Research Article

Determination of Bio active and Pharmaceutical Components of Chloroform Extract of a Herbal Formulation *Nalla Marunthu* by GC – MS Analysis.

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ABSTRACT

The objective of this study to determine the bio active and pharmaceutical components of the Chloroform extract of a poly herbal formulation *Nalla Marunthu.* The phytoconstituents of Chloroform extract of a poly herbal formulation of *Nalla marunthu* were analysed by GC-MS. Our results indicate that the presence of 48 phyto constituents of a poly herbal formulation of *Nalla marunthu*. Medicinal plants have been exhaustively studied for their potential value as a source of drugs. Obviously natural products will continue to be extremely important as sources of medicinal agents.

Keywords: *Nalla marunthu,* phyto constituents, medicinal agents, poly herbal formulation, molecular structure, GC-MS analysis.

INTRODUCTION

The focus on herbal therapy is not only in third world developing countries but also in developed countries. The studies regarding their assessment of risks and benefits, identifying pharmacologically active components/ principles and biological activities; scientific validation and revealing ethno medical values are going on worldwide with regards to thousands of herbs and their extracts, preparations and products which altogether would play vital role in perpetuating, propagating, popularizing and promoting the wider usages of drugs/medicines based on herbs. Herbal plants have been used as a source of valuable medication in virtually all cultures worldwide due to presence of important antimicrobial principles, immune modulatory activities, and maintenance of general health, precious therapeutic properties and healing potentials; thus ensure prevention and cure for several diseases and disorders of humans and animal [1]. In India large number of plant species had been screened for their pharmacological properties but still, a vast wealth of plant species is unexplored. Medicinal plants are at interest to the field of therapeutics, as most of the drug industries depend in part of plants for the production of pharmaceutical compounds [2]. India is endowed with a rich wealth of medicinal plants and it is one of the 12 mega bio-diversity centres having 45,000 plant species. In India around 20,000 medicinal plants have been recorded recently, but more than 500 traditional communities use 800 plant species for curing different disease Natural products, such as plant extracts, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity[3]. According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases .Due to the development of adverse effects and microbial resistance to the chemically synthesized drugs, men turned to ethno pharmacognosy. They found literally thousands of phytochemicals from plants as safe and broadly effective alternatives with less adverse effect. Many beneficial biological activity such as anticancer, antimicrobial, antioxidant, antidiarrheal, analgesic and wound healing activity were reported. In many cases the people claim the good benefit of certain natural or herbal products[4].

Croton bonplandianus belongs to the family of Euphorbiaceae or Caster family. A much branched woody herb and 20-50 cm tall, branches stellate hairy to glabrous. Leaves alternate or subopposite, shortly petiolate; lamina 1.2-3.2 cm long, narrowly ovatelanceolate, apex acute, cuneate at base. Inflorescence terminal, 5-7 cm long, flowers laxly distributed. Male flowers small, white occupies the upper portion of the inflorescence. Female flowers few at the base of the inflorescence. Capsule 0.45 x 0.4 cm, oblong-ellipsoid, shallowly 3-lobed. Actions and uses: Juice of 3-4 leaves is given for 3-4 days to cure cough. Seed paste is applied locally on eczema and ringworm to cure. Latex is used to heal cuts and wounds. EtOH (50%) extract of plant is hypotensive and spasmolytic. The leaf extract shows antiviral activity against tomato spotted with virus coepia. The plant extract is also effective against green gram leaf curl disease[5].

Trianthema portulacastrum Linn. is an herb used in Ayurvedic medicine. *Trianthema portulacastrum* Linn, belonging to the family Aizoaceae, is one of the common weed, which has enormous traditional uses against diseases and some bioactive compounds have been

isolated from this weed. It is an exotic weed and a native of tropical America. It is growing throughout most tropical countries, such as Baluchistan, Ceylon, and India [6]. It is .now naturalized throughout India in cultivated fields, river beds, waste ground, etc [7]. Its infestation is very common in various agricultural and vegetable crops, such as mustard, maize, pigeon pea, mung bean, potato, onion, cotton, soybean, pearl millet, and sugarcane, especially during the rainy seasons. This is not cultivated commercially, but it is found throughout India as a tropical problematic terrestrial weed by virtue of its infestation in plains, river beds, and in wastelands. It also grows automatically in cultivated fields with agriculture and vegetable crops, especially in the rainy seasons[8].

Amaranthus polygonoides is a herb the pharmacological properties of Amaranth products are considered of vital importance . For reducing tissue swelling the leaves are well thought-out to be constructive, and they have a cleansing effect too. The plant has also been used curatively for diarrhea, dysentery, excessive menstrual flow, ulcers and intestinal hemorrhaging. For the treatment of intestinal bleeding, excessive menstruation, diarrhea and other related problems, a tea made from its leaves are used[9].

