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Original Article

GC-MS Analysis of Indrakeeladri Native Medicine used in the Treatment of Stroke

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ABSTRACT

Context: A stroke or cerebrovascular disease is an abrupt onset of neurological deficit that is attributable to a focal vascular cause. Antiplatelet drugs, anticoagulants and thrombolytics are used in ischemic stroke. Indrakeeladri, is a village in Andhra Pradesh in India where traditional local practitioners prescription has long been practiced for treatment of stroke by using unknown and undisclosed formulation (gulikalu) without any name and labeling. Patients who have taken treatment at Indrakeeladri have noticed improvement in their symptoms particularly in speech. **Aims:** We endeavoured to analyze the formulation to know its composition as this analysis might help us in preparation of newer drugs for stroke. **Settings and Design:** GC MS analysis. **Methods and Material:** The sample was powdered and tested for its solubility in different solvents of increased polarity strength and then subjected to Gas Chromatography Mass Spectrometry (GC-MS) analysis in order to know its chemical components. **Results:** The sample was soluble only in water. The GC-MS analysis of the test sample revealed the presence of methyl tetradecanoate, dodecanoic acid methyl ester and methyl 10-methyl undecanoate as the major constituents. These substances are known to have inhibitory effect on blood coagulation. **Conclusions:** Further studies need to be carried out to find out whether these components of native medicine have a role in the treatment of stroke or not.

Key words: Native medicine, Stroke, Methyl tetradecanoate, Dodecanoic acid methyl ester, Methyl 10-methyl undecanoate

Key Messages: The GC-MS analysis of the Indrakeeladri native medicine used in the treatment of stroke revealed the presence of methyl tetradecanoate, dodecanoic acid methyl ester and methyl 10-methyl undecanoate as the major constituents.

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INTRODUCTION

Stroke is a term used to describe an abrupt onset of focal neurologic deficit due to vascular damage in the brain that lasts at least 24 hours.[1,2] Stroke is one of the major cause of disability and the second leading cause of death worldwide; in 2011 itself it caused 6.2 million deaths.[3] Stroke can be either ischemic or hemorrhagic in origin. Eighty percent of strokes are caused due to ischemia to the brain caused by occlusion of the cerebral blood vessels.[4]

Antiplatelet drugs,[5] anticoagulants[6] and thrombolytics are used in the treatment of ischemic stroke. Antiplatelet drugs like Aspirin and Clopidogrel are used as first line agents for secondary stroke prevention. Recombinant plasminogen activator (rt-PA) is the only biologically effective drug approved by FDA for the treatment of ischemic stroke.[6] In spite of many recent advances that are available the rate and degree of recovery is yet unsatisfactory in many cases.[7] Many patients with stroke in India initially approach quack medical practitioners for treatment. A survey conducted in Ludhiana, a city in the North West of India showed that about 48% of chronic stroke patients received alternative treatment.[8] Herbal medicine, Ayurvedic massage, Unani, Siddha, Marma therapy, Reiki therapy, Yoga, Herbal baths, prayer, reflexology and energy transfers are only some method of treatments offered across the country![9]

The prognosis of stroke is dependent on many variables from age of the patient to the size of the affected area of the brain.[10] Some patients recover within minutes to hours while some have a lifelong handicap.[11] Hence unscrupulous practitioners find it easy to administer drugs of unknown value to the gullible public[12] and if by chance, a mild stroke gets recovered by itself, the success is attributed to the obscure formulation.

In Andhra Pradesh, a progressive state situated in the heart of South India, many people seek treatment from a village called Indrakeeladri. It is located in Krishna district, Andhra Pradesh, India's at 16°30'54.6"North and 80°37'22.1"East. The practice of taking this local formulation for stroke has been in vogue for many decades. Some of our patients who have taken treatment at Indrakeeladri for their previous attacks report that they have noticed improvement in their symptoms particularly in speech. The medicine is given in the form of pellets (gulikalu) and

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is advised to be taken twice daily after the meals. During the treatment period there were strict dietary restrictions. Specifically, brinjal, lady's-finger, hemp, roots, tubers, green gram, red gram and egg were not to be taken. Each patient incurred about Rs1000 per month for this treatment.

