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GC-MS analysis, heavy metal content and predication of anti-diabetic activity spectra of a novel polyherbal formulation

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Abstract
Since there is a very huge development in the use of traditional medicine, assurance and potency as well as quality control of herbal medicines and traditional therapies are became more important. Hence, to determine the active principle compounds and its possible biological and pharmacological activities of a novel polyherbal formulation by using GC-MS and PASS (Prediction Activity Spectra for Substances) were carried out. Polyherbal formulation (PHF) was made up of four plants namely *Syzygium cumini, Picrorhiza kurroa, Madhuca indica and Commiphoramukul.* Methanol extract of PHF was used as the sample for GC-MS analysis. The identified compounds by GC-MS areCorynan-17-ol -18,19-didehydro-10-methoxy-, acetate (ester), Hexadecanoic acid methyl ester, 8-octa decenoic acid methyl ester, Octadecanoic acid methyl ester, Carda-4, 20(22)-di enolide and 4-Chlorocholest-4-en-3-one. The biological activities of the identified compounds are predicted with the help of Prediction Activity Spectra for Substances technique. The heavy metal analysis carried out by atomic emission spectroscopy method. The result showed that the PHF not contains high toxic level of heavy metals; (Arsenic -0.022 mg/L; below detectable level, Chromium 0.026 mg/L and Lead 0.006 mg/L; below detectable level).

Keywords: *Anti-diabetic, Commiphora mukul,* Gas chromatography, *Heavy Metal, Madhuca indica,* PASS, *Picrorhiza kurroa, Syzygium cumini*

Abbreviations
PASS- Prediction Activity Spectra for Substances
PFK- Perfluorokerosene
PHF- Polyherbal formulation
SAR- Structure-Activity Relationships

1. Introduction
For the last few years, there has been a global trend for the regeneration of awareness in the traditional system of treatments because of the various side effects/ toxic effects of synthetic compounds. So investigation of medicinal plants using indigenous medical systems has become ever more important for speed up better and effective treatment, especially in the case of Diabetes mellitus. Since there is a very huge development in the use of traditional medicine, assurance and potency as well as quality control of herbal medicines and traditional therapies are became more important concerns for both health authorities and the public in all over the world [Kadir MF *et al*., 2013] [5]. Essential products from medicinal plants, both as isolated compounds or as extracts/formulations, provide unrestricted opportunities for new drug leads [Murugesan A *et al.*, 2014] [9].
Heavy metals are widespread in soil as a result of geo-climatic conditions and environmental pollution. Therefore, their assimilation and accumulation in plants is common (Kofi Annan *et al.*, 2010) [6]. The heavy metal content in the plant was beneficial only to a certain limit. The heavy metal stress in the plant affects the entire cycle of life due their accumulation in the biological species and further bio magnifications to the higher order levels thereby accumulating as much of metal stressors in the environment (Mohammad *et al.*, 2012) [8]. Heavy metals cause stress to the cells in the body and hence affects the oxidation process called as the oxidative damage. In the present study, the concentration of various toxic heavy metallic elements in the PHF were analyzed using Inductively coupled plasma atomic emission spectroscopy (ICP-AES) - Ferkin Elmer Optima 5300 DV.

The diverse and complex chemical structures of compounds provide the basis for modulation of different targets. Since there are thousands of known pharmacological targets are present, studying and understanding of these targets and their mechanisms will be very difficult and hectic job. So a computer aided method could be extremely useful for this purpose. Hence, to determine the active principle compounds and its possible biological and pharmacological activities of a novel anti-diabetic polyherbal formulation by using GC-MS and PASS (Prediction Activity Spectra for Substances) were carried out.

2. Materials and Methods

Polyherbal formulation (PHF)

Polyherbal formulation (PHF) is made up of four plants namely Syzygium cumini, Picrorhiza kurroa, Madhucaindica and *Commiphora mukul*

2.1 Heavy metal analysis

Inductively coupled plasma atomic emission spectroscopy (ICP-AES), also referred to as inductively coupled plasma optical emission spectrometry (ICP-OES), is an analytical technique used for the detection of trace metals. It is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms and ions that emitelectromagnetic radiation at wavelengths characteristic of a particular element. The intensity of this emission is indicative of the concentration of the element within the sample. Emission spectrometry is based on the principle that atoms or irons in an excited state tend, to revert back to the ground state and in so doing emit characteristic wavelength and intensity of that light is proportional to the concentration of that particular element in the sample solution. This technique is used for quantitative and quality determination of the metals and metalloids in the sample.

