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Anti-diabetic activity, characterization and derivation of proposed mechanism of action of *pisoniaalba*

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder that imposes a huge health and economic burden on societies. Because the currently available medications have many drawbacks, it is important to search for alternative therapies. Medicinal plants used in traditional medicines are ideal candidates. Hence, this study was undertaken to investigate the anti-diabetic activity of methanolic extract of *Pisonia alba*(MEPA). The antihyperglycemic activity was assessed using streptozotocin-induced diabetic model. Experimental diabetes was induced by a single intraperitoneal injection of streptozotocin at a dose of 150 mg/kg and animals with fasting blood glucose level (BGL) > 200 mg/dL were considered diabetic. Glibenclamide (5 mg/kg) was used as a standard drug. Fasting BGL and body weight were used to assess the antidiabetic activity; the characterization was performed using the different analytical techniques. The result was analyzed using GraphPad Prism software version 8 and one-way ANOVA followed by Tukey's post hoc test with $p< 0.05$ considered as statistically significant. The MEPA (500 mg/kg) showed a significant BGL reduction in all the three animal models. MEPA showed a significant antihyperglycemic activity in STZ induced diabetic mice, hypoglycemic activity and improvement of oral glucose tolerance in normal animals. The findings of the study strongly are strong evidences for the anti-hyperglycemic potential of the MEPA. MEPAs antihyperglycemic activity by inhibiting the glycogenolytic pathway and by improving peripheral utilization of glucose by acting as insulin mimetic agent. The effect may be attributed to the presence of bio-actives such as scopoletin, coumarin, gallic acid, ellagic acid and epicatechin.

Keywords: Diabetes mellitus, Streptozotocin, α-amylase, Medicinal plant, *Pisonia alba*

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1] Abnormalities in carbohydrate, fat, and protein metabolism are common feature of DM, which are caused by inefficient action of insulin on target tissues primarily on skeletal muscle, body fat and liver [2]. It is associated with acute and chronic complications, which are accountable for the majority of DM-related morbidity and mortality, financial burden and poor quality of life. Moreover, the persistently elevated sugar level induced diabetic complications result in damage of various organs, mainly the eyes, kidneys, nerves, and blood vessels [3]. The global social, economic and health burden of

diabetes is rising at alarming rate with its devastating complications [4]. DM has become one of the leading causes of morbidity and mortality worldwide. The number of patients is predicted to grow to 642 million in the year 2040, with the greatest increase expected in low and middle-income countries [5].

In spite of the fact that several antidiabetic agents have been introduced to the market from natural and synthetic sources, diabetes, as well as its micro and macro complications, continues to be a major medical problem worldwide [6]. The currently available modern drugs used for the treatment of diabetes are often associated with limitations such as inadequate efficacy, high cost, and various side effects [7]. In view of the aforesaid drawbacks of conventional medicines, medicinal plants with claimed antidiabetic activity can be used as an alternative approach in the management of diabetes especially in developing countries due to their cost effectiveness, accessibility, far-reaching cultural acceptability, and lower side effects [8]. More than 1200 species of medicinal plants are used throughout the world by different ethnic people in traditional medicine for their supposed antidiabetic activity.6 Some of these traditional medicines may be advocated to be formulated as active ingredients that can potentially exert health benefits including antidiabetic effects; yet, considerations of possible variations as a result of difference of geographic, climatic and extraction techniques should be taken into account [9, 10].

*Pisonia alba*also known as *Pisonia alba spanoghe, pisoniaumbellifera*, belongs to the family of Nyctaginaceae. It is found on many of the Seychelles Islands that have had habitat restoration and subsequently is a key part of the habitat associated with high biodiversity and a complex food web. It is therefore not as easy as replacing Pisonia with other native tree species; it was discovered by [11] that Pisonia is the most common nest tree for the Seychelles warbler an endemic land bird brought back from near extinction by careful habitat management and translocation, thus showing that careful consideration of the entire island ecosystem is essential. Uses: The leaves are edible. Young leaves are used as a vegetable. Leaves make good cattle feed too and are mostly used to treat rheumatism or arthritis. In traditional Indian medicine, they are used as an anti-diabetic; Leaves, of course, are used by natives as cattle feed; they are cooked and eaten for arthritis; the leaves are also carminative; Leaves are an antidote for snake bites [12].

MATERIALS AND METHODS

Collection and authentication of plant materials

The root of *Pisonia alba*was collected from local area of Gobi, Erode district. Plant collection was done, during the month of January 2018. And the root was authenticated by Dr.P.Jayaraman, professor, PARC, West Tambaram, Chennai.

Preparation of extracts

Preparation of the extract of different whole plant of *Pisonia alba*is done by using petroleum ether, chloroform, ethyl acetate and methanol solvent extraction process is done by percolation process. Preliminary phytochemical investigations for secondary metabolites were conducted on different extracts obtained from *Pisonia alba*and examined for metabolites like carbohydrates, alkaloids, glycosides, tannins, protein and amino acid, saponins.

Pharmacological study

Hyperglycemic study in diabetic models in streptozotocin induced diabetic model

Experimental design for anti-diabetic activity

30 male rats were weighed and randomly distributed into five groups of six rats each. The rats were divided into five groups using random block design to randomly segregate the animals into different experimental groups. The segregated groups were named Group –I to V. Group-I to Group-V were taken as normal control, Diabetic control, Insulin treated, Glibinclamide treated and sample treated groups, respectively. Group III received glibinclimide (5 mg/Kg) for 45 days after the induction of diabetes whereas Group IV received insulin (10 units/kg b.w) for 45 days. The Group V received MEPA at 500 mg/kg for 45 days. (Table: 1).

Table 1: Animal groups, treatment and dosage (n = 6 in each group)

Groups	Treatment	Dosage
I	Control	-
II	Diabetic Control	-
III	Insulin	5U/kg
IV	Standard Glibenclamide treated group	5 mg/kg
V	MEPA	500 mg/kg

CON- Control, PCN-Diabetic control (Only Streptozotocin treated), Gli- Glibenclamide treated group, MEPA- *Pisonia alba* root methanol extract at dose of 500 mg kg-1 BW treated group. Preparation of streptozotocin (STZ) solution

The STZ solution was prepared by dissolving the commercial STZ in freshly prepared citrate buffer (0.1 M, pH 4.5). The concentration of prepared working solution was 20 mg/mL and the prepared solution was used immediately to avoid any chemical degradation [13].