The plant *Indigofera tinctoria* belongs to the family of Fabaceae, its commonly known as "True Indigo'' and called as Neeli or Avuri in Tamil. *I. tinctoria* is a shrub, distributed throughout the India. The different parts of the plants are used for variety of diseases. The seeds of *I. tinctoria* containing galactomannon composed of galactose and mannose. Roots and leaves are used in epilepsy and hydrophophia. The aerial parts of *I. tinctoria* used in treatment of antiproliferative activity in human lung cancer[10]. Different solvent extracts of *I. tinctoria* showed antibacterial activity[11]. Dry powder of *I. tinctoria* used to treatment of asthma[12]. The leaves of *I. tinctoria* used as antiinflamatory traditionally. Indirubin is the active compounds isolated from *I. tinctoria* leaves active as effective anticancer drug[13]. Indigotin is the active compounds isolated from this leaves possess hepatoprotective activity[14].

MATERIALS AND METHODS

Collection of medicinal plants

The poly herbal formulation were used for this study contains the equal amount of aerial parts of the plants such as *Croton bonplandianus, Trianthema portulacastrum, Indigofera tinctoria and Amaranthus polygonoides* were collected from the nearby medicinal gardens, Chennai, India. The parts of the plants were authenticated by botanist and herbal formulations were prepared by as the available litrature.

Plant Materials

The Chloroform extract of a herbal formulation NallaMarunthu were used for this study

Preparation of Plant extracts

The extraction of the poly herbal powder was carried out using known standard procedures. The plant materials were dried in shade and powdered in a mechanical grinder. The powder (25.0 g) of the plant materials were initially defatted with petroleum ether (60-80°C), followed by 900 ml of hydroalcohol by using a Soxhlet extractor for 72 hours at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using Whatman filter paper (No.1) while hot, concentrated in vacuum under reduced pressure using rotary flask evaporator, and dried in a desiccator. The hydroalcoholic extract yields a dark greenish solid residue weighing 5.750 g (23.0% w/w). More yields of extracts were collected by this method of extractions. The extracts were then kept in sterile bottles, under refrigerated conditions, until further use. The dry weight of the plant extracts was obtained by the solvent evaporation and used to determine concentration in mg/ml. The extract was preserved at 2- to 4°C. This crude extracts was used for further investigation for potential of antimicrobial properties.

Chemicals and Reagents

All chemicals were used for this project were purchased from M/s. Sigma Chemicals, USA.

GC-MS analysis of Chloroform extract of Nalla Marunthu.

Preparation of plant extract

25gm of the powdered poly herbal powder were soaked in 95% chloroform for 12hrs. The extracts were then filtered through Whatman filter No.41 along with 2gm sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper was made wet with 95% ethanol along with sodium sulphate. The filtrate was then concentrated by bubbling nitrogen gas into the solution. The extract contained both polar and non-polar phyto-components in the plant material. 2µl of this solution was employed for GC-MS analysis. The herbal powder was extracted with chloroform and analyzed using GC-MS (GC Clarius 500 Perkin Elmer) analyzer. The data were obtained on an Elite-1(100% Dimethyl poly siloxane) column (30 0.25mm 1µmdf). Helium (99.999%) was used as the carrier gas with a flow rate of 1ml/min in the split mode (10:1). An aliquot of 2µl of ethanol solution of the sample was injected into the column with the injector temperature at 250ºC. GC oven temperature started at 110ºC and holding for 2min and it was raised to 200ºC at the rate of 10ºC/min, without holding. Holding was allowed at 280ºC for 9 min with program rate of 5ºC/min. The injector and detector temperatures were set at 250ºC and 280ºC respectively. Ion source temperature was maintained at 200ºC.

