled individually. The time interval among two smelling was kept 2 minutes to nullify the effect of previous smelling.

2.4.2. Physicochemical analysis:

Test for Silicate: 2ml of the sample was shaken well with distilled water.

Action of Heat: 2ml of the sample was taken in a dry test tube and heated gently at first and then strong.

Flame Test: 2ml of the sample was made into a paste with con. HCl taken in a watch glass was introduced into non-luminous part of the Bunsen flame.

Ash Test: A filter paper was soaked into a mixture of extract and dil. cobalt nitrate solution and introduced into the Bunsen flame and ignited.

Chemical analysis:

5ml of sample was taken in a 250ml clean beaker and added with 50ml of distilled water. Then it was boiled well for about 10 minutes. Then it was cooled and filtered in a 100ml volumetric flask and made up to 100ml with distilled water. This preparation was used for the qualitative analysis of acidic/basic radicals and biochemical constituents in it.

Phytochemical analysis by TLC and HPTLC methodology:

Taken 15ml of the sample and 60ml Ethyl acetate was added and kept overnight. Then Ethyl acetate layer was separated and dried over sodium sulphate anhydrous. Filtered and concentrated to 10ml at room temperature. 20µl, 25µl of the above solution were applied on Merck Aluminum plate pre-coated with silica gel $60F_{254}$ of 0.2mm thickness using ATS-IV. The plate was developed in *Toluene: Ethyl acetate* (8.2). The plate was dried and visualized in UV 254 and UV366nm and photographs were taken, the plate was scanned at 254nm before dipping. Then the plate was dipped in vanillin-sulphuric acid and heated at 105ºC till the colour of the spots appeared and photos were taken[12]. The procedure undergone for the analysis of TLC and HPTLC analysis was as per Wagner H and Bladt S, 1996.The experimental procedure was done at SAIF, IIT Madras, Chennai-36.

2.4.3. Elemental analysis by ICP-OES:

The ICP- OES is a trace-level elemental analysis technique that uses the emission spectra of a sample to identify and quantify the elements present. The experimental procedure was done at SAIF, IIT Madras, Chennai-36.

2.4.4. Gas chromatography Mass Spectroscopy (GC-MS):

Gas chromatography mass spectroscopy is a technique that combines the analytical features of gas chromatography and mass spectroscopy which can evaluate the presence of volatile oils and phyto-constituents present in the test drug. GC-MS for Injidravagam was done at SAIF, IITM, Chennai-96.

3. Results and Discussion

The results of organoleptic and Physico-chemical characters of the study drug *Inji dravagam* were described in the table 1, 2 and 3. The biochemical analysis of *Inji dravagam* by acid radical test (table 4)and basic radical test (table 5) shows the presence of **Chloride, Ammonium and Calcium**. The results of TLC and HPTLC analysis are discussed in table 6, figure 2, 3(i) and 3(ii). The heavy metal analysis of the trial drug by ICP-OES confirmed the absence of heavy metals such as lead,nickel, copper, cadmium, arsenic and mercury (table 7) which are in below detection limits. The presence of other elements such as calcium, iron, potassium, magnesium, phosphorus and zinc (table 7) validated the therapeutic value of *Inji dravagam***.** An antacid used for the treatment of peptic ulcer disease mainly contains calcium compound as a natural linker enhancing the integrity of gastrointestinal mucosa[13]. The presence of both calcium and magnesium in this drug *Inji dravagam* antagonizes with each other as per the classical therapy in which the noxious effects of calcium compounds was antagonized by magnesium sulphate[14] and also the presence of magnesium reduces the parietal cell mass, stimulates the production of prostaglandins, and blocks indomethacin action in indomethacin induced ulcers thus exhibiting gastroprotective and antiulcer property[15]. The decrease in the phosphorus levels in blood signifies the recurrence of PUD which can be therapeutically corrected by this drug *Inji dravagam* due to the presence of phosphorus[16] and hence the recurrence of this disease can be avoided. The compound zinc as zinc acexamate (ZAC) possesses to have an inhibitory effect on gastric lesions found in the experimental models which was due to the various protective activities present in the zinc[17]. Thus the drug *Inji dravagam* is proved to have various beneficial and therapeutic effects on peptic ulcer disease due to the present of these elements. Through GC-MS analysis, the name, molecular weight and structure of