Preparation of Glibenclamide solution

Glibenclamide (Daonil®, 5 mg), an oral hypoglycemic drug was dissolved in distilled water (82.33 mL) to give a concentration of 60 µg/mL and administered orally at a dose of 600 µg/ kg daily for a period of 45 days. Induction of diabetes in rats-The streptozotocin at the dose of 45 mg/kg of the body weight was injected to rats of each group intra peritoneally, except for the normal control group. After 30 hours of streptozotocin injection, glucose solution was supplemented to avoid any mortality due to hypoglycemia induced by sudden hyperinsulinemia. The diabetic state in the animals was confirmed by measuring the fasting blood glucose levels after 72 h of STZ injection. The animals with the blood glucose level above 200 mg/dl were considered diabetic. After the confirmation of diabetic state, the treatment was started.

Collection of blood samples

To evaluate the blood glucose level at different interval of treatment in various groups the blood was collected from the tail vein using the artificial rat restrainer. About 100 µl of blood was collected at each sampling. After the treatment for 45 days all the animals were decapitated. Blood was collected through cardiac puncture into the tubes coated with a pre-coagulant. The coagulated blood was centrifuged to separate serum. The separated serum was used for various biochemical analyses.

Oral glucose tolerance test (OGTT) in Diabetic rats

The experiment was performed in diabetic control and MEPA treated groups at the end of the treatment. The two groups were overnight fasted and oral glucose load (2 gm/kg) was administered. The blood glucose level in the both the groups was analyzed at 0, 30, 60, 120 and 160 min after the administration of glucose.

Histological examinations

For histological examinations, small pieces of liver, kidney were fixed in Bouin's solution for 24 h, dehydrated through graded concentration of ethanol, embedded in paraffin wax, sectioned at 5 µm thickness and stained with Mayer's hematoxylin and eosin (HandE) and photographed in complex microscope with camera.

Biochemical analysis

Liver marker enzymes (ALP, SGPT & SGOT), total protein, albumin, urea, bilirubin, total cholesterol, tri-glycerides were analyzed using respective standard kits from Medline diagnostics. Estimation of liver glycogen content- 1 gm of liver was weighed and transferred to 10 mL test tube. 3 mL of KOH (30 %) was added and the contents were boiled on a water bath for 20 min to digest the liver and to release glycogen. The contents were cooled to room temperature and 0.2 mL of Na2SO4 was added. Then the glycogen was precipitated by adding 95 % ethanol. The contents were centrifuged to completely sediment the glycogen. The precipitate was dissolved in distilled water and the solution was made up to 10 mL. 1 mL of the glycogen solution was taken into 10 mL test tube and 1 mL of HCl (1.2 N) was added and heated on a boiling water bath for 2 H. The contents

were neutralized using the NaOH of the same strength (14).

Isolation of active constituent

Thin layer chromatography

The principle of separation is adsorption; one or more compounds are spotted on a thin layer of adsorbent coated on a chromatographic plate. The mobile phase solvent flows through because of capillary action (against gravitation force). The component moves according to their relative affinity towards stationary phase, travel slower. The component with lesser affinity towards the stationary phase, travel faster. The components with greater affinity towards the stationary phase travel slower. Thus, the components are separated.

The migration of spot on chromatograms is indicated by the term R_f value. The $R_f$values must be in the range 0.1-1(15)

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Procedure

Preparation of the plate

100 gm of silica gel – G was weighed and made in to a homogenous suspension with 200 ml of distilled water to form slurry. The slurry was poured into a TLC applicator which was adjusted to 0.25mm thickness on flat glass plates of different dimensions (10×5, 20×5, 20×10, etc) the coated plates were allowed to dry in air followed by heating at 100-105°C for 1 hour, cooled and protected from moisture. The plates were stored in dry atmosphere before using the plates were activated at 110°C for 10 minutes.

Development of chromatography

About 2mm of adsorbent from the edge of the plate has been removed to give sharply defined edges. The MEPA was dissolved in respective solvent and made up to 10 ml. Then with the help of capillary tube extract was spotted on TLC plate which was developed in TLC chamber, previously saturated with different solvent system. The solvent front was allowed to rise to distance of about 12 cm from the base line and the plate was removed from the chamber and allowed to dry in air [16-19].

HPTLC analysis

High performance thin layer chromatography (HPTLC) is an invaluable quality assessment tool for the evaluation of botanical materials. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. Additionally, numerous samples can be run in a single analysis thereby dramatically reducing analytical time. With HPTLC analysis can be viewed sing different wavelengths of light thereby providing a more complete profile of the plant than is typically observed with more specific types of analyses. (19)

Analysis of phyto-constituents using hplc &gc-mschromatogram

From the phytochemical study, it is obvious that out of the selected 15 phytochemical, ten of them showed positive results indicting the presence of the phytochemical. Since, it is a preliminary screening the test was only to find the presence or absence but did not quantify them. One of the constraints was the cost factor involved. Therefore, the study moved to the analysis for phyto-constituents usingHPLC.

GC-mschromatogram

To know the bioactive compounds present in the root of *Pisonia alba* the Methanol extract was concentrated residues designated as PAS. The GC–MS analysis of bioactive compounds from the methanolic extract of the root of *Pisonia alba*was done using Agilent Technologies GC systems with GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped with HP-5MS column (30 m in length × 250 µm in diameter × 0.25 µm in thickness of film). Spectroscopic detection by GC–MS involved an electron ionization system which utilized high energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1 mL/min. The initial temperature was set at 50 – 150 °C with increasing rate of 3 °C/min and holding time of about 10 min. Finally, the temperature was increased to 300 °C at 10 °C/min. One microliter of

the prepared 1% of the extracts diluted with respective solvents was injected in a splitless mode. Relative quantity of the chemical compounds present in each of the extracts of *Pisonia alba*was expressed as percentage based on peak area produced in the chromatogram.

Identification of Compounds

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

STATISTICAL ANALYSIS

All the values estimations were expressed as mean ± standard error of mean (S.E.M) and were analyzed for significance by ANOVA and groups were compared by Tukey-Kramer multiple comparison test, using InStat v.2.02 software (GraphPad Software Inc.).Differences between groups (p Value) were considered significant at P <0.05 level.