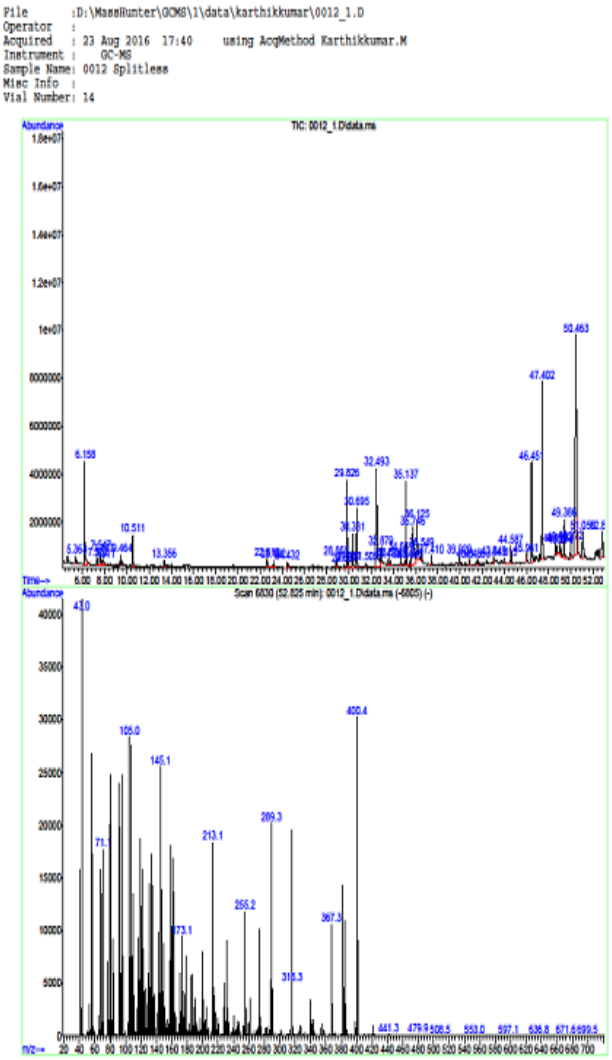
The mass spectrum of compounds in samples was obtained by electron ionization at 70 eV and the detector was operated in scan mode from 45-450amu (atomic

mass units). A scan interval of 0.5 seconds and fragments from 45 to 450 Da was maintained. The total running time was 36 minutes[15].

Identification of components

Identification was based on the molecular structure, molecular mass and calculated fragments. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown component was compared with the spectrum of the component stored in the NIST library version (2005), software, Turbomas 5.2.

RESULTS AND DISCUSSION



Plants have many phytochemicals with various bioactivities, including antioxidant, anti-inflammatory and anticancer properties. Some studies have reported that extracts from natural products such as fruits, vegetables and medicinal herbs have positive effects against cancer compared with chemotherapy or hormonal treatments. The antidiuretic, anti-inflammatory, anticancer, antiviral, antimalaria and antibacterial activities of the medicinal plants are due to the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, phlobatannins and reducing sugars. Medicinal plants are used for discovering and screening of the phytochemical constituents for the manufacturing of new drugs. The phytochemical analyses of the medicinal plants have commercial interest in research institutions and pharmaceutical companies for manufacturing new drugs for the treatment of various diseases[16].

Today approximately 80% of the world's population relies on traditional plant based medicines for primary health care. The remaining 20% of the world's population also depends on plant products for health care. About 25% of prescription drugs dispensed in the United States contain plant extracts or active ingredients derived from plants[17]. Out of a total of 520 new drugs approved for commercial use between 1983 and 1994, 30 were new natural products and 127 were chemically modified natural products. Despite the great successes already achieved in natural products chemistry and drug development, we have barely begun to tap the potential of our molecular diversity. Only an estimated 5% to 15% of the 250,000 species of higher terrestrial plants in existence have been chemically and pharmacologically investigated in systematic fashion. The Chloroform extract of a polyherbal formulation *Nallamarunthu* contains 48 various phytocontituents[18]. such as S-Tetrachloroethane, 2.2,2-Dichloropropane,1,1,3-Trichloro-2 propanone, Pentachloroethane,1.2-[(7-Methylenebicyclo[3.3.1]non-2-en-3-yl)oxy]ethyl acetate #,1.2-Chlorobutyryl chloride, Avlothane, Propanal, 2,3-dichloro-2-methyl,Phenol, 2,4-bis(1,1-dimethylethyl), Phenol, 4-chloro-2,6-bis(1,1-dimethylethyl), Cetene, α-Hexadecene, tert-Hexa decanethiol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol,2-Pentadecanone, 6,10,14-trimethyl,3,7,11,15-Tetramethyl-2-hexadecen-1-ol ,7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dion,n-Hexadecanoic acid,Eicosene, (E),13-Tetradecenal,1.1,1-Dimethyltetradecyl hydrosulfide #Phytol, 1-Hexadecyn-3-ol, 3,7,11,15-tetramethyl, 5.3,7,11,15-teramethyl-2-hexadecene-1-ol-, (2E,7R,11R),2-Pentadecanone, 6,10,14-trimethyl Formula, 9,12,15-Octadecatrienoic acid, (Z,Z,Z), Octadecanoic acid,1-Heneicosyl formate,tert-Hexadecanethiol,n-Tetracosanol-1,1.1,1-Dimethyltetradecyl hydrosulfide #,Heptadecane, 2,6,10,15-tetramethyl,9-Hexacosene, Octatriacontylpenta fluoropropionate, Eicosane, Octacosyltrifluoroacetate, 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E), Tritetracontane, Ibogamine-18-carboxylic acid, 12-methoxy-, methyl ester,Hexacosyl acetate, Stigmasta-4,7,22-trien-3α-ol, Octadecanal, Cholesta-4,6-dien-3-ol, (3β),1-Heptacosanol, Vitamin E, Campesterol.

