Vol 7|Issue 2| 2017 | 43-46.

e-ISSN 2248 - 9169
Print ISSN 2248 - 9150

International Journal of
Experimental Pharmacology

www.ijepjournal.com

PHARMACOGNOSTICAL STANDARDIZATION, GC-MS ANALYSIS AND *IN VITRO* SCREENING OF AN OFFICIAL SIDDHA FORMULATION- "NOCHI KUDINEER"

G.Lalithambigai, M.Sakthiganapthi, G. Prakash Yoganandam*, V.Gopal

Department of Pharmacognosy, College of Pharmacy, Mother Theresa Post Graduate and Research Institute of Health Science, (A Govt. of Puducherry Institution), Puducherry-6, India.

ABSTRACT

The present study deals with pharmacognostical evaluation of an official Siddha formulation "Nochi Kudineer". The ingredients of this formulation were collected, authenticated by a taxonomist and Pharmacognostic evaluation such as macroscopy, microscopy, fluorescence powder analysis; physio-chemical constants were done as a botanical way of standardization. Preliminary phytochemical screening reveals the presence of carbohydrates, alkaloids, glycosides, and saponins. Further the isolated ethanolic extract is subjected to GC-MS studies to find out the exact phytomolecule structure and their formula. From the GC-MS instrument analysis, it was confirmed that the extract consists of Piperine ($C_{17}H_{19}NO_3$) and Piperidine,1-[5-(1,3-benzodioxal-5-yl)-1-oxopentyl]- ($C_{17}H_{23}NO_3$). This kind of identification might be valuable to lay down the Pharmacopoeial standards of herbal drugs. A descriptive in-vitro anti-inflammatory method by protein denaturation was performed from the extract of "Nochi kudineer" A statistically significant anti-inflammatory activity was from the aqueous extracts of Nochi kudineer and it may be developed as a potential lead to combat the inflammatory disorders.

Keywords: Nochi kudineer, Piperine, GC-MS.

INTRODUCTION

Siddha system of medicine has been proved to be very effective in controlling various diseases [1]. This system is also popular in providing prophylactic medicine for diseases such as viral fever, Dengue, inflammation etc. Siddha Pharmacopoeia describes 32 types of internal medicines, of which Kudineer is one of the most important polyherbal formulations equally referred to Khashayas in Ayurveda [2,3]. These Kudineer formulations lack patient's compliance in the present day life style and hence are unpopular. Further quality control studies at par with modern medicine formulations are yet to be carried out.

Inflammation is a bodily response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed physiological functions. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. It is the body response to inactivate or destroy the invading organisms, to remove the irritants and set the stage for tissue repair. It is triggered by the release of chemical mediators from injured tissue and migrating cells [4]. The commonly used drug for management of inflammatory conditions are non-steroidal anti-inflammatory drugs, which have several adverse effects especially gastric irritation leading to formation of gastric ulcers [5]. Natural products have contributed significantly towards the development of modern medicine. Recently traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their active therapeutic principles. The rich wealth of plant kingdom can represent a novel source of newer compounds with significant anti-inflammatory activities. The major merits of herbal medicine seem to be their perceived

Corresponding Author

G. Prakash Yoganandam
Email id: gprakashyoga@gmail.com

efficacy, low incidence of serious adverse effects, and low cost. The present study was conducted to evaluate the possible in vitro anti-inflammatory effect of aqueous extract of Nochi kudineer against the denaturation of protein. Denaturation of protein is a well-documented cause of inflammation [6,7].

Materials and methods:

Nochi kudineer- Preparation

1. Tender leaves of nochi koluntu – 1 handful
2. Milaku – 8 gm
3. Puntu – 4 gm
4. Kammaru vertrilai – 10 nos.

Gently pound the drugs and make decoction. The decoction should be prepared by boiling 25 gms of the drug mixture with 500 ml of water till reduced to 125 ml [8].

Preliminary Phytochemical Screening

The different qualitative chemical tests can be performed for establishing profile of given extract for its chemical composition [9,10].

Protein denaturation method

In vitro protein denaturation was adopted by Sakat *et al*.[11]. The anti-inflammatory activity of Nochi kudineer was studied by using inhibition of protein denaturation method. The reaction mixture (5ml) consist of 0.2 ml of egg albumin (from fresh hen's egg), 2.8ml phosphate buffered saline (pH: 6.4) and 2ml of varying concentration of extracts. Similar volume of double distilled water served as control. Then the mixtures were incubated at 37±2 °C in an incubator for 15 minutes and then heated at 70 ºC for 5 minutes. After cooling, their absorbance was measured at 660nm by using vehicle as blank. Diclofenac at the final concentration of (1mg/ml) was used as reference drug and treated similarly for determination of absorbance. The Percentage inhibition of protein denaturation was calculated.

GC-MS Analysis [12]

The given sample was extracted with ethanol and analysed through Gas Chromatography –Mass Spectrometry for identification of different compounds.