2.2 Gas Chromatography-Mass spectrometry (GC-MS)

Methanol extract of PHF was used as the sample for GC-MS analysis. The solution (µl) was injected with the help of a micro syringe into injection port of GC system. Chromatographic separation was carried out with JEOL GCMATE II GC-MASS SPECTRO METER; it is a combination of Agilent technologies (Gas chromatographic system) and Jeol GC mate II (Mass spectrometry). Gas chromatographic system has HP-5 column to program temperature of maximum 250˚C. High pure Helium is carrier gas. A typical Mass spectrometer consists of Tungsten filament as electron source which works with 70eV, a double focusing analyzer and photo multiplier tube as detector with resolution of maximum 5000. The precise mass is determined by comparing the unknown mass of the sample peak with the known mass of a reference peak. The reference sample used here: Perfluorokerosene (PFK) for calibration.

2.3 Identification of components

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight, structure of the components in the test material was ascertained.

2.4 Prediction Activity Spectra for Substances (PASS)

This computer system can predict biological activity based on structural formula of a chemical compound to reveal novel biological activities of selected compounds, their mechanisms and related side effects. PASS estimates the probabilities of a particular substances belonging to the active and inactive sub-sets from the SAR Base (Structure-Activity Relationships Base) [4, 10]. Many study reports are available about this approach to predict the various activities of natural products [13, 1].

The PASS can predict several thousand pharmacological effects; biochemical mechanisms of action; specific toxicities; and metabolic terms on the basis of structural formulae of drug like substances with average accuracy. This can be further validated in vitro as well as in vivo assays [2, 10].

2.5 External files of substances

The structure of these chemical compounds were obtained from PubChem (http://pubchem.ncbi.nlm.nih.gov/) and Chem Spider (http:// www.chemspider.com/), and each chemical compound was drawn with chemical drawing tools such as Advanced Chemistry Development (ACD)/Chem Sketch and saved in the " MOL files "format. The biological activity spectra of these phyto-constituents were obtained by PASS version (version 9.1, http://www.ibmc.msk.ru/PASS).

2.6 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. The PASS prediction results were interpreted for the particular compound if (i) Pa > 0.7, the chance to find the activity experimentally is high; but the chance of the substance being the analogue of a known pharmaceutical agent is also high (ii) 0.5 < Pa <0.7,the chance to find the activity experimentally is less but the compound is probably not so similar to known pharmaceutical agents; and (iii) Pa < 0.5, the chance to find the activity experimentally is less, but the chance to find a structurally new compound, that is, new chemical entity is more [12, 7].

3. Results and Discussion

3.1 Heavy metal analysis

Environment, pollution, atmosphere, soil, harvesting and handling are some of the factors, which play a major role in contamination of medicinal plants by metals and also by microbial growth. Therefore it is necessary to measure and establish the levels of metallic elements in the herbal plants as these elements when consumed at higher levels become toxic. The PHF analyzed for the presence of important heavy metals such as Arsenic, Chromium and Lead are analyzed in this study. The result showed that the PHF not contains high toxic level of tested heavy metals. (Table- 1). The heavy metal content did not exceed the limit given according to the WHO guidelines (2007) [14].

Table 1: Heavy Metal Analysis of Polyherbal Formulation

NO.	COMPOUND	WAVE LENGHT	CONENTRATION	NORMAL LEVEL
1	Arsenic (As)	188.979	-0.022 mg/kg (BDL)	0.053 mg/L
2	Chromium (Cr)	267.716	0.026 mg/L	0.007 mg/L
3	Lead (Pb)	220.353	0.006 mg/L (BDL)	0.042 mg/L

BDL- Below Detectable Level

3.2 GCMS

The GC-MS analysis in the methanolic extract of PHF showed the presence of rich variety of phytochemical compounds, are Corynan-17-ol-18,19-didehydro-10-methoxy-, acetate (ester), Hexadecanoic acid methyl ester, 8-octa decenoic acid methyl ester, Octadecanoic acid methyl ester, Carda-4, 20(22)-di enolide and 4-Chlorocholest-4-en-3-one. The active principle compounds, their Molecular weight (MW), Area of Concentration (%), Molecular formula (MF), And Retention Time (RT) are presented (Table 2 and Figures 1-7).