Table 1: Organoleptic character of *Inji dravagam*

S. No	Parameters	Results
1.	Colour	Pale yellow
2.	Odour	Aromatic odour
3.	Taste	Pungent
4.	State of matter	Liquid

Table 2: Physical characters of Injidravagam

S.No.	Parameters	Result
1	pH	5.02
2	Specific Gravity	0.9981
3	Volatile matter	Negligible
4	TLC	Refer figure:2
5	HPTLC	Refer figure:3(i), (ii)

Table 3: Chemical characters of *Inji dravagam*

S.No	Experiment	Observation	Inference
1.	Physical Appearance of extract	Colourless	---
2.	Test for Silicate	No Sparingly soluble	Absence of Silicate
3.	Action of Heat	No White or brown fumes evolved.	Absence of Carbonate or
4.	Flame Test	No bluish green flame.	Absence of copper
5.	Ash Test	No Appearance of yellow colour flame	Absence of sodium

Table 4: Results of Acid radicals studies of *Inji dravagam*

S.No	Parameter	Observation	Result
1	Test for Sulphate	-	Negative
2	Test for Chloride	Cloudy appearance present	**Positive**
3	Test For Phosphate	-	Negative
4	Test For Carbonate	-	Negative
5	Test For Nitrate	-	Negative
6	Test for Sulphide	-	Negative
7	Test For Fluoride &oxalate	-	Negative
8	Test For Nitrite	-	Negative
9	Test For Borax	-	Negative

Table 5: Results of basic radicals studies of *Inji dravagam*

S.No	Parameter	Observation	Result
1	Test for Lead	-	Negative
2	Test for Copper	-	Negative
3	Test For Aluminium	-	Negative
4	Test For Iron	-	Negative
5	Test For Zinc	-	Negative
6	Test for Calcium	Cloudy appearance and white precipitate obtained	**Positive**
7	Test For Magnesium	-	Negative
8	Test For Ammonium	Mild brown colour appears.	**Positive**
9	Test For Potassium	-	Negative
10	Test For Sodium	-	Negative
11	Test For Mercury	-	Negative
12	Test For Arsenic	-	Negative

Table 6: TLC and HPTLC analysis

S.No	254nm		366nm		Dipped in Vanillin-Sulphuric Acid	
	Colour	**Rf**	**Colour**	**Rf**	**Colour**	**Rf**
1	Green	0.56	Blue	3.0	Grey	0.04
2	Green	0.84	Blue	6.8	Grey	0.1
3	Green	099			Grey	0.53
4	Green				Grey	0.89

Figure 2: TLC Photodocumentation of DTL sample 1510357

UV254nm **UV366nm** **Derivatisedm with sulphuric acid**

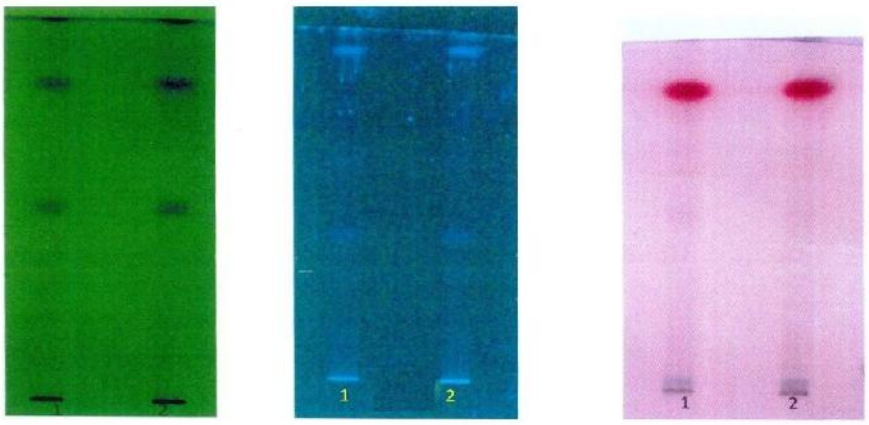
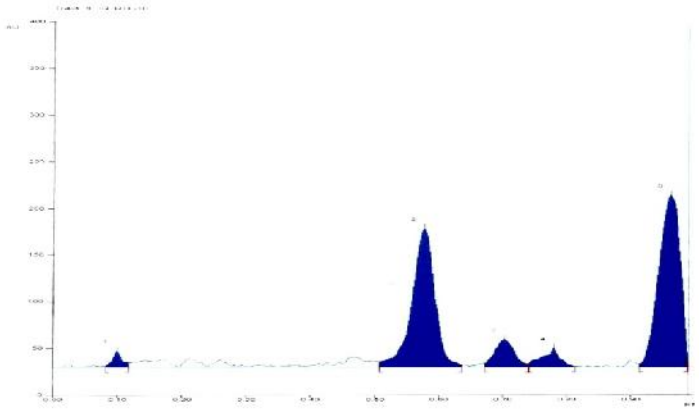