Anti diabetic activity

Streptozotocin induced diabetes model

Streptozotocin-induced diabetes in laboratory animals presents a cheap and reliable experimental model to assess the anti-diabetic potential and also to elucidate the mechanism of action of particular drug in controlling diabetes. Many synthetic compounds/plant products are assigned with the anti-diabetic potential using the streptozotocin induced diabetic models. Streptozotocin is a cytotoxic compound which targets pancreatic beta cells resulting in insulin deficiency. The resulting hyperglycemia due to lack of insulin leads to various negative pathological conditions which are similar to conditions developed in diabetic subjects. The model can used to study the insulin secretagogue, insulin potentiating and insulin mimetic actions of any pipeline drug. Hence, the study was planned to evaluate anti-diabetic potential of MEPAin the streptozotocin induced diabetic model. The study was conducted for 45 days after the induction of diabetes. During the study period, various parameters such as bodyweight, food and water intake, urine output (indirect) and fasting blood glucose level were assessed. The parameters analyzed and their results are presented in subheadings below. The chapter concludes with the conclusions drawn from the results of various experiments.

Body and organ weights

A significant ($P \leq 0.05$) reduction in the body weights of the diabetic control group was observed. The extent of decrease in body weights was significantly low in insulin treated and MEPA treated groups when compared to diabetic control group. The weight loss in the MEPA treated group was comparatively low both on the 15th and 45th day of the study. Mean organ weights of all the experimental groups are presented in Fig:1. There was a decrease in weights of various organs, when compared to normal control group; in STZ treated group was observed. In the treated groups weights of the organs are increased and the improved weights of organs can be comparable to normal control group.

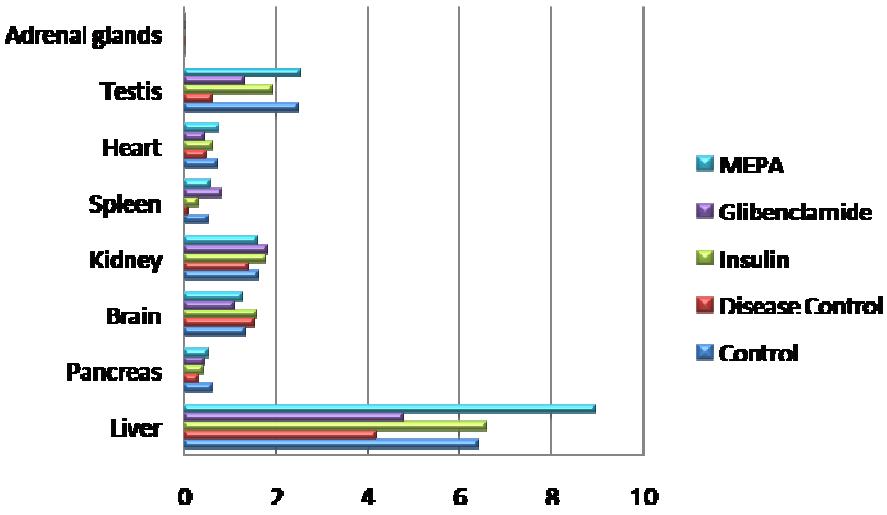


Figure 1 Organ weights of experimental groups (g)

Water and food intake

Food and water intake of various groups are presented in Fig:2. As expected, the animals of diabetic control groups consumed higher amount of food and water, compared to other groups. Among the treated groups, food and water intake was comparatively low in the MEPA group.

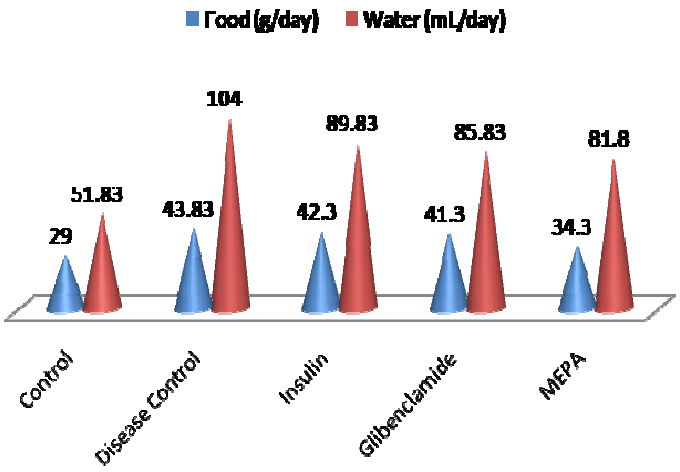


Figure 2: Water and food consumption in various experimental groups

Changes in fasting blood glucose

Levels of fasting blood glucose in all the experimental groups are presented in Fig:3. There was significant ($P \leq 0.05$) increase in the fasting serum glucose in STZ treated groups compared to normal control group. Among the treated groups, there was a significant ($P \leq 0.05$) and gradual decrease in blood glucose level at various intervals, when compared to untreated diabetic control group.

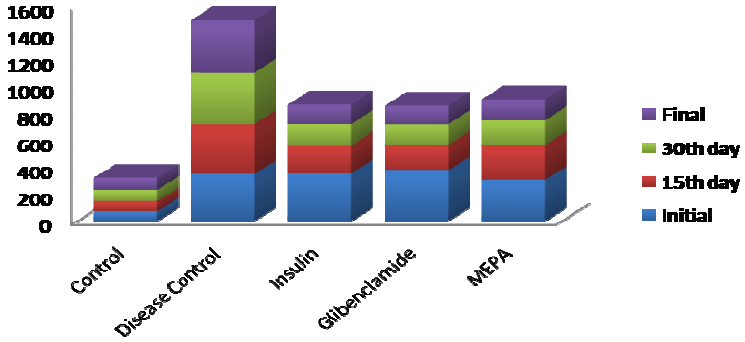


Figure 3: Fasting blood glucose (mg/dL) of the groups at various intervals of the study

Liver marker enzymes

From the Table 1, it can be observed that there is an elevation in liver marker enzymes viz., ALP, AST and AST in serum of diabetic rats. The activities of liver marker enzymes has significantly increased in diabetic control group when compared to control and all the other treated groups. It can be observed that treatment with glibenclamide, insulin and MEPA has decreased the activities of ALT, AST and ALP, and the effect was more profound in MEPA treated group.

Lipid profile

The lipid parameters of diabetic, normal control and treated groups are also given in Table 1. Results indicate that there is severe hyperlipidemia in the diabetic control group. MEPA treatment resulted in an increase in serum HDL and a decrease in triglycerides. Insulin and glibenclamide treatment also showed improvements in the lipid status and the effect was more in group treated with insulin.

