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Research Article

ANALYSIS FOR PHYTOCEUTICALS AND BIOINFORMATICS APPROACH FOR THE EVALUATION OF THERAPETIC PROPERTIES OF WHOLE PLANT METHANOLIC EXTRACT OF M*UKIA MADERASPATANA* (L.) M.ROEM. (CUCURBITACEAE) – A TRADITIONAL MEDICINAL PLANT IN WESTERN DISTRICTS OF TAMIL NADU, INDIA.

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ABSTRACT

Mukia maderaspatana (L.) M.Roem. (Cucurbitaceae), is a locally used traditional medical plant species, distributed in the low hills of Western Ghats, Tamil Nadu and Kannur and Calicut districts of Kerala, India. The present investigation deals with Gas Chromatography-Mass Spectrometry (GC-MS) analysis of methanolic extract of whole plant of this species to determine the phytochemicals. The study revealed the presence of eight phytochemical compounds of medicinal importance which includes one compound of alkaloid group, Acetamid, 2-cyano-N-(1,1-Dimethylethyl)-, five compounds of terpenoid group, 2-Hydroxytetracosanolide (essential oil), Nonacosane, Didodecyl phthalate, Trans-2-methyl-3propionyl-cyclopentanone and Solanesol (fatty alocohols), one compound of ketone group, Z, Z-6, 28-Heptatriactontadien-2-one and one compound of phehyl group, phosphine Triphenyl-. In bioinformatics approach, by using the software, Prediction Activity Spectra for Substances (PASS), molecular formula, pharmacological effects and drug likeness were determined for all the eight compounds scientifically which confirm the traditional usage of *M. maderaspatana.*

Keywords: *Mukia maderaspatana,* methanolic extract, phytochemistry, bioinformatics.

INTRODUCTION

The complex and diverse chemical structures of natural compounds provide the basis for modulation of different biological targets[1]. Multitargeted actions of natural compounds could lead to additive/synergistic or antagonistic effects[2]. Since there are several thousands of known pharmacological targets and natural products exhibit pleiotropic action interacting with multiple targets, computer-aided methods could be extremely useful for the evaluation of natural products[3]. *Mukia maderaspatana* (L.) M.Roem. (Cucurbitaceae) ia a slender, scabrous climber. It is useful in vitiated conditions of pitta, burning, sensation, dyspepsia, flatulence, colic, constipation, ulcers, cough, asthma, neuralgia, nostalgia, odontalgia and vertigo[4]. Decoctions of leaves of this plant is being used by siddha practitioners in Tamil Nadu for the treatment of hypertension[5]. The plant is also reported to have the activities like hepatoprotective, antiheumtic, antiflatulent, anti-inflammatory, anticancer, antidiabetic, diuretic and stomachic and used for toothache and recommended in vertigo and biliousness also[6,7]. Inspite of this diverse uses, this species has not been analysed scientifically so far. Hence, to determine the active principal compounds of *M. maderaspatana,* phytochemical analysis and prediction of molecular formula and drug likeness by using the computer programme, Prediction Activity Spectra for Substances (PASS) were carried out.

MATERIALS AND METHODS

Collection of the plant materials

The study species, *M. maderaspatana* collected from dry deciduous forests of Maurthamalai the western ghats, Tamil Nadu, India, was dried for 20 days at room temperature and powdered for further analysis.

Preparation of the plant extract

100g powdered whole plant material was exhaustively extracted by using methanol sovent in soxhlet apparatus for 24hr for getting maximum yield of soluble compounds[8]. The crude extract was filtered and concentrated under vacuum and controlled temperature with a rotary evaporator and residues were freeze dried. The extract was stored at -8°C in deep freezer until further use.

Gas Chromatography-Mass spectrometry (GC-MS)

Five ml of methanol extract was evaporated to dryness and reconstituted in 1ml methanol. The extracts were then subjected to GC-MS analysis. Chromatographic separation was carried out with CEGC 8000 top MSMD 8000 Fyson instrument with Db 35 mr column (10mx0.5mm, 0.25mm film thickness). Heating programs were executed from 100-250°C at 3 minutes by using helium as a carrier gas with a flow rate of 1ml/min in the split mode (1:50). An aliquot (2ml) of oil was injected into the column with the injector heater at 250°C.

Analytical conditions

Injection temperature at 250°C, interface temperature at 200°C, quadruple temperature at 150°C and ion source temperature at 230°C were maintained. Injection was performed in split less mode.