Table: 2- GC MS Analysis of Polyherbal Formulation

No	Compounds	Retention time(min)	Area of Peak (%)	Molecular formula	Molecular mass
1	Corynan-17-ol, 18,19-didehydro-10-methoxy-,acetate (ester)	17.02	10.35	$C_{22}H_{28}N_2O_3$	368.47
2	Hexadecanoic acid, methyl ester (Palmitic Acid)	17.23	16.29	$C_{17}H_{34}O_2$	270.45
3	8-octa decenoic acid, methyl ester	18.97	53.04	$C_{19}H_{36}O_2$	296.40
4	Octadecanoic acid, methyl ester (Stearic Acid)	19.2	13.84	$C_{17}H_{34}O_2$	270.45
5	Carda-4, 20(22)-di enolide	23.07	3.56	$C_{30}H_{44}O_9$	548.66
6	4-Chlorocholest-4-en-3-one	26.15	2.92	$C_{27}H_{43}ClO$	419.08

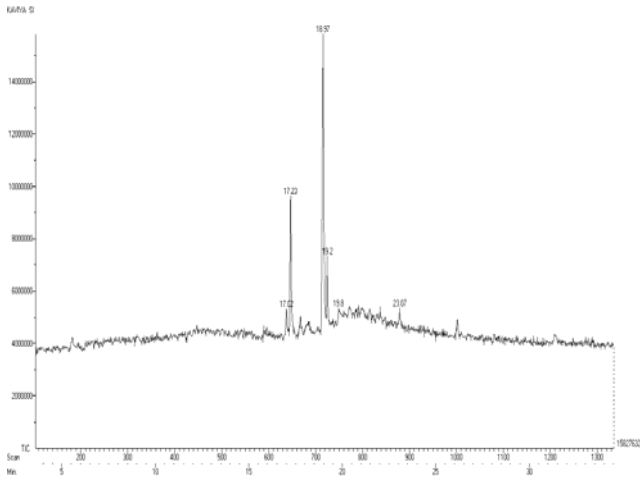

Fig 1: Chromatogram obtained from the GC-MS of PHF

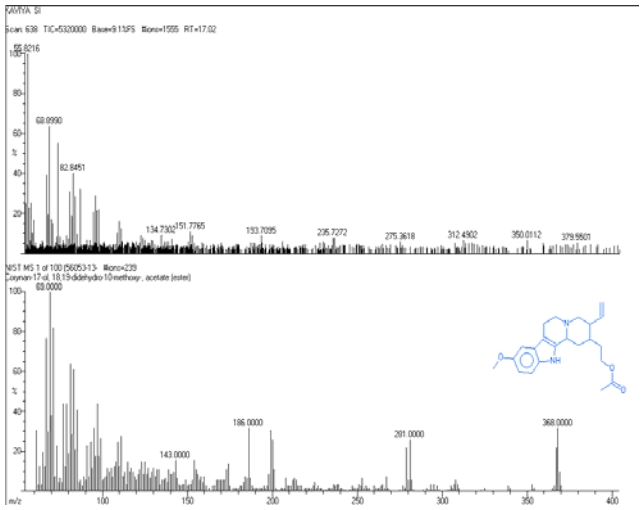

Fig 2: Chromatogram of Corynan-17-ol-18,19-didehydro-10-methoxy-acetate (ester)