Most of the patient's approached our tertiary care center, Govt. General Hospital (GGH), Guntur, Andhra Pradesh, India for various reasons. Some,

i. Could not afford the cost of treatment.
ii. Did not get recovery from the attack.
iii. Could not follow the dietary restrictions or
iv. Accessed our centre for acute care only- they had intended to approach Indrakeeladri village subsequently.

The patients were neither informed about the composition of the medicine, their source or preparation or the mechanism of action. Was the composition of this native medicine similar to currently used drugs? Do any components of this medicine help in stroke recovery? The drugs prescribed by them have never been studied scientifically. Hence we decided to analyze the drugs to know their composition as this might help us in preparation of a new drug for stroke.

MATERIALS &METHODS

Sample collection

The drug sample (gulikalu) was collected from patients who visited GGH who were treated previously at Indrakeeladri for stroke.

Solubility

The sample was powdered and tested for its solubility in different solvents of increased polarity index like hexane, benzene, chloroform, ethyl acetate, ethanol, methanol and water.[13, 14, 15]

GC-MS analysis

The Gas Chromatography Mass Spectrometry (GC-MS) analysis was done in order to know the components of the sample as it was readily available. The components were analyzed by GC-MS (SHIMADZU QP 2010) employing the electron impact (EI) mode at an ionizing potential of 70 eV with a 30 m × 0.32 mm film thickness and 1.8 µm capillary column (Resteck-624 MS) packed with 5% phenyl dimethyl silicone at an ion source temperature of 200°C. For further analysis, GC/MS settings were as follows: the initial column temperature was set at 45°C and held for 4 min; the temperature was raised to 50°C and then increased up to 175°C at a rate of 10^0C / min for 2 minutes, and then finally programmed to 240°C at a rate of 25°C / min, and kept isothermal for 2 minutes. Helium was used as carrier gas with a flow rate of 1.491 ml / min with a split ratio of 1:10. During sample analysis the column oven temperature was maintained at 280°C.[16-20]

RESULTS

Solubility

When the sample was powdered and checked for its solubility in different solvents of increased polarity strength, it was soluble only in water and very poorly in methanol.

Identification of Compounds

Interpretation of GC-MS spectrum was done using the database of National Institute Standard and Technology (NIST) which consists of more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known component inherent in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained. The GC-MS analysis revealed the presence of many compounds. (Figures 1-7, table 1) and the major component was methyl tetradecanoate (76.12%).

DISCUSSION

Ischemic strokes are caused either by local thrombus formation or by embolic phenomenon, resulting in occlusion of a cerebral artery. Atherosclerosis, particularly of the cerebral vasculature, is an important cause, although 30% are cryptogenic. Emboli can arise either from intra- or extra cranial arteries. These cause 20% of all ischemic strokes.[1,2] The major signs and symptoms of stroke are a sudden onset weakness on one side of the body, inability to speak, loss of vision or vertigo.[32] Standard of care for ischemic stroke involves infusion of a thrombolytic agent that dissolves the clot formed in the cerebral blood vessel. Antiplatelet agents such as aspirin and clopidogrel are used to decrease the clot forming propensity of the patient's blood which is the cause of the stroke attack in the first place. Hence they prevent further ischemic strokes and help in reperfusion of the affected brain tissue.[33]

Stroke patients of Andhra Pradesh have been using this native medicine since many decades with the belief that it would cure the disease. It is a matter of interest to know the reason why even educated and sophisticated people and sometimes even professional doctors have also preferred using this medicine. Physically or chemically these compounds do not resemble the drugs used therapeutically in allopathic medicine for treatment of stroke, such as thrombolytic agents and antiplatelet drugs.

The GC-MS analysis of the Indrakeeladri drug sample for stroke revealed the presence of methyl tetradecanoate (76.12%), dodecanoic acid methyl ester (17.14%) and methyl 10-methyl undecanoate as the major constituents.

Some of these compounds have antiplatelet activity and affect blood coagulation pathways by various mechanisms. Methyl tetradecanoate is one of the components of Aggreceride A which is a platelet aggregation inhibitor from Streptomyces, but its mechanism of action is not yet known.[34] Tetradecanoic acid methyl ester sodium salt (C14-MES) decreases fibrinogen levels and prolongs prothrombin time and also decreases serum triglyceride levels.[35] These characteristics hint at a potential role of the formulation for the treatment of ischemic stroke.

