Isolation and characterization of compounds from *Argemone mexicana* L. (Papaveraceae) with Peroxisome Proliferator-Activated Receptor (PPAR) Modulatory Effects

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ABSTRACT

Background: *Argemone mexicana* is used as emetic, demulcent, and laxative in folkloric medicine. This study aimed at isolating the chemical compounds and exploring the PPAR modulatory effects of isolated phytochemicals.

Objective: The study was carried out to isolate the chemical compounds and to explore the PPAR modulatory effects of the isolated phytochemicals.

Method: The leaves were collected, authenticated, and voucher specimen deposited at the University of Lagos herbarium, Nigeria. The leaves extracted with ethanol and extracts subjected to column chromatography on sephadex LH-20 and Silica gel. Structure elucidation were achieved by analyses of their 1-Dimensional and 2-Dimensional NMR. Reporter gene assay were performed on the extracts and isolated compounds for activation of PPARα and PPARγ.

Result: Nine compounds were isolated from the ethanol extract of the plant, and were proposed to be: **1**(1-(β-d-ribofuranosyl)-1H-1,2,4-triazone), **2a**((Z)-2-(2,4-dihydroxy-2,6,6-trimethylcyclohexylidene) acetic acid), **2b**(cis-3,6-dihydroxy-a-ionone), **3**((E)-4-(r-1',t-2',c-4'-trihydroxy-2',6',6'- trimethylcyclohexyl) but-3-en-2-one), **4**(Rhein), **5**(Tamarexetin), **6**(41-methoxylquercetin 3-β-D-glucoside), **7**(Tamarixetin-3-O-β-D- robinoside) and **8**(Palmitic acid). The extracts were found to activate PPARα with a fold induction of more than 2.0 over the vehicle control but a lower induction was observed with PPARα. The isolated compounds showed lower activation of PPARα and PPARγ.

Conclusion: Three flavonoids, palmitic acid, Rhein, a triazole and three substituted cyclohexane were isolated, while the extract showed PPARα activation, the isolated compounds did not. These effect might be due to the synergistic effect of the constituents, thus the extract has more promising effect.

Key words: Argemone, flavonoids, substituted cyclohexane, Phytochemistry, tamarexetin

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Isolement et caractérisation de composés issus d'*Argemone mexicana* L. (Papaveraceaeayant des effets modulateurs sur le récepteur activé par les prolifératrices de peroxysomes (PPAR)

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RÉSUMÉ

Contexte: L'*Argemone mexicana* est utilisé comme émétique, émollient et laxatif en médecine traditionnelle. Cette étude visait à isoler les composés chimiques et à explorer les effets modulateurs des PPAR des composés phytochimiques isolés.

Méthode: Les feuilles ont été collectées, authentifiées et les spécimens de référence ont été déposés à l'herbier de l'Université de Lagos, au Nigéria. Les feuilles ont été extraites avec de l'éthanol et les extraits ont été soumis à une chromatographie en colonne sur du Sephadex LH-20 et de gel de silice. L'élucidation de la structure a été réalisée par des analyses de leur RMN 1 et 2 dimensions. Des tests de gènes rapporteurs ont été effectués sur les extraits et les composés isolés pour l'activation de PPARα et PPARγ.

Résultat: Neuf composés ont été isolés de l'extrait éthanolique de la plante et ont été proposés comme étant : **1** (1-(β-d-ribofuranosyl)-1H-1,2,4-triazone), **2a**((Z)-2-(2,4-dihydroxy-2,6,6-triméthylcyclohexylidène) acide acétique), **2b**(cis-3,6-dihydroxy-a-ionone), **3**((E)-4-(r-1',t-2',c-4'-trihydroxy-2',6',6'- triméthylcyclohexyl) but-3-en-2-one), **4**(Rhein), **5**(Tamarexétine), **6**(41-méthoxyl q uercétine 3-β-D-glucoside), **7**(Tamarixétine-3-O-β-D- robinoside) et **8**(acide palmitique). Les extraits se sont avérés activer PPARα avec une induction de plus de 2,0 fois supérieure à celle du témoin du véhicule, mais une induction plus faible a été observée avec PPARα . Les composés isolés ont montré une activation plus faible de PPARα et PPARγ.

Conclusion: Trois flavonoïdes, l'acide palmitique, le Rhein, un triazole et trois cyclohexanes substitués ont été isolés. Si l'extrait a montré une activation de PPARα, les composés isolés n'en ont pas montré. Ces effets pourraient être dus à l'effet synergique des constituants, l'extrait ayant donc un effet plus prometteur.