GC Programme: Column BR -5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30m x 0.25mm ID X 0.25 µm df. Equipment Scion 436 – GC Bruker, Carrier gas 1ml per min, Split 10:1, Detector TQ, Quadrupole Mass Spectrometer, Software MS Work Station 8, Sample injected 2µl, Oven temperature Programme, 110°C hold for 3.50 min, Up to 200°C at the rate of 10°C / min- No hold, Up to 280° C at the rate of 5°C / min – 12 min hold, Injector temperature 280° C, Total GC running time 40.50 min. **MS Programme:** Library used NIST Version -11
Inlet line temperature 290°C, Source temperature 250° C, Electron energy 70 Ev. Mass scan (m/z) 50-500 amu, Solvent Delay 0 – 3.5 min, Total MS running time 40.50 min

Fig.1. Habit of *Piper nigrum*

Fig.2. Habit of *Piper nigrum*

Fig.3 Habit of *Piper betle*

Fig.4 Habit of *Allium sativum*

RESULTS:

Phytochemical analysis:

The aqueous extract of Nochi kudineer has showed the presence of alkaloids, carbohydrates, glycosides, phytosterols, tannins, flavonoids.

Table 1. Results of Preliminary phytochemical screening of Nochi kudineer

Name of the Phytocompound	Aqueous extracts
Carbohydrates	++
alkaloids	+++
Tannins	++
Flavonoids	++
Saponins	+
Glycosides	+++
Phytosterols	++
Proteins	-
Amino acids	-
Phenols	++
Anthraquinone	-
Terpenoids	-

Highly (+++); Moderate (++); Mild(+); Absence(-); Present (+)

Fig 1. GC-MS/MS Chromatogram analysis of Nochi kudineer

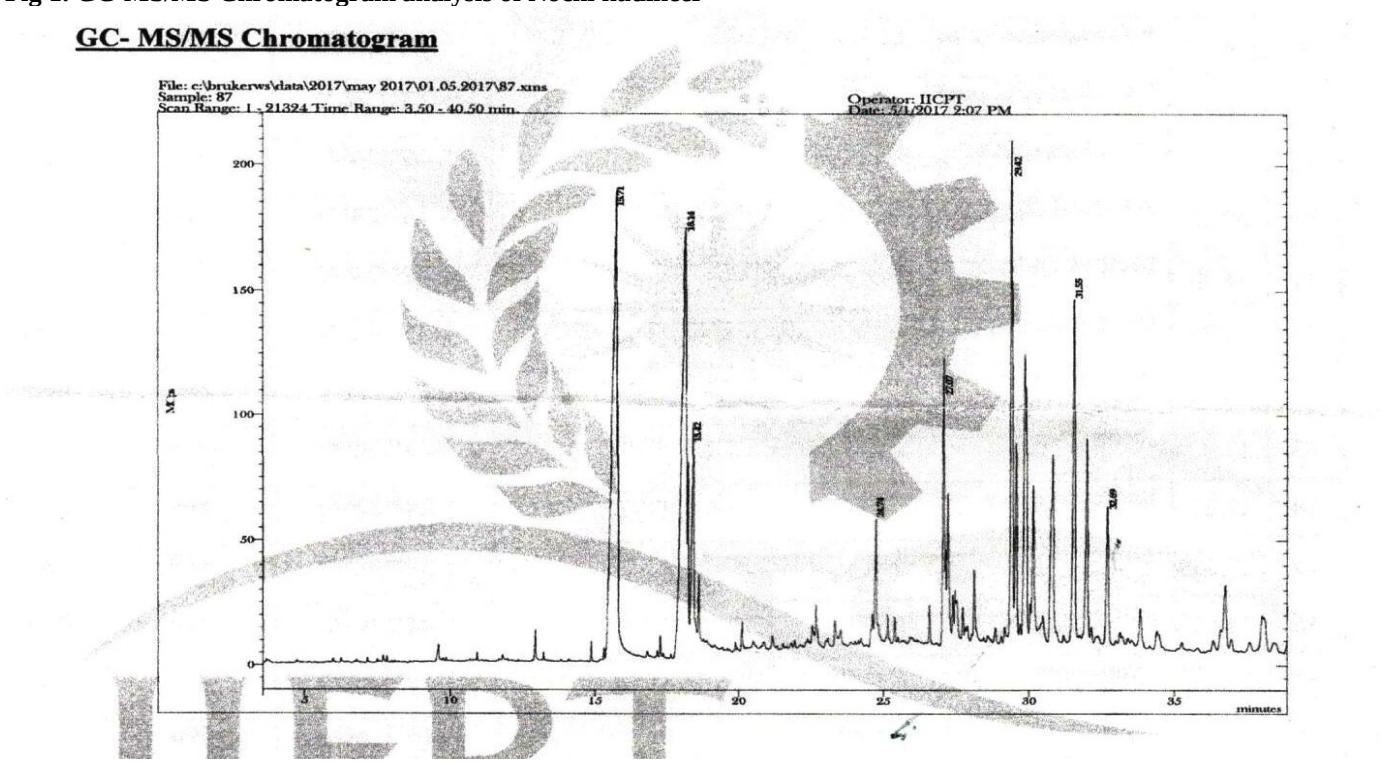

Table 2. Anti-inflammatory activity of Aqueous extract of Nochi kudineer on protein denaturation