Table 1: GC-MS analysis of Chloroform extract of *Nalla maruthu.*

Peak	Retention Time	Molecular Formula	Name of the Compounds
1	5.363	C_4H8Cl2	Butane,1,1-dichloro
2	6.158	$C_2H_2Cl_4$	S-Tetrachloroethane
3	7.302	$C_3H_6Cl_2$	2.2,2-Dichloropropane
4	7.547	$C_3H_3Cl_3O$	1,1,3-Trichloro-2-propanone
5	7.777	C_2HCl_5	Pentachloroethane
6	7.911	$C_{14}H_{20}O_3$	1.2-[(7-Methylenebicyclo[3.3.1]non-2-en-3-yl)oxy]ethyl acetate #
7	9.464	$C_4H_6Cl_2O$	1.2-Chlorobutyryl chloride
8	10.511	$C_4H_6Cl_2O$	Avlothane
9	13.356	$C_{14}H_{22}O$	Propanal, 2,3-dichloro-2-methyl
10	22.612	$C_{14}H_{22}O$	Phenol, 2,4-bis(1,1-dimethylethyl)
11	23.192	$C_{14}H_{21}ClO$	Phenol, 4-chloro-2,6-bis(1,1-dimethylethyl)
12	24.432	$C_{16}H_3$	Cetene
13	28.860	$C_{16}H_{32}$	α-Hexadecene
14	29.581	$C_{16}H_{34}S$	tert-Hexadecanethiol
15	29.826	$C_{20}H_{40}O$	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
16	29.867	$C_{18}H_{36}$	2-Pentadecanone, 6,10,14-trimethyl
17	30.331	$C_{20}H_{40}O$	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
18	30.695	$C_{20}H_{40}O$	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
19	31.505	$C_{17}H_{24}O_3$	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dion
20	32.493	$C_{16}H3_{2O}2$	n-Hexadecanoic acid
21	32.879	$C_{20}H_{40}5$	Eicosene, (E)
22	33.473	$C_{14}H_{26}O$	13-Tetradecenal
23	33.674	$C_{16}H_{34}S$	1.1,1-Dimethyltetradecyl hydrosulfide #
24	34.669	$C_{20}H_{40}O$	Phytol
25	35.048	$C_{20}H_{38}O$	1-Hexadecyn-3-ol, 3,7,11,15-tetramethyl
26	35.137	$C_{20}H_{40}O$	5.3,7,11,15-teramethyl-2-hexadecene-1-ol-, (2E,7R,11R)
27	35.538	$C_{18}H_{36}O$	2-Pentadecanone, 6,10,14-trimethylFormula
28	35.746	$C_{18}H_{30}O_2$	9,12,15-Octadecatrienoic acid, (Z,Z,Z)
29	36.125	$C_{18}H_{36}O_2$	Octadecanoic acid
30	36.549	$C_{22}H_{44}O_2$	1-Heneicosyl formate
31	37.410	$C_{16}H_{34}S$	tert-Hexadecanethiol
32	39.929	$C_{24}H_{50}O$	n-Tetracosanol-1
33	40.843	$C_{16}H_{34}S$	1.1,1-Dimethyltetradecyl hydrosulfide #
34	41.593	$C_{21}H_{44}$	Heptadecane, 2,6,10,15-tetramethyl
35	43.049	$C_{26}H_{52}$	9-Hexacosene
36	44.015	$C_{41}H_{77}F_5O_2$	Octatriacontylpentafluoropropionate
37	44.587	$C_{20}H_{42}$	Eicosane
38	45.961	$C_{30}H_{57}F_3O_2$	Octacosyltrifluoroacetate
39	46.451	$C_{30}H_{50}$	.2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)
40	47.402	$C_{43}H_{88}$	Tritetracontane
41	48.613	$C_{22}H_{28}N_2O_3$	Ibogamine-18-carboxylic acid, 12-methoxy-, methyl ester
42	48.962	$C_{28}H_{56}O_2$	Hexacosyl acetate
43	49.074	$C_{29}H_{46}O$	Stigmasta-4,7,22-trien-3α-ol

44	49.386	$C_{18}H_{36}O$	Octadecanal
45	49.972	$C_{27}H_{44}O$	Cholesta-4,6-dien-3-ol, (3β)
46	50.463	$C_{27}H_{56}O$	1-Heptacosanol
47	51.050	$C_{29}H_{50}O_2$	Vitamin E
48	52.825	$C_{28}H_{48}O$	Campesterol

CONCLUSION

Based on the results of the present study, it may be concluded that the important phytochemicals are present in the chloroform extract of *Nalla marunthu* may play an important role in various diseases in near future. However, further studies are required to establish their efficacy using human cell lines and also to observe any adverse effects in normal cells.

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