Mots clés: Argémone, flavonoïdes, cyclohexane substitué, phytochimie, tamarexétine

1.0 INTRODUCTION

Traditional medicines have played a vital role in health systems, and are used to treat various acute and chronic conditions with minimal toxic effect or none. In Africa, plants are used as primary health medication because of their potential pharmacological properties. The efficacy of plant-based drugs has been paid great attention because of their fewer side effects, lower cost and easier availability comparing with synthetic drugs[1] (Prasathkumar *et al*., 2021)

About 80 % of the population in Africa still relies mainly on plant drugs because they are believed to be safe, acceptable, affordable, compatible and suitable for the treatment of various diseases[2] (Van Wyk & Prinsloo 2018). Herbal plants are often used as a natural remedy to cure various health problems including tuberculosis, cancer, diabetes mellitus, heart diseases, wound healing, asthma, pharyngitis, hypertension etc. Plants rich in bioactive, phytomedicinal compounds such as alkaloids, flavonoids, tannins and polyphenols have been used to cure illnesses because of their various pharmacological properties.[1]

According to a WHO survey of 41 sub-Saharan African nations, just 22 % provide emergency in-patient care for serious Non-communicable diseases (NCDs including diabetes), while 37 % have minimal out-patient care.[3] Diabetes is a chronic disease characterized by raised blood glucose level, known as hyperglycaemia, caused by insufficient insulin production from the pancreas or the body's inability to use insulin (insulin resistance). Long-term, uncontrolled high blood sugar will damage human organs and body parts. Diabetes is a major cause of blindness, kidney failure, heart disease, stroke, and lower limb amputation. Africa had diabetes-related expenditure of US$ 13 billion, accounting for 1 % of global diabetes-related expenditure.[4] More than half (54 %) of people living with diabetes in the African region are undiagnosed.

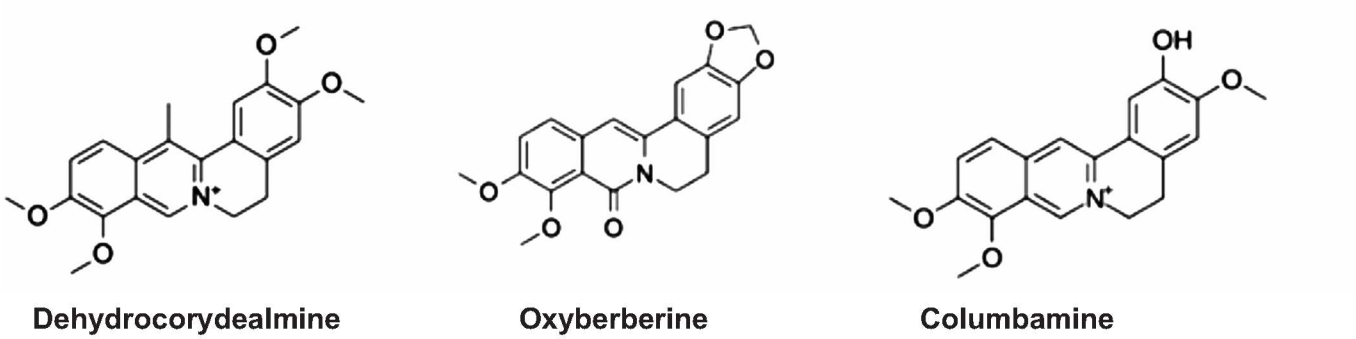
The main challenge with diabetes in Africa is that the prevalence is rapidly increasing at an unprecedented rate,[5] as sub-Saharan Africa contains some of the world's poorest countries which are economically unable to cope with the burden. Thus, the need for more research in medicinal plants that have folkloric use as an anti diabetics.

Argemone mexicana L. (Papaveraceae) known as mexican prickly poppy is a well-known weed in the agricultural and waste lands whose seed are used as emetic, demulcent, laxative and antidote in snake poisoning. It is a prickly, glabrous, branching annual herb with yellow juice and showy yellow flowers, as shown in Figure 1, and it is naturalized throughout up to an altitude of 1500 m.[6] The plant is used in different parts of the world for the treatment of several ailments including tumors, warts, skin diseases, inflammations, rheumatism, diabetes, jaundice, leprosy, microbial infections, and malaria. Interestingly, the plant is the source of a diverse kind of chemical constituents although alkaloids are mostly abundant.[7]

It is also used in the treatment of jaundice, leprosy, piles, dysentery and warts. The whole plant has analgesic, antispasmodic, possibly hallucinogenic and sedative properties.[8] This study aimed at isolating the chemical compounds responsible for the pharmacological effects of *Argemone mexicana*.