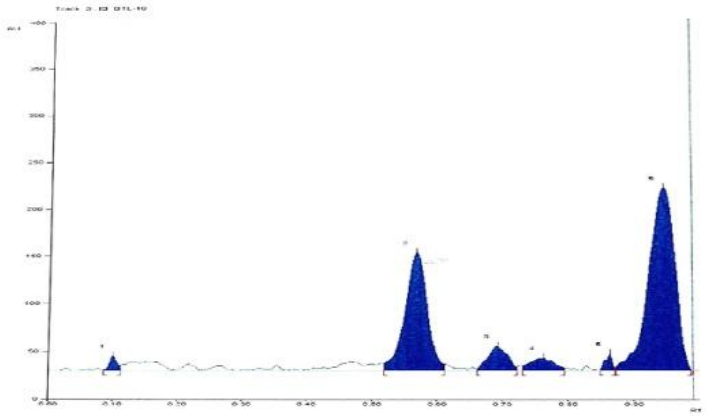
Figure 3(i): HPTLC Fingerprint profile of DTL sample 1510357 AT 254 nm at 20µl



Track 3-ID DTL 10

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.09 Rf	0.9 AU	0.10 Rf	15.7 AU	4.10 %	0.11 Rf	5.7 AU	152.1 AU	1.40 %	unknown *
2	0.52 Rf	7.7 AU	0.57 Rf	122.6 AU	31.91 %	0.61 Rf	6.9 AU	3418.5 AU	31.45 %	unknown *
3	0.66 Rf	2.0 AU	0.69 Rf	24.9 AU	6.50 %	0.72 Rf	4.2 AU	616.0 AU	5.67 %	unknown *
4	0.73 Rf	5.3 AU	0.76 Rf	13.0 AU	3.38 %	0.80 Rf	2.8 AU	394.7 AU	3.63 %	unknown *
5	0.85 Rf	0.2 AU	0.87 Rf	17.2 AU	4.49 %	0.87 Rf	2.7 AU	152.0 AU	1.40 %	unknown *
6	0.87 Rf	3.3 AU	0.95 Rf	190.6 AU	49.63 %	0.99 Rf	0.7 AU	6135.2 AU	56.45 %	unknown *