Kidney markers

The serum levels of bilirubin and urea are presented in Table 1. It can be noted that the urea levels were within the normal range in all groups, however, it was lowest in the diabetic control group compared to the treated groups. As expected, serum bilirubin was elevated beyond the normal range in the diabetic control group. Treatment with INS, GLI and MEPA favorably influenced the bilirubin levels towards the normal values as observed in the control group.

Table 1 Liver and kidney health markers in various groups

Group	ALP (U/L)	SGPT (ALT) U/L	SGOT (AST) U/L	T.prog/dL	Albumin g/dL	Urea mg/dL	T. Bilirubin mg/dL	T.Cholesterol(mg/dL)	TGL (mg/dL)
Control	125.5^{a} ±9.7	10.5^{a} ±0.77	44.13^{a} ±0.45	6.11^{cd} ±0.108	1.13^{b} ±.087	46.94^{c} ±2.85	0.125^{a} ±0.010	45.8^{a} ±1.015	92.24^{a} ±3.11
Disease Control	205.75^{c} ±4.8	39.9^{c} ± 0.90	81.03^{d} ±1.93	4.11^{a} ±0.12	2.58^{d} ±0.21	37.86^{b} ±2.46	0.65^{b} ±0.020	72.81^{d} ±4.52	136.7^{d} ±7.70
Insulin	$133.^{75a}$ ±12.7	41.56^{c} ±1.45	72.31^{c} ±1.42	$5.87b^{c}$ ±0.27	0.53^{a} ±0.24	33.9^{a} ±2.84	0.121^{a} ±.08	53.31^{c} ±3.15	114.51^{c} ±5.58
Glibenclamide	180.5^{b} ±14	16.1^{a} ±0.91	55.01^{b} ±2.73	5.19^{ab} ±0.10	1.34^{c} ±0.13	34.43^{ab} ±2.47	0.151^{a} ±0.006	54.11^{bc} ±2.91	107.86^{b} ±8.63

MEPA	191^{b} ±11.1	25.08^{b} ±1.87	51.9^{ab} ±1.12	6.15^{d} ±0.14	1.42^{c} ±0.33	34.43^{a} ^{b} ±2.76	0.14^{a} ±0.02	49.61^{b} ±1.76	100.83^{b} ±3.32

The values are the mean of results from each group with SD (where n=6).Values carrying different superscripts letters a,b,c…in columns differ significantly (*P≤ 0.05*).

Glycogen content in liver

The glycogen content of the groups is given in fig:4.. A severe decrease in the glycogen content of liver was noted in diabetic control group when compared to normal control. The groups treated with MEPA and insulin had near normal glycogen levels.

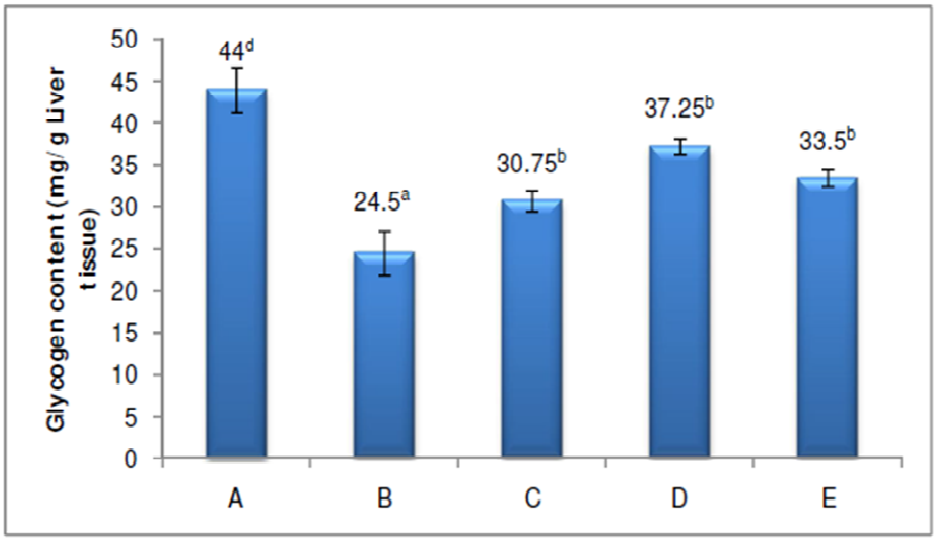


Figure 4 Glycogen content in liver

A-Normal control, B-Diabetic Control, C-Glibenclamide, D-Insulin and E-MEPA treated (The values are mean values for 6 rats in each group with ± SD.Values carrying different superscripts letters a,b,c…differ significantly (*P≤ 0.05*).

Histopathology of liver and kidney

The histopathological sections of the liver from various groups are shown in Figure 5. There was much distortion in the normal lobular architecture of the liver in the untreated diabetic control group. The treatment with MEPA has improved the health of hepatocytes towards normal physiology.

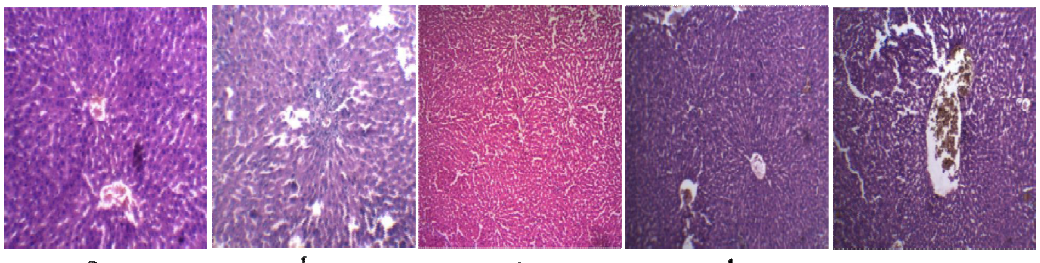


Figure 5: Liver sections of the groups.

a-Normal control, b-Diabetic Control, c-Glibinclamide, d-Insulin and d-Mahagoni Aqueous extract. Photographs shown are taken at 10X resolution

The kidney sections of the contro group showed normal renal tubules indicating normal glomurelus (Figure 6). There were no observable changes in the kidney histology between the normal control and STZ induced diabetic groups.