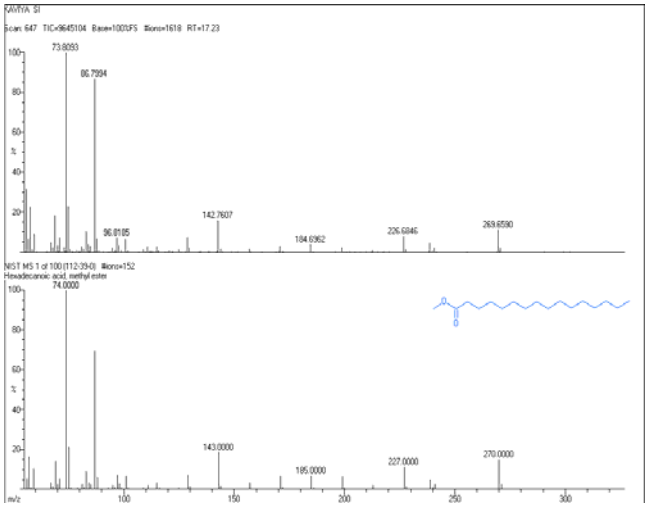

Fig 3: Chromatogram of Hexadecanoic acid, methyl ester

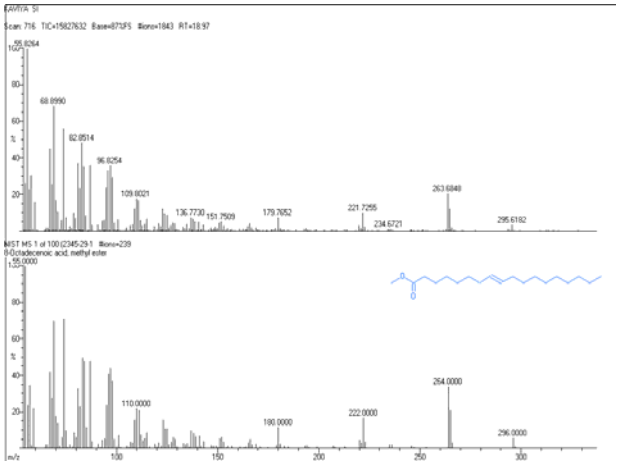

Fig 4: Chromatogram of 8-octa decenoic acid, methyl ester

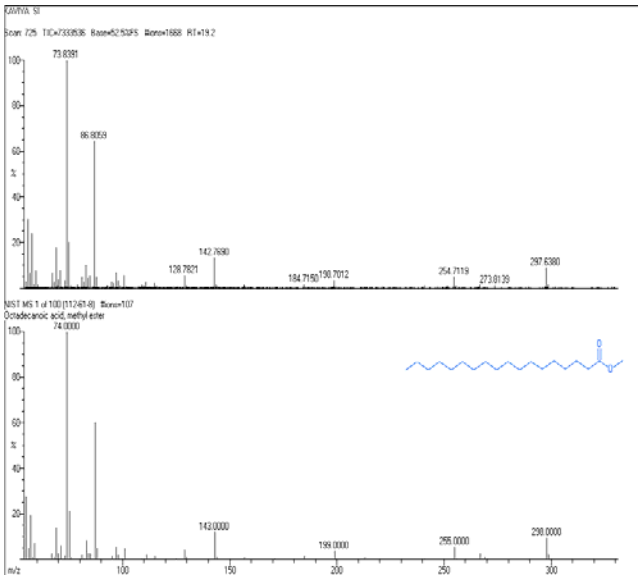

Fig 5: Chromatogram of Octadecanoic acid, methyl ester

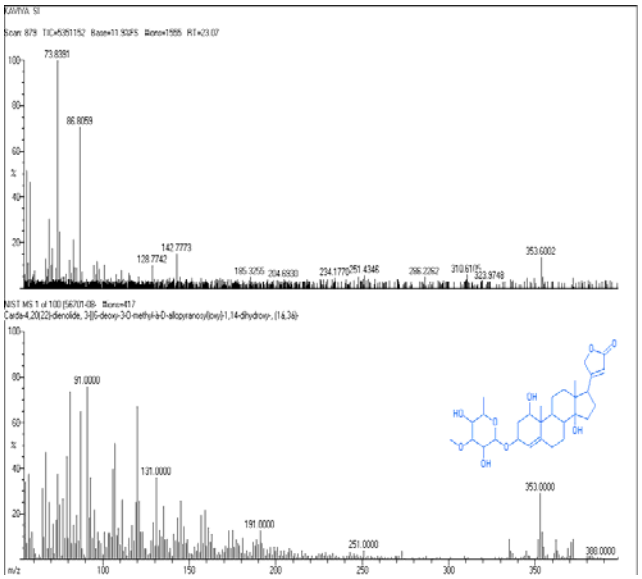

Fig 6: Chromatogram of Carda-4, 20(22)-di enolide

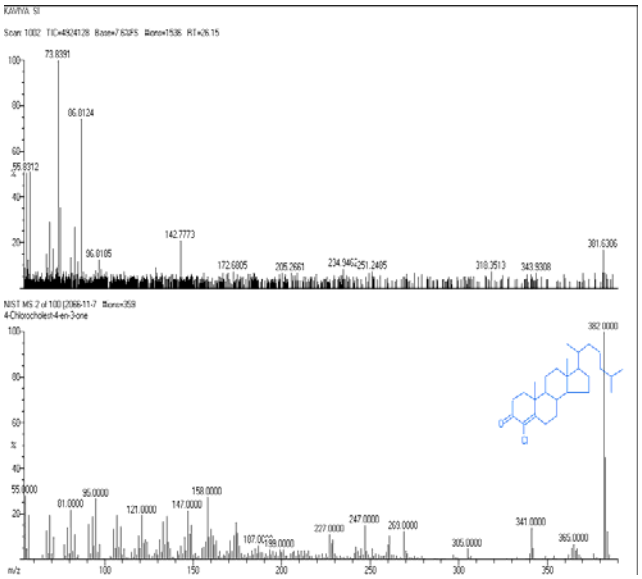

Fig 7: Chromatogram of 4-Chlorocholest-4-en-3-one

1. Corynan-17-ol, 18,19-didehydro-10-methoxy-, acetate (ester)

The mass spectrum of the compound showed the molecular ion peak at m/z 368.47, corresponding to the molecular formula $C_{22}H_{28}N_2O_3$ indicating eight degrees of unsaturation in the molecule