Many of these compounds have other medicinal properties. Esters and amides of myristic acid have antibacterial activity.[36] Methyl tridecanoate is one of the main active components of Acacia pennata which is useful in the prevention of Alzheimer's disease. This is due to the inhibition of β-amyloid aggregation.[37] Methyl 12-methyl tridecanoic acid inhibits acetyl cholinesterase and is used as a pesticide.[38] Lauric acid has very strong bactericidal activity against *P. acnes and is used in the treatment of acne*.[39] Oils rich in lauric acid decrease the ratio of total to HDL cholesterol.[40]

2,8,9-Trioxa-5-aza-1-silabicyclo (3.3.3) undecane is one of the compounds detected in the formulation. It belongs to a group of compounds called silatranes. Silatranes have been used to heal wounds and stimulate hair-growth. They also possess pharmacological properties like anti-inflammatory, anticancer, antibacterial and fungicidal activities.[41] Presence of these compounds in the sample may solely or collectively have beneficial effect on stroke. Some of the derivatives of these components are distributed in marine sources, plant sources, animal sources and further investigation is necessary for proper documentation.[42]

Further studies need to be carried out to find out whether these components of native medicine have a definitive role in the treatment of stroke or not. If it is proved that these components can be used in the treatment of stroke, it can lead to discovery of newer drugs which improve the outcome of the stroke patients.