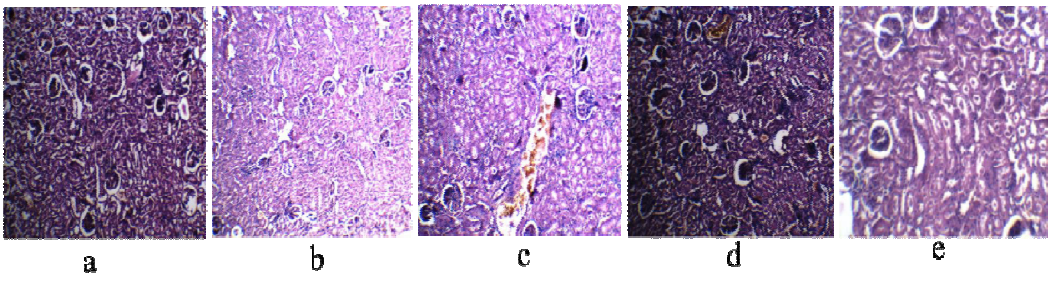


Figure 6 Kidney sections of the groups

a-Normal control, b-Diabetic Control, c-Glibenclamide, d-Insulin and d-MEPA. Photographs shown are taken at 10X resolution

Oral glucose tolerance study

This test was performed in the animals 3 days prior to their sacrifice to study the impact of MEPA treatment on oral glucose tolerance and compared with the DC group. Figure 7 shows the blood glucose levels in MEPA treated and diabetic control group after a glucose load (10 g/kg BW). It was observed that the blood glucose levels at all-time intervals were significantly low in MEPA treated group, thus the area under the glucose curve was also significantly low compared to untreated diabetic control group ($P \leq 0.05$).

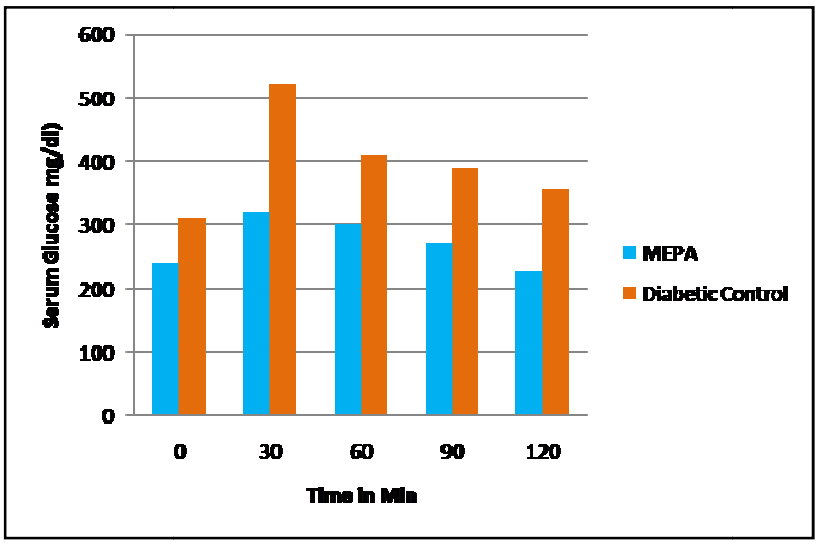


Figure 7 Oral glucose tolerance in diabetic control and MEPA treated groups

Isolation of active constituent

Thin layer chromatography & HPLC

The difference spots developed in each solvent system were identified by means of UV light, iodine chamber or corresponding spray reagent and their R_f values are given in the Table – 2, Fig: 8.

Table 2: Solvent System of TLC

Solvent system	Number of spot	Colour of spot	Detector	R_f value
Benzene: Ethyl acetate (9:1)	2	Pink	Anisaldehyde spray	0.884
		Violet		0.732
Hexane: Ethyl acetate (9:1)	1	Violet	Anisaldehyde spray	0.682
Chloroform: Methanol (10:1)	1	Pink	Anisaldehyde spray	0.938
Toluene: Ethyl acetate: Formic acid: Methanol (3: 3: 0.8 : 0.2)	1	Brown	Iodine chamber	0.582

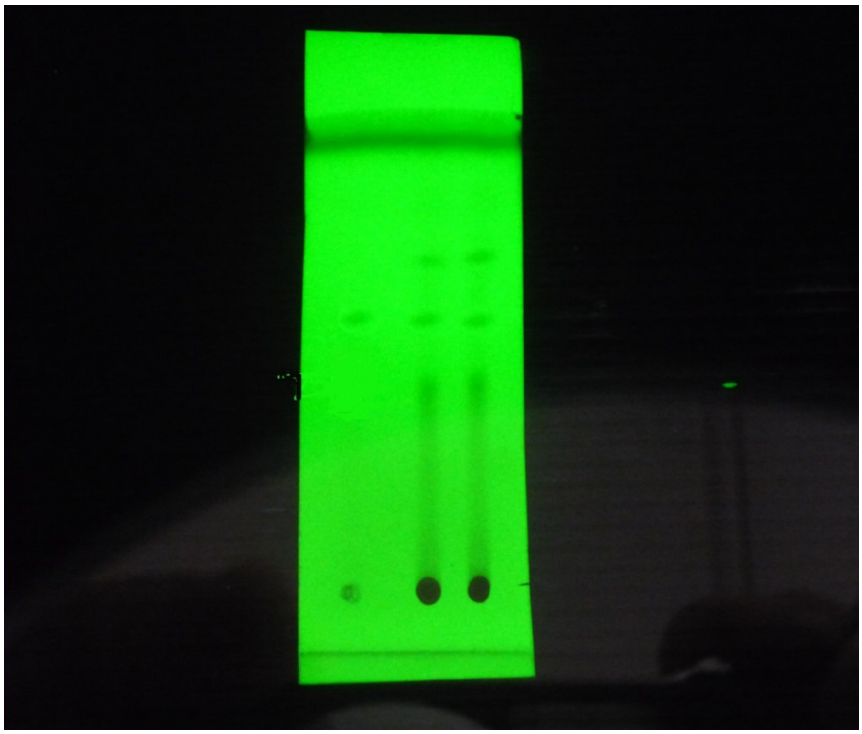


Figure 8: TLC OF MEPA

Of the various solvent systems tried that containing toluene: ethyl acetate: formic acid: methanol in the ratio of 3: 3: 0.8: 0.2 was found to be the most suitable one. In this system, ellagicacid was resolved (Rf = 0.41) (Figure 3) in the presence of other compounds in the sample extract (Figure 09 & 10). The identity of the bands of ellagicacid in the sample extracts was confirmed by overlaying their UV absorption spectra with those of the standard ellagicacid using a CAMAG TLC Scanner 3.