Figure 3(ii): HPTLC Fingerprint profile of DTL sample 1510357 AT 254 nm at 25µl



Track 4 ID DTL-10

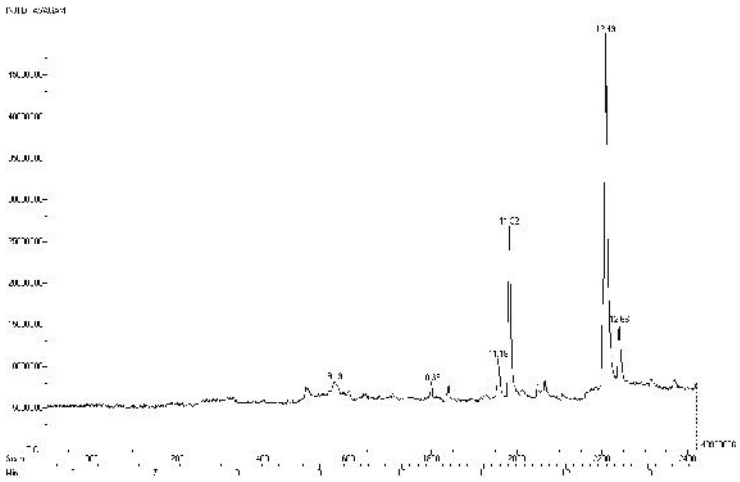
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.08 Rf	1.1 AU	0.10 Rf	17.0 AU	4.22 %	0.12 Rf	4.9 AU	215.1 AU	1.98 %	unknown *
2	0.51 Rf	6.0 AU	0.58 Rf	149.2 AU	37.09 %	0.64 Rf	2.6 AU	4371.6 AU	40.25 %	unknown *
3	0.67 Rf	4.2 AU	0.70 Rf	30.0 AU	7.45 %	0.74 Rf	3.6 AU	747.2 AU	6.88 %	unknown *
4	0.74 Rf	4.0 AU	0.78 Rf	20.4 AU	5.07 %	0.81 Rf	0.5 AU	466.0 AU	4.29 %	unknown *
5	0.91 Rf	3.6 AU	0.96 Rf	185.7 AU	46.18 %	0.99 Rf	10.5	061.1 AU	46.60 %	unknown *

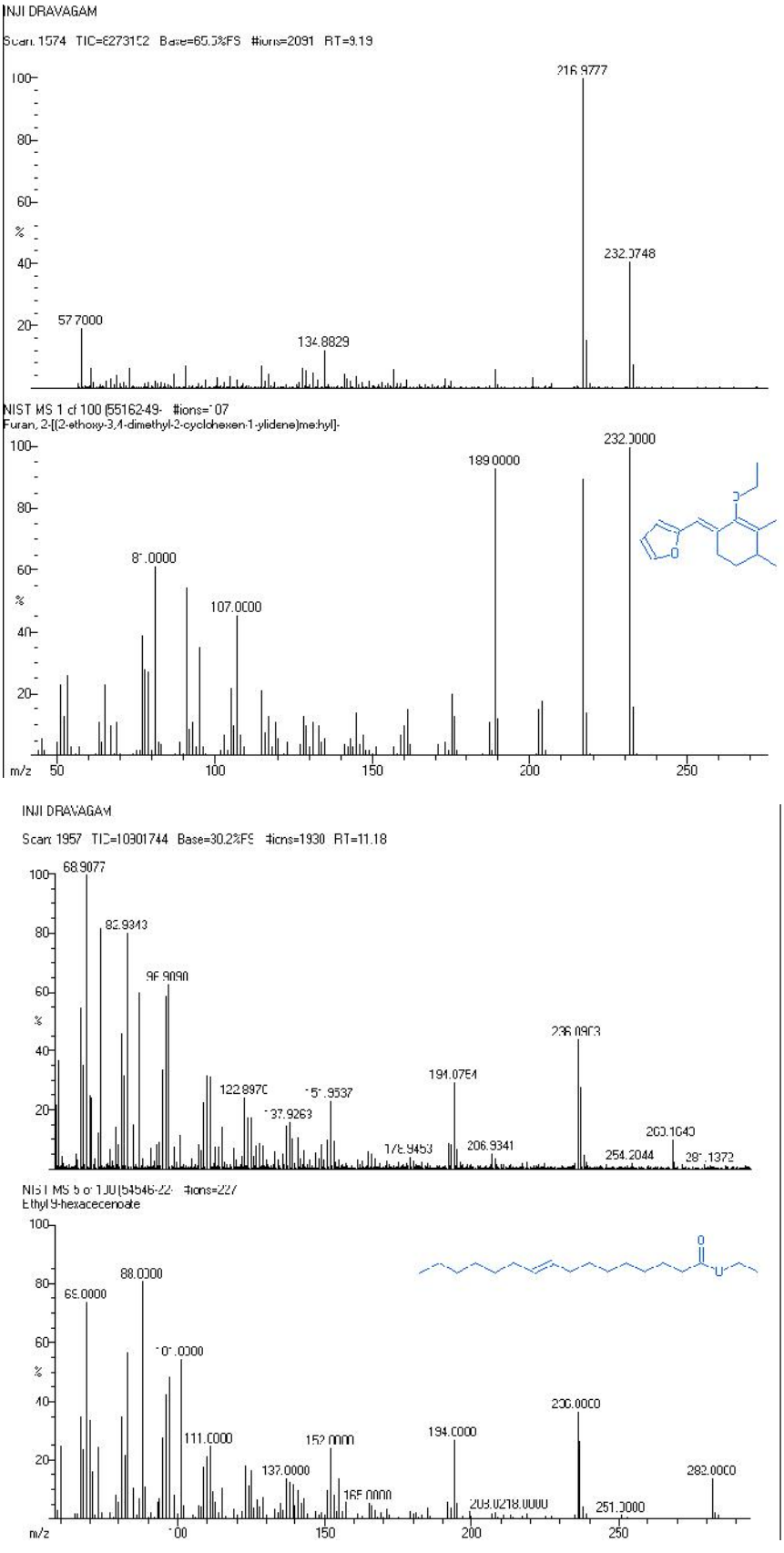
Table 7: ICP-OES study results of *Inji dravagam*