Data analysis

The mass spectra of compounds in samples were obtained by electron ionization (EI) at 70ev, and the detector was operated in scan mode from 20 to 600 atomic mass units (amu). Identifications were based on the molecular structure, molecular mass and calculated fragmentations. Resolved spectra were identified for phytochemicals by using the standard mass spectral database of WILEY and NIST[9,10].

Prediction Activity Spectra for Substances (PASS)

This computer system can predict biological activity based on structural formula of a chemical compound. The PASS approach is based on the suggestion, Activity=Function (Structure). Thus, "comparing" structure of a new substance with that of the standard biologically active substances, it is possible to find out whether a new substance has a particular effect or not. PASS estimates the probabilities of a particular substances belonging to the active and inactive sub-sets from the SAR Base (Structure-Activity Relationships Base)[11,12].

External files of substances

PASS uses Sdfile (.sdf) or MOLfile (.mol) formats as an external source of structure and activity data to prepare both SAR Base and the set of substances to be predicted[13]. SD files can be exported either from ISIS/Base 2.0+ (MDL Information Systems, Inc.) or from another moleculaar editor which has the option of SD file's export. MOLfiles can be prepared by ISIS/Draw. Molecular properties and 3D structure of compound were determined by using .sdf format which is obtained from Pubchem database (NCBI)[14]. The .mol generates 3D images using ArgusLab[15].

Algorithm of prediction

The result of prediction is returned in the form of a table containing the list of biological activity with the appropriate probability values (i.e) the values defining the likelihood for a given activity type to be either revealed (Pa) or not revealed (Pi) for each activity type from the predicted biological activity spectrum. Their values vary from 0.000 to 1.000. Only those activity types for which Pa > Pi are considered possible[16].

RESULTS AND DISCUSSION

The GC-MS analysis in the methanolic extract of *M. maderaspatana* showed the presence of rich variety of phytochemical compounds (Table 1 and Figures 1-9). The results revealed the presence of following eitht compounds: one compound of alkaloid group, Acetamid, 2-cyano-N-(1,1-Dimethylethyl)-, five compounds of terpenoid group, 2-Hydroxytetracosanolide (essential oil), Nonacosane, Didodecyl phthalate, Trans-2-Methyl-3-propionyl-cyclopentanone and Solanesol (fatty alcohols), one compound of ketone group, Z,Z-6, 28-Heptatriactontadien, one compound of phenyl group, Phosphine Triphenyl-. It has been reported already that these phytochemicals belonging to different secondary metabolites such as alkaloids, terpenoids and phenyl compounds have high medicinal properties[17,18,19]. The terpenoids in general are used as antibacterial, antiheoplastic, anti-carcinogenic, antimalarial, anti-ulcer and hepaticidal and diuretic and other pharmaceutical functions[20, 21]. Similarly in *Andrographis paniculata* by using GC-MS 13 compounds of different secondary metabolites have been identified[22]. In other medicinal plants such as *Nerium oleandar* and *Thevetial peruviana* through GC-MS studies, presence of many kinds of saturated and unsaturated fatty acids have been reported[23].

Table 1:Phytochemical compounds of the methanolic extract of the whole plant of *Mukia maderaspatana* using GC-MS analysis.

S.No.	Phytochemical compounds	Molecular formula	Retention time/min.	Molecular weight (m/z)
1.	Acetamid, 2-cyano-N- (1,1-imethylethyl)-	$C_7H_{12}ON_2$	3.210	140
2.	2-Hydroxytetracosanolide	$C_{24}H46O3$	13.446	382
3.	Z,Z-6,28-Heptatriactontadien-2-one	$C_{37}H_{70}O$	18.370	530
4.	Phosphine Triphenyl-	$C_{18}H_{15}P$	23.103	262
5.	Nonacosane	$C_{29}H_{60}$	26.695	408
6.	Didodecyl Phthalate	$C_{32}H_{54}O_4$	27.415	502
7.	Trans-2-Methyl-3-propionyl-Cyclopentanone	$C_9H_{14}O_2$	30.227	154
8.	Solanesol	$C_{45}H_{74}O$	30.557	630