Ellagic acid is an organic heterotetracyclic compound resulting from the formal dimerisation of gallic acid by oxidative aromatic coupling with intra molecular lactonisation of both carboxylic acid groups of the resulting biaryl. It is found in many fruits and vegetables, including raspberries, strawberries, cranberries, and pomegranates. It has a role as an antioxidant, a food additive, a plant metabolite, an EC 5.99.1.2 (DNA topoisomerase) inhibitor, an EC 5.99.1.3 [DNA topoisomerase (ATP-

hydrolysing)] inhibitor, an EC 1.14.18.1 (tyrosinase) inhibitor, an EC 2.3.1.5 (arylamine N-acetyltransferase) inhibitor, an EC 2.4.1.1 (glycogen phosphorylase) inhibitor, an EC 2.5.1.18 (glutathione transferase) inhibitor, an EC 2.7.1.127 (inositol-trisphosphate 3-kinase) inhibitor, an EC 2.7.1.151 (inositol-polyphosphate multikinase) inhibitor, an EC 2.7.4.6 (nucleoside-diphosphate kinase) inhibitor, a skin lightening agent, a fungal metabolite and an EC 2.7.7.7 (DNA-directed DNA polymerase) inhibitor. It is an organic heterotetracyclic compound, a cyclic ketone, a lactone, a member of catechols and a polyphenol. It derives from a gallic acid.

Ellagic acid is present in several fruits such as cranberries, strawberries, raspberries, and pomegranates. In pomegranates, there are several therapeutic compounds but ellagic acid is the most active and abundant. Ellagic acid is also present in vegetables. Ellagic acid is an investigational drug studied for treatment of Follicular Lymphoma (phase 2 trial), protection from brain injury of intrauterine growth restricted babies (phase 1 and 2 trial), improvement of cardiovascular function in adolescents who are obese (phase 2 trial), and topical treatment of solar lentigines. Ellagic acid's therapeutic action mostly involves antioxidant and anti-proliferative effects.

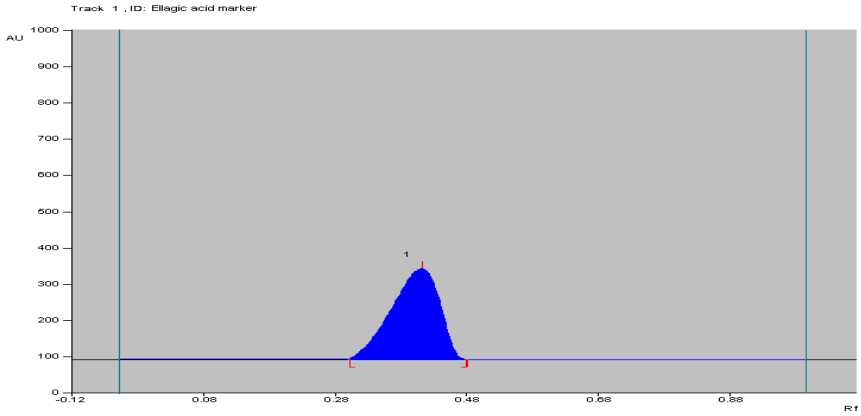


Figure 9: R_f value of marker compound

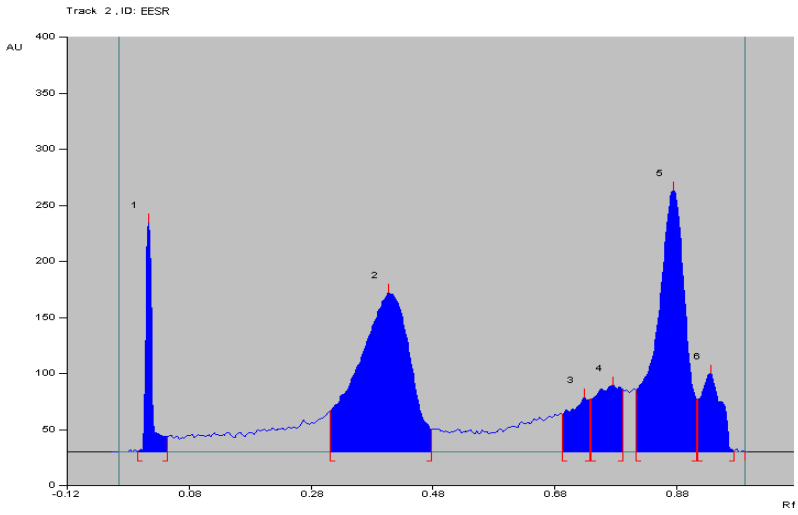


Figure 10: R_f values of MEPA

Analysis of the phyto-constituent of the various extracts of them epausing HPLC

In this study many phyto- constituents were discovered from the MEPA. However, the highest peak percentage area & retention time were observed in methanolic medium which is expressed in the following pages. Hence, the methanol extract was selected for further studies in the second phase by GC-MS Analysis. (Figure:11).

Sample Information

Sample Name	: Methanol (S) NK
Data Filename	: LC 3008007.lcd
Method Filename	: Plant extract-MU-5.lcm
Report Filename	: Default.lcr
Date Acquired	: 8/30/2013 8:04:26 PM

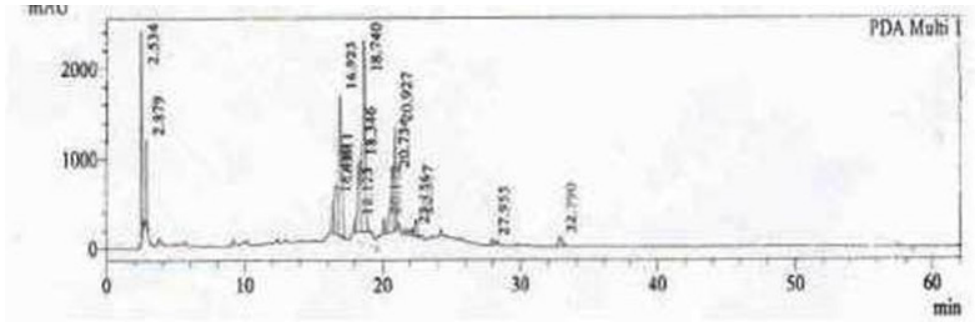
Vail#: 25
Injection Volume: 20 uL

Column Info: Phenomenex Gemini NX C 18 (250 X 4.6 mm, 5µ)
Column no : IICMS/LCC/005