2. Hexadecanoic acid methyl ester

The mass spectrum of the compound showed the molecular ion peak at m/z 270.45, corresponding to the molecular formula $C_{17}H_{34}O_2$. This compound also known as palmitic acid and it is a saturated fatty acid

3. 8-Octadecenoic acid methyl ester

The mass spectrum of the compound showed the molecular ion peak at m/z 296.49, corresponding to the molecular formula $C_{19}H_{36}O_2$ indicating two degrees of unsaturation in the molecule.

4. Octadecanoic acid, methyl ester

The mass spectrum of the compound showed the molecular ion peak at m/z 270.45, corresponding to the molecular formula $C_{19}H_{36}O_2$. This compound also known as stearic acid. It is a saturated fatty acid

5. Carda-4, 20(22)-di enolide and 4-Chlorocholest-4-en-3-one

The mass spectrum of the compound showed the molecular ion peak at m/z 548.66, corresponding to the molecular formula $C_{30}H_{44}O_9$ indicating four degrees of unsaturation in the molecule

6. 4-Chlorocholest-4-en-3-one

The mass spectrum of the compound showed the molecular ion peak at m/z 419.08, corresponding to the molecular formula $C_{27}H_{43}C_{IO}$ indicating two degrees of unsaturation in the molecule

3.3 PASS

In order to find out the structure and specific activity of the compounds which are identified by GC-MS analysis, it is under gone for prediction of activity by using PASS software. Type of biological activities predicted by PASS includes the pharmacological effects, toxicity, molecular mechanisms and drug likeness of compounds etc. The activities more or less directly related to the diabetes and lipid metabolism are presented in Tables 3-7. Pa and Pi values of each activity was also studied using PASS

Table 3: Prediction of various probable activities of Corynan-17-ol, 18, 19-didehydro-10-methoxy-, acetate (ester)

No	Pa	Pi	Activity
1	0.404	0.091	Lipid metabolism regulator
2	0.295	0.134	G-protein-coupled receptor kinase inhibitor
3	0.275	0.057	Growth hormone agonist
4	0.237	0.087	Antidiabetic symptomatic
5	0.192	0.018	Diabetic retinopathy treatment

Table 4: Prediction of various probable activities of HexadecAnoic acid, methyl ester

No	Pa	Pi	Activity	No	Pa	Pi	Activity
1	0.914	0.003	Superoxide dismutase inhibitor	10	0.586	0.029	Calcium channel (voltage-sensitive) activator
2	0.910	0.003	Lipoprotein lipase inhibitor	11	0.500	0.037	Hypolipemic
3	0.894	0.005	G-protein-coupled receptor kinase inhibitor	11	0.466	0.004	Anti-hyperlipoproteinemic
4	0.831	0.005	Lipid metabolism regulator	13	0.359	0.011	Pancreatic disorders treatment
5	0.822	0.010	Glucose oxidase inhibitor	14	0.330	0.012	Chloride channel activator
6	0.753	0.004	Insulin promoter	15	0.283	0.009	HMG CoA synthase inhibitor
7	0.723	0.007	Cholesterol antagonist	16	0.323	0.070	Antidiabetic
8	0.709	0.007	HMOX1 expression enhancer	17	0.200	0.012	Insulin sensitizer
9	.632	0.010	Lactase inhibitor	18	0.085	0.015	Lipase inhibitor

Table 5: Prediction of various probable activities of 8-octa decenoic acid, methyl ester