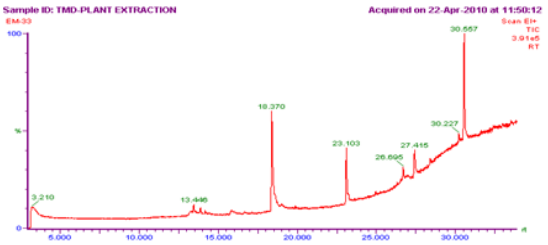

Fig. 1. Gas chromatogram of the methanolic extract of *Mukia maderaspatana*

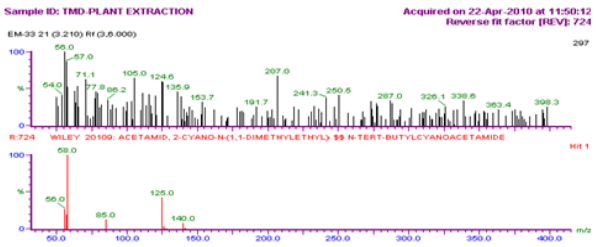

Fig -2

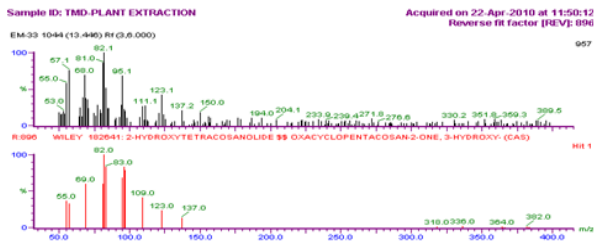

Fig -3

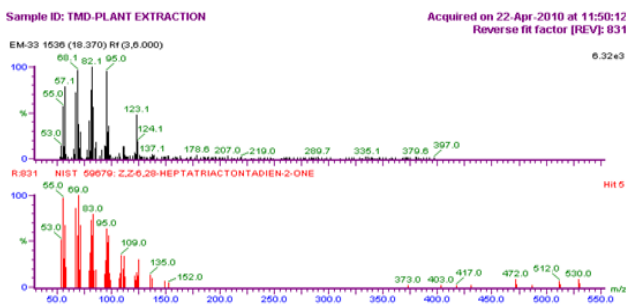

Fig -4

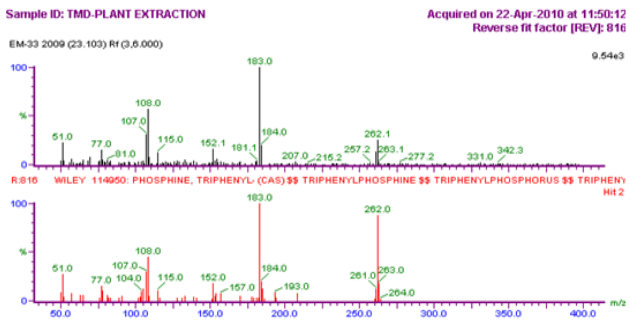

Fig -5

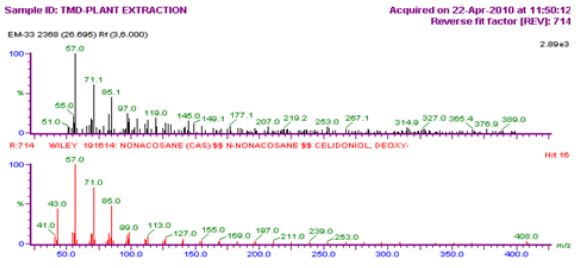

Fig -6

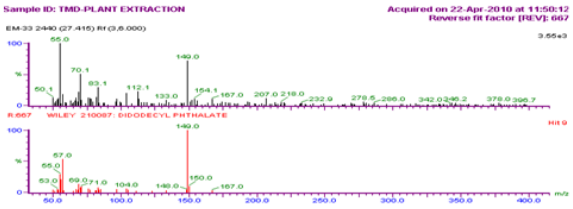

Fig -7

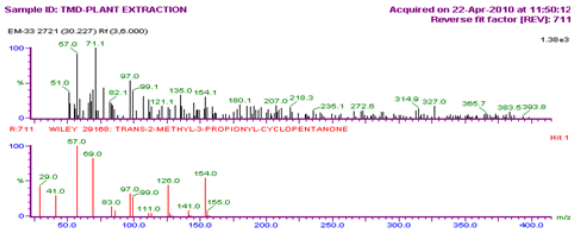

Fig -8

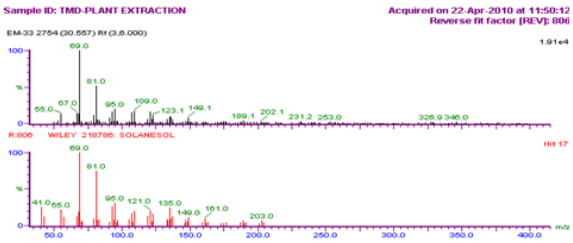

Fig -9

Figs. 2-9. Mass spectra for compounds in methanolic extract of *Mukia maderaspatana.* Fig. 2. Acetamid, 2-cyano-N- (1,1-Dimethylethyl)- Fig. 3. 2-Hydroxytetracosanolide Fig. 4. Z,Z-6,28-Heptatriactontadien-2-one Fig. 5. Phosphine Triphenyl- Fig. 6. Nonacosane Fig. 7. Didodecyl Phthalate Fig. 8. Trans-2-Methyl-3-propionyl-Cyclopentanone Fig. 9. Solanesol.