Method

Mobile Phase A :0.01M Ammonium acetae pH 5.0 with acetic acid
Mobile phase B : Acetonitrile :Methanol (30:70)
<<LC Program>>

Time	Unit	Command	Value	Comment
0.01	Pumps	B.Conc	10	
5.00	Pumps	B.Conc	10	
10.00	Pumps	B.Conc	30	
20.00	Pumps	B.Conc	50	
24.00	Pumps	B.Conc	78	
40.00	Pumps	B.Conc	78	
45.00	Pumps	B.Conc	80	
50.00	Pumps	B.Conc	80	
55.00	Pumps	B.Conc	10	
62.01	Controller	Stop		



1 PDA Multi 1 / 254nm 4nm

PeakTable

Ch1 254nm 4nm

Peak#	Ret. Time	Area	Area %	Name
1	2.53	9778241	8.75	
2	2.88	2787130	2.49	
3	16.47	3466028	3.10	
4	16.61	8216992	7.35	
5	16.92	19798700	17.72	
6	18.12	1144156	1.02	
7	18.35	9896183	8.86	
8	18.74	27316485	24.45	
9	20.11	1578058	1.41	
10	20.73	9519265	8.52	
11	20.93	12863964	11.51	
12	22.14	921767	0.82	
13	22.40	1847875	1.65	
14	27.95	834868	0.75	
15	32.79	1773083	1.59	
Total		111742794	100.00	

Figure 11: Analysis of the presence of phyto-constituents in the MEPA using HPLC.

Analysis of phyto-constituents present in the mepausing gc-mschromatogram

GC-MS analysis of the crude MEPA indicates the 46 peaks components at different retention time and different percentage area Interpretation of mass spectrum GC-MS was conducted using the database of the National Institute Standard and Technology (NIST) having more than 62,000 patterns. The comparison of the mass spectrums with the data base gave more than 90% match as well as confirmatory compound of the structure match. The peak compounds were compared with the spectrum of the known phyto-constituent stored in the NIST library. The molecular weight, molecular structure and formula were ascertained by using the NIST, Pub Chem, Chem Spider and Chemicalbook. (Figure:12)

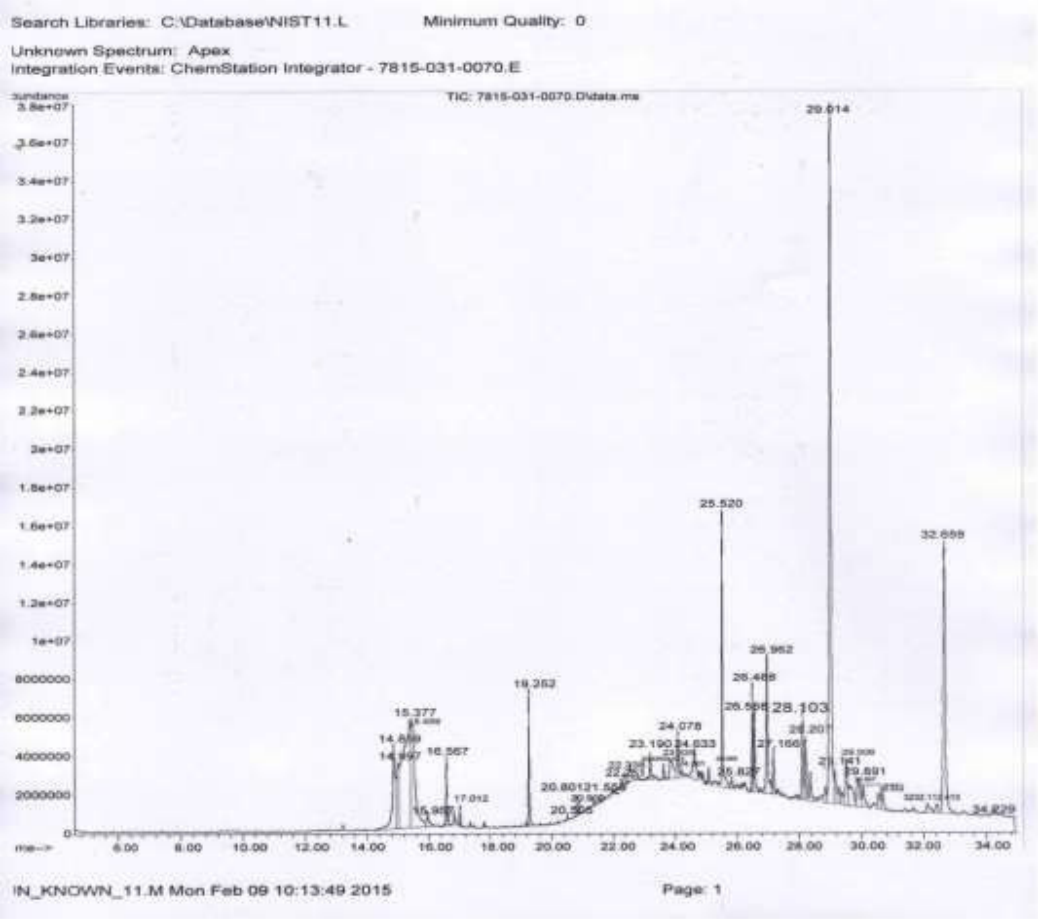


Figure 12: Phyto-components identified in the MEPA by GC-MS Peak

From the preceding table, it can be observed that many active chemicals are present in the methanolic extract of root of *Pisonia alba*. These active principles help might be responsible for the biological activity exerted by the MEPA.

DISCUSSION

Streptozotocin is a cytotoxic compound which induces diabetes by damaging the beeta-cells of pancreas through mechanism involving the damage to cell membrane of beeta-cells resulting in degeneration. beeta- cell degeneration results in insulin insufficiency and hypeglycemia. The Streptozotocin induces diabetes which is similar to diabetes mellitus with non-ketosis hyperglycemia. The symptoms include dyslipidemia, loss of protein mass and body weight, damage to liver and other organs resulting in major disturbance of central metabolic balance [20].