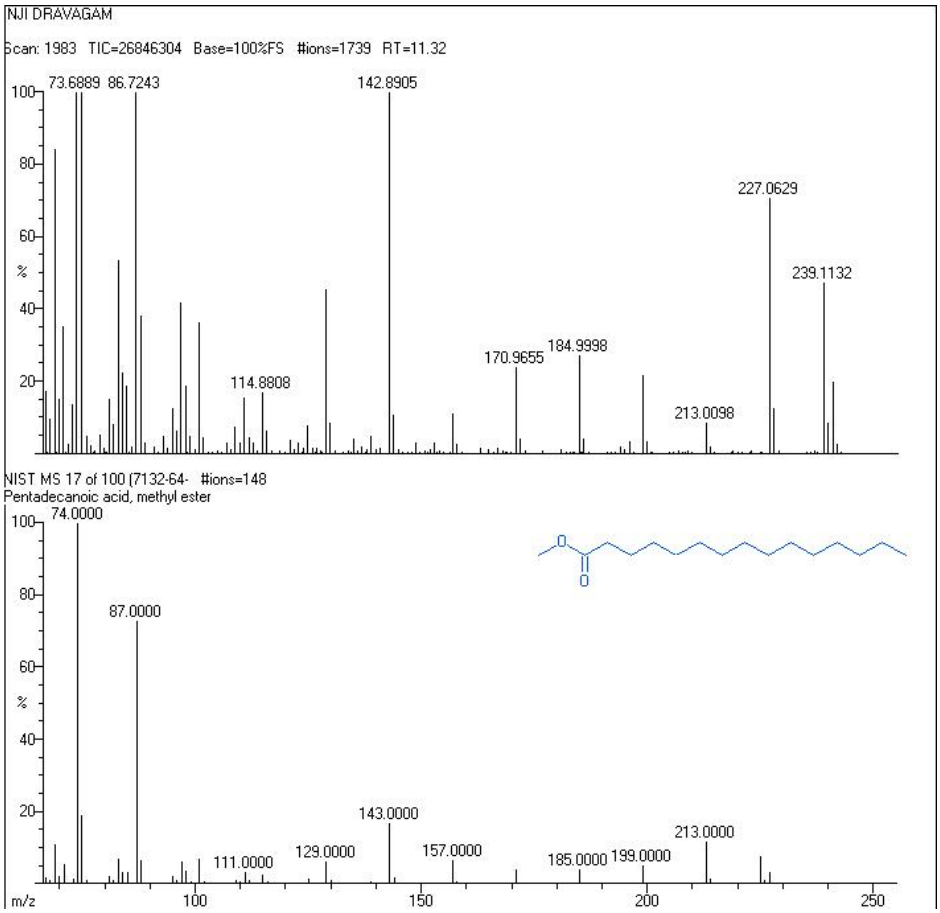
S.No	Elements	Wavelength in nm	Inji Dravagam mg/L
1.	Arsenic	As 188.979	BDL
2.	Calcium	Ca 315.807	12.760mg/L
3.	Cadmium	Cd 228.802	BDL
4.	Copper	Cu 327.393	BDL
5.	Iron	Fe 238.204	08.346mg/L
6.	Mercury	Hg 253.653	BDL
7.	Potassium	K 766.491	23.821mg/L
8.	Magnesium	Mg 285.213	11.153mg/L
9.	Nickel	Ni 231.604	BDL
10.	Phosphorus	P 213.617	19.341
11.	Lead	Pb 220.353	BDL
12.	Zinc	Zn 206.200	01.258mg/L