Concentration of Aqueous extract(µg/ml)	% inhibition
200 µg/ml	31.51±0.88
400 µg/ml	38.04±0.83
600 µg/ml	42.38±0.97
800 µg/ml	54.34±1.77
1000 µg/ml	61.93±0.90

Table 3. Anti-inflammatory activity of diclofenac sodium on protein denaturation

Diclofenac sodium (1mg/ml)	% inhibition
200 µg/ml	27.23±2.56
400 µg/ml	36.10±2.46
600 µg/ml	45.55±1.99
800 µg/ml	60.57±18.97
1000 µg/ml	73.38±2.31

DISCUSSION

Herbal medicine is the use of plant extracts to treat various types of diseases. Medicinal plants exist in many local varieties depending on the regional flora. Many modern drugs were originally extracted from plant sources, they are now made synthetically, and many other drugs are descended from plant substances. The preliminary phytochemical screening tests may be useful in the detection of the bioactive phytocompounds may lead to the discovery and development drugs. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacological active phytochemical compounds. Phytochemical investigations on the Nochi kudineer revealed the presence of various phytoconstituents such as triterpenoids, steroids, flavonoids, tannins, saponins, vitamins, sugars, vanillin, ursolic acid. These phytochemicals have various health benefits such as antioxidant, anti-microbial, anti-inflammatory, cancer preventive, anti-diabetic and anti-hypertensive effect. For example, saponins have hypotensive and cardio-depressant properties. Glycosides are naturally cardioactive drugs used in the treatment of congestive heart failure and cardiac arrhythmia.

CONCLUSION

Inflammation is the symptomatic alert for any disorders in our biological system. It can be treated by using many conventional anti-inflammatory drugs, eg, diclofenac. They are known to produce various toxic metabolites which intern causes many unwanted side effects. In the traditional medical practice, lots of herbs and their combination used to treat pain and its symptoms. One of the very popular, official siddha medicines is "NOCHI KUDINEER". This work enlightens the ingredients, method of kudineer preparation, phytochemical constituents and medicinal uses of Nochi kudineer. This might be useful to resolve the controversies lies in the preparation and standardization of Nochi kudineer in the field of Pharmacognosy, Phytochemistry and Pharmacology.

ACKNOWLEDGEMENT

The authors are very much thankful to the Dean, MTPG&RIHS and the staff members in the department of Pharmacognosy for their support and encouragement in bringing this review work successfully.

REFERENCES

1. Vaidhya Rathnam K. and Murugesa Mudhaliar S. *Siddha* Materia Medica (Medicinal plant division), 2nd edition, *Indian Medicine and Homeopathy*, Chennai, 2008, 627.
2. Shanmugavel. Siddha Maruthuva Noi Naadal Thirattu, 2nd part, Indian medicine and Homeopathy, 2003, p.1, 23.
3. Kirtikar KR and Basu BD. *Indian Medicinal Plants*, Bishen Singh Mahendra Pal Singh, Dehradun, 1984.
4. Anonymous. *New medical dictionary*. New delhi: Oxford and IBH Publishing Co.Pvt.LTD., 2007, p.376.
5. Tripathi KD. *Essentials of medical pharmacology.* New Delhi: Jaypee Brothers Medical Publishers Ltd.; 2008, p.189
6. Leelapraksh G, Mohan Dass S. *In vitro* anti-inflammatory activity of methanol extract of *Enicostemma axillare*. *International Journal of Drug Development & Research*, 3, 2010, 189-196.
7. Ingle PV, Patel DM. C-reactive protein in vzrious disease condition – an overview, Asian J Pharm Clin Res. 2011;4(1):9-13.
8. The Siddha formulation of India, Citta Vaittiya Tirattu, pp.291.
9. Kokate CK, Purohit AP, Gokhale SB. Analytical Pharmacognosy,23rd ed. Pune, Nirali Prakashan, 2003, 111-12.
10. Raaman N. Qualitative Phytochemical Screening. Phytochemical Techniques, New Delhi, *New India Publishing Agency*, 2000, 19-24.
11. Sakat S, Juvekar AR, Gambhire MN. In vitro anti-oxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn, *International Journal of Pharma and Pharmacological Science*, 2(1), 2010, 146-155.
12. Srinivasan K, Kumaravel S. Unraveling the Potential Phytochemical Compounds of *Gymnema sylvestre* Through GC-MS Study, *International Journal of Pharmacy and Pharmaceutical Sciences*, 8(1), 2016, 1-4.