Mahagoni treatment to diabetic rats decreased fasting blood glucose level and the effect was comparable to the anti-diabetic drug glibenclamide. The improvement in glycemia was also supported by the decrease of daily water and food intake by the diabetic rats. In addition to reduction in the hyperglycemic state, the MEPA group showed better oral glucose tolerance as indicated by the oral glucose tolerance test performed at the end of treatment. Oxidative stress is worsened in the case of hyperglycemia and literature indicates the direct role of oxidative stress in the development of secondary complications of diabetes. Increased oxidative stress is claimed to be triggered directly by the hyperglycemia [21]. In the present study, it was observed that the induction of diabetes by streptozotocin resulted in oxidative stress, wherein the innate antioxidant system is weakened. In the serum of diabetic control rats, there was large depletion of antioxidants such as glutathione and anti-oxidant enzymes viz., SOD and catalase. Increase in serum lipid peroxides was observed as a consequence of reduced anti-oxidants and increased oxidants in the serum of diabetic control rats. MEPA treatment also improved the antioxidant levels and decreased the lipid peroxides, indicating overall amelioration of oxidative stress in the diabetic state. The observed beneficial phenomenon is consistence with the former reports on the anti-oxidant changes in the streptozotocin induced models by treatment with the medicinal plants [21-25].

In the normal physiology the glucose in the plasma will be taken up by muscle and adipose tissue, which forms the major reserve source of energy. Insulin plays a major role in glucose uptake into muscle and adipose tissue, lack of insulin or insufficient insulin results in failure of glucose uptake into muscle and adipose tissue resulting in decreased stored energy. Decreased stored energy force the body to utilize the protein mass in the muscle resulting in the loss of overall bodyweight. In the diabetic control rats there was a drastic fall in the body weight was observed during the study, whereas treatment with the MEPA decreased the loss of body weight in the diabetic rats indicating the insulin mimetic or insulin potentiating activity of the components in the extract of plant.

Glycogen is the gluco-polymer which is stored in the liver and is used at the time of starvation to provide instant energy to the body. In severe diabetes, due to the inactive glycogen synthetic pathways and overactive breakdown pathways, glycogen content of the liver will be depleted. MEPA treatment improved the liver glycogen content in sever diabetic rats indicating the effect on MEPA on the pathways inducing glycogenesis. Prior reports in STZ induced diabetic models concord with our observations [26, 27].

The hyperlipidemia associated with diabetic state, a risk factor for coronary heart disease, is attributed to the hyperglycemia-induced lypolysis [28]. The lypolysis is due to activated lipoprotein lipases on the fat depots as a consequence of depleted insulin level. The plasma lipid profile in the MEPA treated group was restored towards normal values indicating positive effect of the MEPA on overall lipid metabolism [29, 30]. Although urinary output was not recorded, the wetting of the soft bed used in the cages was more marked in the diabetic control group, indicating the presence of polyuria, a well-known symptom of uncontrolled diabetes.

The findings of the study strongly are strong evidences for the anti-hyperglycemic potential of the MEPA. MEPAs antihyperglycemic activity by inhibiting the glycogenolytic pathway and by improving peripheral utilization of glucose by acting as insulin mimetic agent. The effect may be attributed to the presence of bio-actives such as scopoletin, coumarin, gallic acid, ellagic acid and epicatechin.

Based on the above analytical work it concluded that the *Pisonia alba* root containing the following components. Thus might be having the below mentioned biological activity.

Table3: Medicinal properties of Phyto-constituent present in the MEPA.

S. No.	Components	Reported activity
1	Benzenemethaneamine	Anti –diabetic activity
2	3,7,11,15-Tetramethyl-2-Hexadecenyl Acetate	Cancer-preventive
3	Tetradecanoic acid	Antioxidant, Lubricant, Hypercholesterolemic, Cancer- preventive, Cosmetic
4	Vitamin E	Antiaging, Antialzheimeran, Antidermatitic, Antidiabetic, Antioxidant, Antitumor, Cancer-preventive, Hypocholesterolemic, Immunostimulant
5	Hexadecanoic acid	Antioxidant, Flavor, 5-AlphaReductase-inhibitor, Antifibrinolytic, Hemolytic, Lubricant, Nematicide, Antialopecic
6	Octadecanoic acid	Lower LDL Cholesterol level
7	γ-Tocopherol	Anticancer, Antioxidant, Antitumor, Antiinflammatory, Hypocholesterolemic, Cardioprotective
8	Stigmasterol	Antihepatotoxic, Antiviral, Antioxidant, Cancerpreventive, Hypocholesterolemic
9	Campesterol	Antioxidant, Hypocholesterolemic
10	Oleic Acid	Antiinflammatory, Antiandrogenic Cancer preventive, Dermatitigenic Hypocholesterolemic, 5-Alpha reductase inhibitor, AnemiagenicInsectifuge,
11	Phytol	Antimicrobial, Anti-cancer, Anti-inflammatory, Hepato-protective, Anti – androgenic
12	Nondecanoic acid	Anti-tumor
13	Stigmast-4-en-3-one	Hypoglycaemic activity

Based on the HPTLC reports the compound could be Ellagic acid. it is an organic heterotetracyclic compound resulting from the formal dimerisation of gallic acid by oxidative aromatic coupling with intra molecular lactonisation of both carboxylic acid groups of the resulting biaryl. It is found in many fruits and vegetables, including raspberries, strawberries, cranberries, and pomegranates.

The ellagic acid having antioxidant nature, a food additive, a plant metabolite it could produce the biological activity in any of the following mechanism

- An EC 5.99.1.2 (DNA topoisomerase) inhibitor,
- An EC 5.99.1.3 [DNA topoisomerase (ATP-hydrolysing)] inhibitor,
- An EC 1.14.18.1 (tyrosinase) inhibitor,
- An EC 2.3.1.5 (arylamine N-acetyltransferase) inhibitor,
- An EC 2.4.1.1 (glycogen phosphorylase) inhibitor,
- An EC 2.5.1.18 (glutathione transferase) inhibitor,
- An EC 2.7.1.127 (inositol-trisphosphate 3-kinase) inhibitor,
- An EC 2.7.1.151 (inositol-polyphosphate multikinase) inhibitor,

- An EC 2.7.4.6 (nucleoside-diphosphate kinase) inhibitor,
- a skin lightening agent, a fungal metabolite
- An EC 2.7.7.7 (DNA-directed DNA polymerase) inhibitor.

Many active principles are found in GC-MS chromatogram, some of its biological activity is explored but many of those activities in not yet got its scientific identity. The further progression of this work might include further characterization to find out the exact compound present in the MEPA and exploration of those hidden biological potential and to manufacture a new drug with multiple disease target.

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