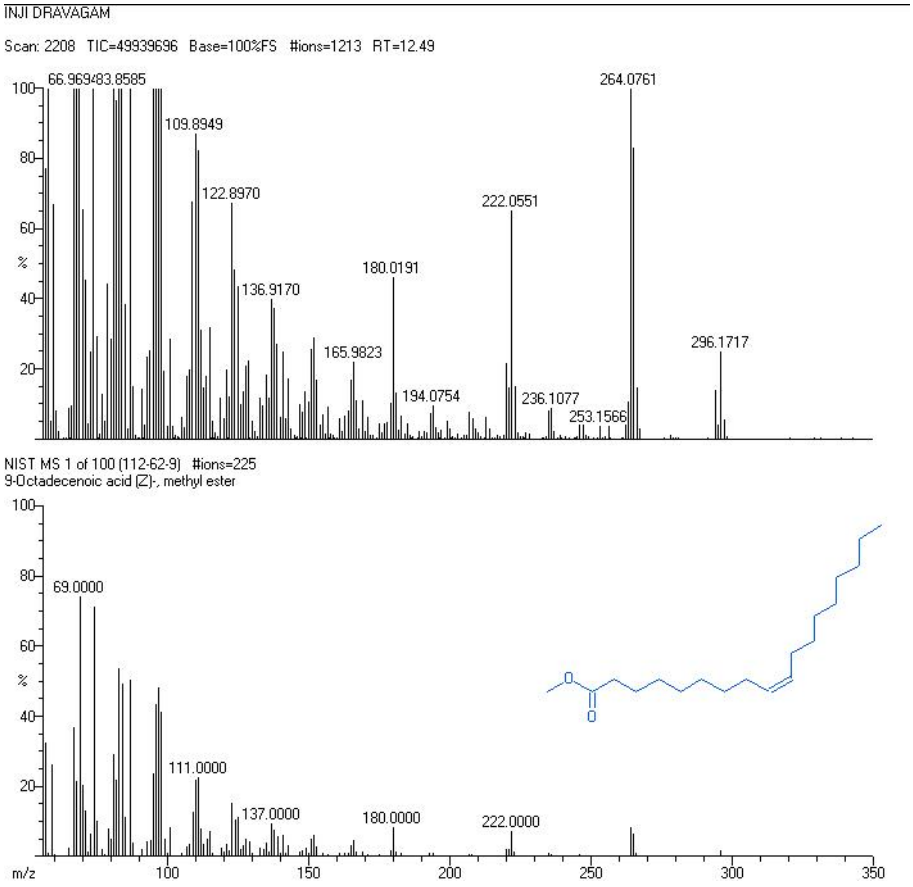
*** BDL – Below Detection Limit**

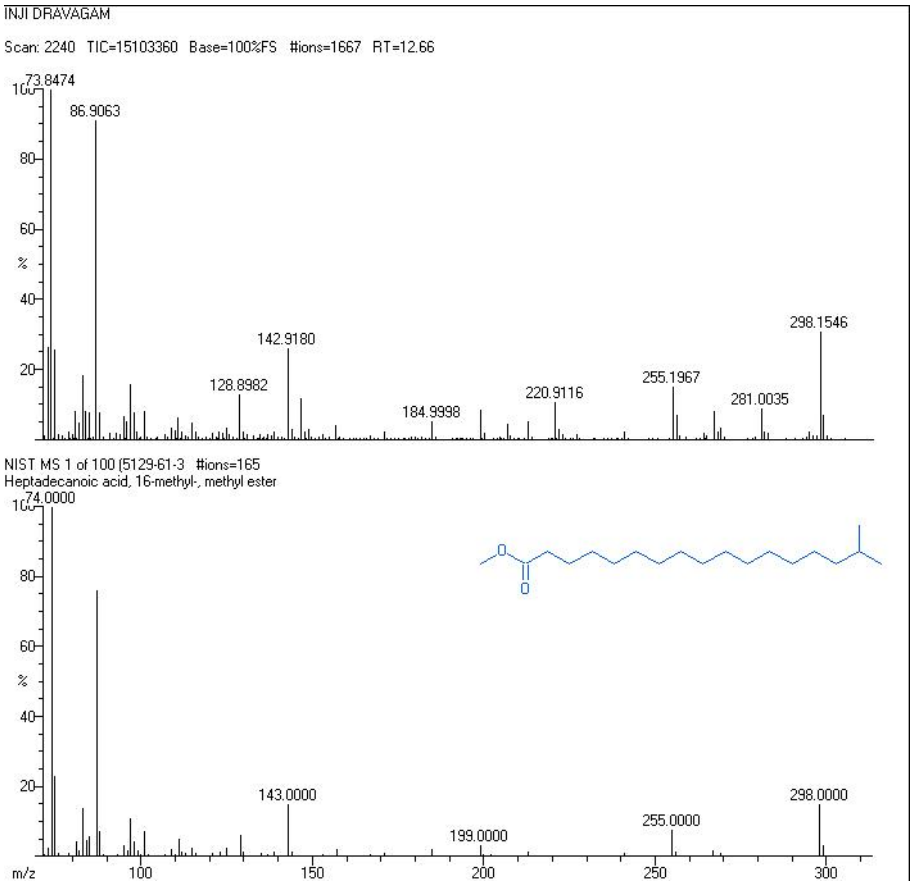
Figure 4: Gas chromatography of *Injidravagam*











different phytochemical compounds present in the test drug *Inji dravagam* was estimated. The Figure 4 (table: 8) shows the characteristic gas chromatogram of *Inji dravagam*. In this sample,6 compounds were identified. The following are Furan [2-(2-ethoxy-3,4-dimethy l- 2- cyclohexo- 1-cylidene) methyl], 5-Otadecenal, Ethyl 9-hexadecenoate, Pentadecandoic acid [methyl ester], 9-octadecenoic acid(Z) [methyl ester]and Heptadecanoic acid 16-methyl- methyl ester.

Table 8: Compounds identified in the trial drug *Inji dravagam* using GCMS

S.No	Rt	Name of the compound	Molecular formula	Molecular weight
1	9.19	Furan,2(2-ethoxy-3,4-dimethyl-2-cyclohexo-1-cylidene	$C_{15}H_{20}O_2$	232.0748
2	10.36	5 Otadecenal	$C_{18}H_{34}O$	252.2545
3	11.18	Ethyl 9-hexadecenate	$C_{18}H_{34}O_2$	281.1372
4	11.32	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	239.1132
5	12.49	9-Octadecenoic[Z], methyl ester	$C_{19}H_{36}O_2$	296.1717
6	12.66	Heptadecanoic acid, 16-methyl- methyl ester	$C_{19}H_{38}O_2$	298.1546

4. Conclusion

From the literature evidence, physico chemical analysis, bio chemical analysis, qualitative studies and quantitative studies it has been proved that the trial drug *Inji dravagam* has therapeutic benefits and a safety profile. Thus it can be concluded that the drug *Inji Dravagam* can be used in the management of Gunmam (peptic ulcer disease) and the disorders related Gastro intestinal tract. Further studies like toxicological and pharmaceutical studies for this drug will be published in the next article.

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