No	Pa	Pi	Activity	No	Pa	Pi	Activity
1	0.849	0.004	Lipid metabolism regulator	15	0.380	0.019	Free radical scavenger
2	0.824	0.011	G-protein-coupled receptor kinase inhibitor	16	0.376	0.022	Lipotropic
3	0.815	0.005	Phosphatidylcholine-retinol Oacyltransferase inhibitor	17	0.349	0.015	Cholesterol synthesis inhibitor
4	0.789	0.005	Lipoprotein lipase inhibitor	18	0.329	0.004	Diabetic nephropathy treatment
5	0.755	0.004	HMOX1 expression enhancer	19	0.340	0.022	Antidiabetic symptomatic
6	0.752	0.005	Cholesterol antagonist	20	0.310	0.014	Anti-hyper-lipoproteinemic
7	0.728	0.005	Lactase inhibitor	21	0.370	0.080	Insulin promoter
8	0.672	0.008	TNF expression inhibitor	22	0.326	0.056	Lipid peroxidase inhibitor
9	0.583	0.014	Hepatoprotectant	23	0.269	0.030	Antioxidant
10	0.582	0.025	Hypolipemic	24	0.242	0.090	Calcium channel activator
11	0.569	0.026	Superoxide dismutase inhibitor	25	0.178	0.027	Antidiabetic (type 1)
12	0.571	0.035	Calcium channel activator	26	0.165	0.020	Insulin sensitizer
13	0.448	0.016	Cardioprotectant	27	0.157	0.013	Anti-hyper-triglyceridemic
14	0.450	0.025	Calcium regulator				

Table 6: Prediction of various probable activities of Octadecanoic acid

No	Pa	Pi	Activity	No	Pa	Pi	Activity
1	0.750	0.008	Lipoprotein lipase inhibitor	9	0.421	0.026	Diabetic neuropathy treatment
2	0.740	0.009	Lipid metabolism regulator	10	0.384	0.011	Cholesterol oxidase inhibitor
3	0.738	0.010	Superoxide dismutase inhibitor	11	0.356	0.004	HMGCoA synthase inhibitor
4	0.686	0.009	Cholesterol antagonist	12	0.335	0.017	Cholesterol synthesis inhibitor
5	0.637	0.014	Calcium channel (voltage-sensitive) activator	13	0.299	0.006	Diabetic nephropathy treatment
6	0.492	0.023	Antihyper- cholesterolemic	14	0.186	0.014	Insulin sensitizer
7	0.489	0.021	Hepatoprotectant	15	0.104	0.009	Lipase inhibitor
8	0.487	0.032	Insulin promoter				

Table 7: Prediction of various probable activities of Carda-4, 20(22)-enolide

No	Pa	Pi	Activity	No	Pa	Pi	Activity
1	0.627	0.016	Cholesterol antagonist	5	0.289	0.025	Antioxidant
2	0.420	0.028	Hepatoprotectant	6	0.238	0.037	Cholesterol synthesis inhibitor
3	0.310	0.089	Hypolipemic	7	0.218	0.048	Growth stimulant
4	0.292	0.015	Nitric oxide antagonist				

Table 8: Prediction of various probable activities of 4-Chlorocholest-4-en-3-one

No	Pa	Pi	Activity	No	Pa	Pi	Activity
1	0.895	0.003	Cholesterol antagonist	8	0.424	0.024	Diabetic neuropathy treatment
2	0.845	0.004	Anti-hypercholesterolemic	9	0.413	0.029	Hepatoprotectant
3	0.637	0.015	HMOX1 expression enhancer	10	0.188	0.037	HMG CoA synthase inhibitor
4	0.603	0.002	Cholesterol synthesis inhibitor	11	0.262	0.160	Lipid metabolism regulator
5	0.621	0.024	Lipoprotein lipase inhibitor	12	0.066	0.005	HMG CoA reductase inhibitor
6	0.592	0.024	Hypolipemic	13	0.242	0.240	Insulin promoter
7	0.417	0.005	Anti-hyperlipoproteinemic				

4. Conclusion

Since plants and animals are essential sources of micronutrients for human beings and become toxic when consumed at higher level. So it necessary to monitor the levels in biological materials that are required by humans for both dietary and medicinal purposes (Kofi Annan *et al.*, 2010 and S. Gajalakshmi *et al.*, 2012) [6, 3]. The present study result showed that the PHF not contains high toxic level of any tested heavy metals such as Arsenic, Chromium and Lead and is safe for the administration into the body.

GC-MS analysis isolates the six different compounds of medicinal importance from the methanol extract of the PHF. Prediction of biological activity of these compounds by using the PASS software was given an idea about the various possible activities of the isolated compounds; more importantly in the case of diabetes mellitus and related

complications. However, PASS is not being able to give an accurate prediction as they are based on two-dimensional (2D) structure of the molecule and does not calculate the molecular energy levels. Hence, it is necessary to confirm with further *in vitro* and *in vivo* studies.

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