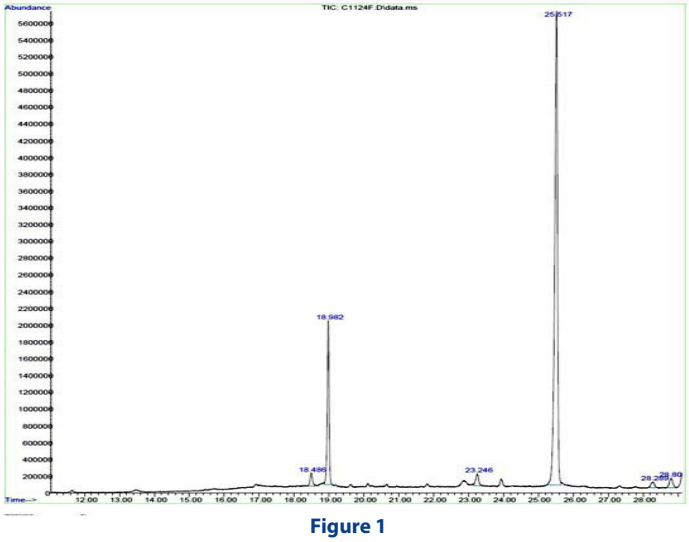

Figure 1

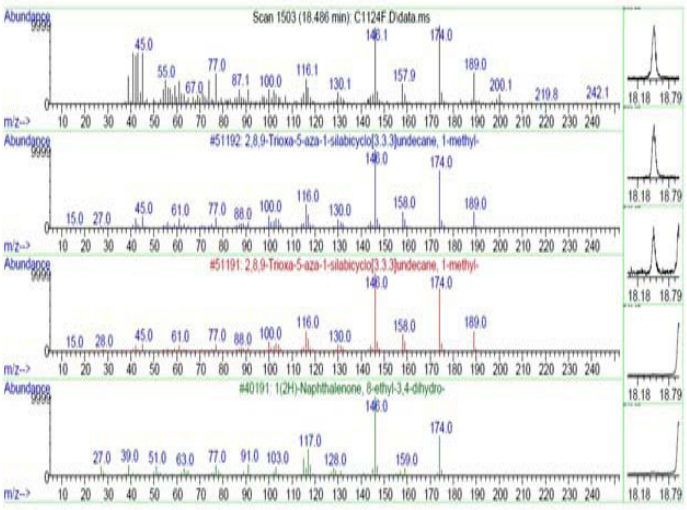

Figure 2

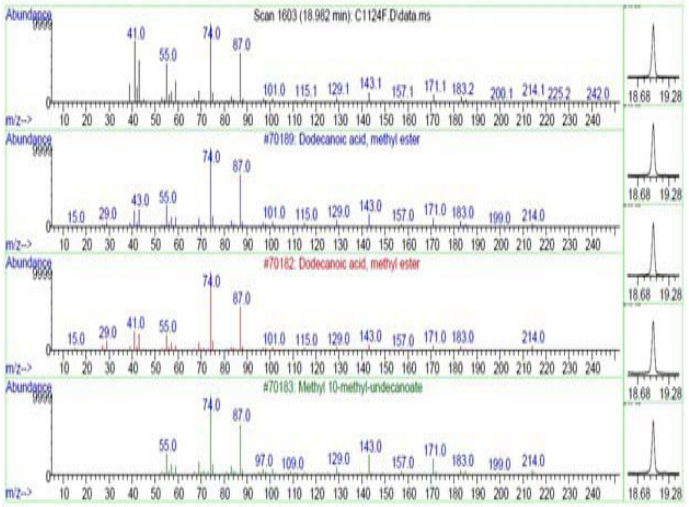

Figure 3

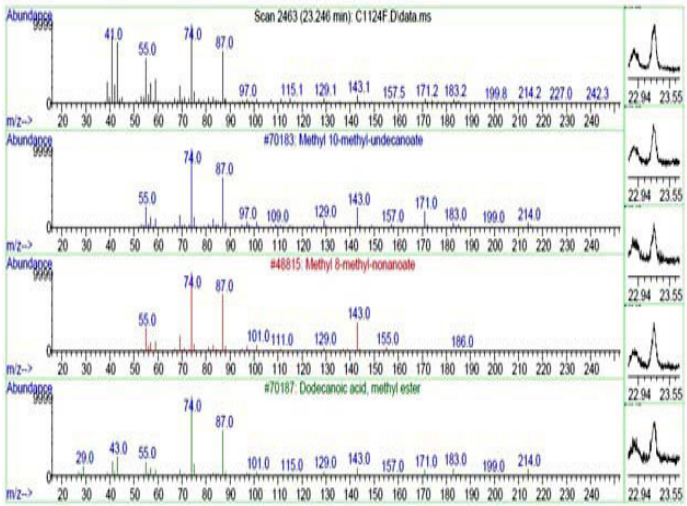

Figure 4

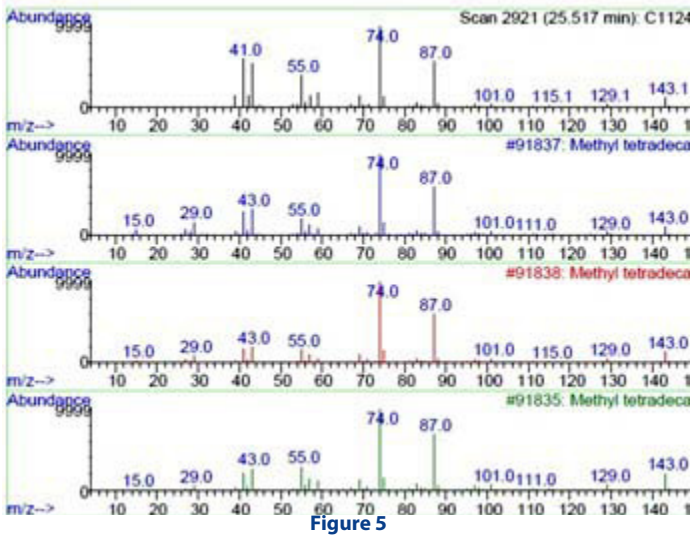

Figure 5

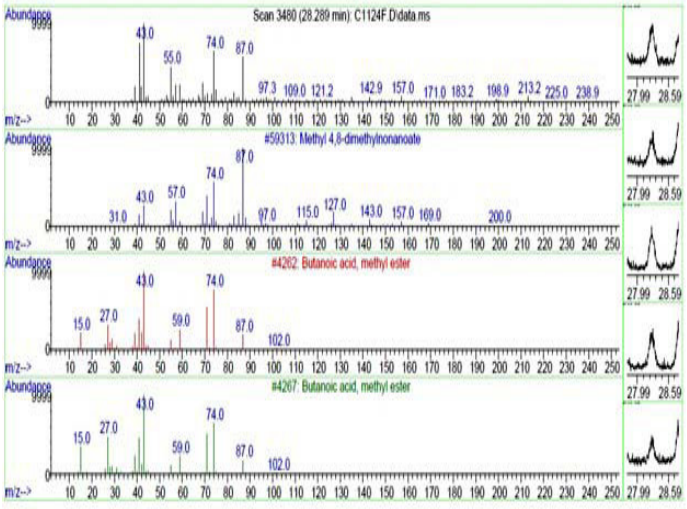

Figure 6

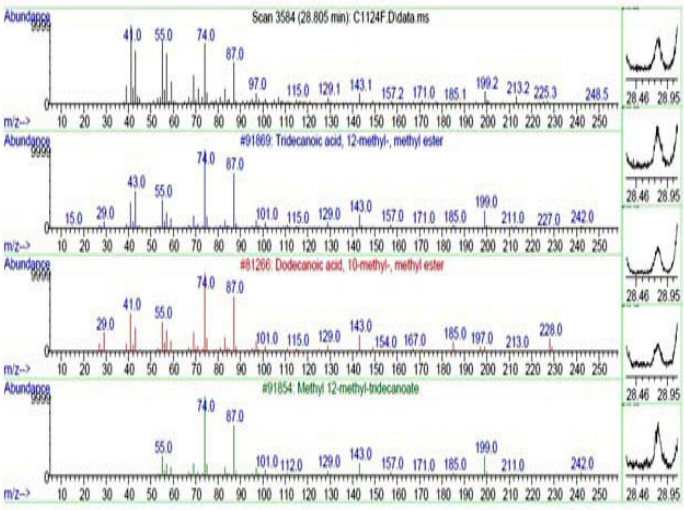

Figure 7:

Figure 1-7: GC-MS Spectrum of Indrakeeladri

Table 1: Compounds analyzed by GC-MS in INDRAKEELADRI drug sample used for stroke

S. No.	RT	Name of the compound	MW	MF	Area (%)	CAS#	Reference
1	18.486	2,8,9-Trioxa-5-aza-1-silabicyclo[3.3.3]undecane, 1-methyl-	189.3	C 7H 15NO 3Si	1.82	002288-13-3	20
2	18.486	1(2H)-Naphthalenone, 8-ethyl-3,4-dihydro	174.3	C 12H 14O	1.82	051015-33-9	21,22
3	18.982	Dodecanoic acid, methyl ester	214.3	C 13H 26O2	17.14	000111-82-0	23
4	18.982	Undecanoic acid, 10-methyl-, methyl ester	214.3	C 13H 26O2	17.14	5129-56-6	24
5	23.246	Undecanoic acid, 10-methyl-, methyl ester	214.3	C 13H 26O2	1.99	5129-56-6	24
6	23.246	Methyl 8-methyl-nonanoate	186.3	C 11H 22O2	1.99	5129-54-4	25
7	23.246	Dodecanoic acid, methyl ester	214.3	C 13H 26O2	1.99	000111-82-0	23
8	25.517	Methyl tetradecanoate	242.4	C15H30O2	76.12	000124-10-7	26
9	28.289	Methyl 4,8-dimethylnonanoate	200.3	C12H24O2	1.08	013758-80-0	27
10	28.289	Butanoic acid, methyl ester	102.1	C5H10O2	1.08	000623-42-7	28