In order to find out the structure and specific activity of these compounds it is under gone for prediction of activity by using PASS software. Type of biological activity predicted by PASS includes the pharmacological effects, toxicity, molecular mechanisms and drug likeness of compounds are presented in Table 2. It is found that the drug likeness of three compounds viz. Solanesol, Z, Z-6, 28-Heptatriactontadien-2-one and Nonacosane are 0.991, 0.874 and 0.738 respectively, which reveals more than 70% probability of being a drug. The high drug likeness for the compound, Solanesol proved the probability of being a drug. The anti-hypertensive activity was shown by the compounds, Phosphine Triphenyl-, Nonacosane and Didodecyl phthalate, the vasodialator activity was expressed by the compounds, Z, Z-6, 28-Heptatriactontadien-2-one, Nonacosane and Didodecyl Phthalate, the diuretic and uric acid excretion stimulant activity was expressed by the compounds, Phosphine Triphenyl- and Didodecyl phthalate, the saluretic activity was shown by the compounds, Phosphine Triphenyl- and Nonacosane and the angiotensin ATZ receptor antogonist activity was expressed by the compounds, Nonacosane and Didodecyl phthalate. By using PASS, prediction biological activity and drug likeness have been predicted for six phytochemical compounds in the threatened medicinal herb, *Exacum bicolor* (Roxb.)[24]. In the similar fashion, predicted thirty molecular mechanisms of action of compounds which cause antihypertensive effect on basis of the structural formulae by the computer programme PASS[25].

Table 2:Predicted activities of compounds identified from GC-MS analysis in *Mukia maderaspatana* by using PASS.

Compound name	Molecular formula	Hydrogen bond donor	Hydrogen bond accepter	Activity			Drug likeness
				Pharmacological effects	Side effects and toxicity	Molecular mechanisms	
Z,Z-6,28-Heptatriactontadien-2-one	$C_{37}H_{70}O$	0	1	Vasodilator	Carcinogenic	Dopamine D2 agonist	0.874
Phosphine, Triphenyl-	$C_{18}H_{15}P$	0	0	Diuretic, Antihypertensive, Uric acid excretion stimulant and Saluretic	Teratogen	Potassium channel activator, Calcium channel antagonist, Calcium antagonist, Beta adrenoreceptor agonist and Metalloproteinase inhibitor	0.004
Nonacosane	$C_{29}H_{60}$	0	0	Antihypertensive,Vasodilator, Angiotensin AT2 receptor antagonist and Saluretic	Embryotoxic, Carcinogenic and Teratogen	Nitric oxide agonist, Adenosine A1 receptor antagonist, Dopamine D2 agonist, Dopamine agonist, Endothelin B receptor antagonist, Endothelin converting enzyme inhibitor, Dopamine D1 agonist, Renin inhibitor, Phosphodiesterase I	0.738

						inhibitor, Angiotensin antagonist, Angiotensin II receptor antagonist, Nitric oxide donor, Calcium antagonist, Potassium channel Angiotensin AT1 receptor antagonist, Adenosine A2 receptor antagonist and Phosphodiesterase V inhibitor	
Didodecyl Phthalate	$C_{32}H_{54}O_4$	0	4	Vasodilator, Antihypertensive,Angiotensin AT2 receptor antagonist, Uric acid excretion stimulant and Diuretic	Teratogen, Carcinogenic and Embryotoxic	Nitric oxide agonist, Calcium channel antagonist, Calcium antagonist, Adenosine A1 receptor antagonist, Beta adrenoreceptor antagonist, Beta 1 adrenoreceptor antagonist, Nitric oxide donor, Beta adrenoreceptor agonist, Endothelin converting enzyme inhibitor, Endothelin B receptor antagonist, Dopamine D2 agonist, Angiotensin antagonist and Angiotensin II receptor antagonist	0.418
Solanesol	$C_{45}H_{74}O$	1	1	Nil	Teratogen and Embryotoxic	Nil	0.991

Pa and pi values of each activity was also studied using PASS (Table 3). The predicted spectra of biological activity also express the side effects and toxicity of the compounds. Attentions have to be paid to both undesirable side effects and toxicity. Using the PASS approach, the problems of the compounds (side effects and toxic effects) can be solved. In the present study, PASS predicted the embrytoxicity, carcinogenicity and teratogenicity for the compounds Z, Z-6, 28-Heptatriactontadien-2-one, Phosphine Triphenyl-, Nonacosane, Didodecyl phthalate and Solanesol where these compounds could be used as drug by controlling the side effects. Moreover, it was shown that the algorithm used in PASS can successfully be applied to discriminating the so-called 'drug-like' compounds from 'drug-unlike' substances[26].