11	28.805	Tridecanoic acid, 12-methyl-, methyl ester	242.4	C 15H 30O2	1.85	005129-58-8	29
12	28.805	Dodecanoic acid, 10-methyl-, methyl ester	228.4	C 14H 28O2	1.85	005129-65-7	30,31

RT: Retention time; MW: Molecular weight; MF: Molecular formula, CAS: Chemical Abstract Service

CONCLUSION

Indrakeeladri drug sample for treatment of stroke revealed the presence of methyl tetradecanoate, dodecanoic acid methyl ester and methyl 10-methyl undecanoate as the major constituents. Their role in treatment of stroke has to be studied further.

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COMPETING INTERESTS

We declare that we have no competing interests.

AUTHORS' CONTRIBUTIONS

Drafting of themanuscript: Sridhar, Ram Dhishana, Ramaiah. *Critical revision of themanuscript for importantintellectual content*: Sundarachary, Sridhar, Ram Dhishana, Ramaiah, Lalitha.*Technical,or material support*: Sridhar, Ram Dhishana, Lalitha, Ramaiah,. *Study supervision*: Sundarachary.

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27. National Center for Biotechnology Information. PubChem Compound Database; CID=31284.
28. National Center for Biotechnology Information. PubChem Compound Database; CID=560158.
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30. National Center for Biotechnology Information. PubChem Compound Database; CID=21204.
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