Table 3: Predicted Pa and Pi values for the GC-MS identified compounds of *Mukia maderaspatana* by using PASS.

S.No.	Compound Name	Activity		Pa	Pi
1.	Z, Z-6, 28-Heptatriactontadien-2-one	Pharmacological Effects	Vasodilator	0.382	0.118
		Molecular Mechanisms	Dopamine D2 agonist	0.136	0.128
		Side Effects and Toxicity	Carcinogenic	0.258	0.125
2.	Phosphine, Triphenyl	Pharmacological Effects	Diuretic	0.553	0.006
			Antihypertensive	0.385	0.030
			Uric acid excretion stimulant	0.238	0.072
			Saluretic	0.065	0.048
		Molecular Mechanisms	Potassium channel activator	0.345	0.005
			Calcium channel antagonist	0.169	0.054
			Calcium antagonist	0.108	0.039
			Beta adrenoreceptor agonist	0.059	0.017
			Metalloproteinase inhibitor	0.027	0.025
		Side Effects and Toxicity	Teratogen	0.507	0.035
			Antihypertensive	0.424	0.023
3.	Nonacosane	Pharmacological Effects	Vasodilator	0.355	0.136
			Angiotensin AT2 receptor antagonist	0.088	0.037
			Saluretic	0.073	0.037
		Molecular Mechanisms	Nitric oxide agonist	0.395	0.053
			Adenosine A1 receptor antagonist	0.229	0.015
			Dopamine D2 agonist	0.227	0.014
			Dopamine agonist	0.225	0.034
			Endothelin B receptor antagonist	0.131	0.021
			Endothelin converting enzyme inhibitor	0.150	0.081

			Dopamine D1 agonist	0.067	0.005
			Renin inhibitor	0.121	0.070
			Phosphodiesterase I inhibitor	0.103	0.058
			Angiotensin antagonist	0.043	0.007
			Angiotensin II receptor antagonist	0.041	0.007
			Nitric oxide donor	0.061	0.030
			Calcium antagonist	0.087	0.057
			Potassium channel activator	0.169	0.152
			Angiotensin AT1 receptor antagonist	0.029	0.013
			Adenosine A2 receptor antagonist	0.092	0.089
			Phosphodiesterase V inhibitor	0.091	0.091
		Side Effects and Toxicity	Embryotoxic	0.482	0.054
			Carcinogenic	0.366	0.052
			Teratogen	0.395	0.083
			Vasodilator	0.653	0.021
		Pharmacological Effects	Antihypertensive	0.478	0.016
			Angiotensin AT2 receptor antagonist	0.112	0.021
			Uric acid excretion stimulant	0.193	0.165
			Diuretic	0.163	0.150
			Nitric oxide agonist	0.495	0.020
			Calcium channel antagonist	0.352	0.010
			Calcium antagonist	0.210	0.018
			Adenosine A1 receptor antagonist	0.183	0.032
			Beta adrenoreceptor antagonist	0.130	0.024
4.	Didodecyl Phthalate	Molecular Mechanisms	Beta 1 adrenoreceptor antagonist	0.092	0.037
			Nitric oxide donor	0.074	0.021
			Beta adrenoreceptor agonist	0.054	0.022
			Endothelin converting enzyme inhibitor	0.135	0.112
			Endothelin B receptor antagonist	0.082	0.066
			Dopamine D2 agonist	0.137	0.123
			Angiotensin antagonist	0.027	0.014
			Angiotensin II receptor antagonist	0.024	0.014
			Teratogen	0.314	0.131
		Side Effects and Toxicity	Carcinogenic	0.277	0.110
			Embryotoxic	0.258	0.177
5.	Solanesol	Side Effects and Toxicity	Teratogen	0.446	0.058
			Embryotoxic	0.369	0.084

CONCLUSION

GC-MS analysis isolates the eight different compounds of medicinal importance from the methanol extract of the whole plant species, *M. maderaspatana*. Prediction of biological activity of these compounds by using the PASS software was successful to some extent. The presence of various bioactive compounds and the confirmation of therapeutic properties justifies the use of whole plant for various ailments by traditional practioners.

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