Figure 1: *Argemone mexicana* showing the flower in its natural habitat (7°21'40"N 4°11'00"E)

Chemical constituents isolated so far isolated compounds belong to the class of alkaloids; besides, terpenoids, flavonoids, phenolics, long-chain aliphatic compounds, and few aromatic compounds are found to be other constituents of this plant. Examples are columbamine, Protopine, Dihydrochelerythrine, dehydrocorydalmine, oxyberberine, Columbamine etc (Figure 2). The alkaloids, dehydrocorydalmine and oxyberberine, isolated from A. mexicana, were found to exhibit antifungal activities against some fungal strains such as *Helminthosporium sp., Curvularia sp., Alternaria cajani, Bipolaris sp.* and *Fusarium udum*.9



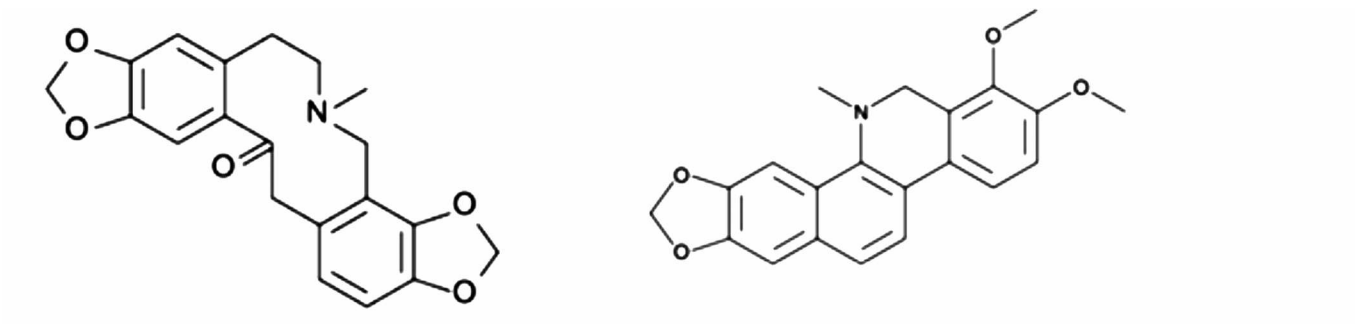


Figure 2: Some compounds previously isolated from *Argemone mexicana*

Argemone mexicana has demonstrated significant pharmacological properties.[9] Studies show its crude extracts and chemical constituents exhibit antimicrobial, anti-inflammatory, and analgesic activities. The plant also promotes wound healing, with tensile strength in treated wounds surpassing that of control groups.[10] Its cytotoxicity is evident against human cancer cell lines through MTT assay.[11] Antioxidant potential has been reported in ethanol extracts of *A. mexicana* roots, showing high free radical scavenging activity, while leaf extracts also exhibit superoxide anion scavenging.[12] The anticancer activity of ethanol and methanolic extracts is observed against various cell lines, with flavonoids contributing to apoptotic effects.[13] The antidiabetic effects of aqueous and hydro-alcoholic extracts significantly lower blood glucose in diabetic rats, with doses being as effective as metformin.[14] The antihepatotoxic properties of aqueous stem extracts in Wistar rats indicate reduced liver enzyme levels upon administration.[15] Additionally, anti-malarial activity is observed, with aqueous extracts acting against *Plasmodium falciparum*.[16]

2.0 METHODS

The leaves of *Argemone mexicana* were collected from Ikire, Osun (7°21'40"N 4°11'00"E), and authenticated by a botanist, Dr. Nodza George and voucher specimen deposited at the University of Lagos, Herbarium, Nigeria (voucher number LUH 6173) . The leaves (1.80 kg) were oven-dried at 40 °C pulverized and extracted with ethanol (50 L). Extracts were filtered using Whatman no 1. filter paper and concentrated using rotary evaporator to obtain ethanol extract (47.18 g). Standard methods were used for preliminary phytochemical screening of the different extracts to know the nature of phytoconstituents present within them.[17,18]

The ethanol extract was subjected to column chromatography (CC) starting with reverse phase silica gel to separate the component in the extract based on their molecular weight. A total of nine fractions (A-H) were obtained and subjected to further CC, pooling like fractions together based on their thin layer chromatography profile (TLC).

Fraction A crystallizes out as sugar and were not investigated further. Fraction B (1.34 g) was further separated by column chromatography (CC) over Sephadex LH-20 (2.0 cm X 108 cm) eluted with methanol (MeOH) to obtain 10 fractions (B1-B10). Subfraction B8 (30.10 mg) was subjected to reverse` phase column chromatography (103 X 2cm) and eluted with gradient of methanol: water (10:0 to 0:10) to obtain 12 sub fractions B4 a-l. Subfraction B4 f was identified as **compound 1**.

Fraction C (0.96 g) was subjected to Sephadex LH-20 (107 X 3 cm) eluting with methanol to obtain 7 sub fraction C 1-7. C5 (633 mg) was further subjected to silica gel CC (82 X 2 cm) and eluted with MeOH:ethylacetate:DCM (15:8:4) to obatin 15 subfractions (C5a-o). Subfraction C5e was obtained as **compound 2** which occur as as a mixture of two **compounds 2a** and **2b** while C5g was identified as **compound 3**.

Fraction D (0. 42 g) was subjected to Sephadex LH-20 CC (99.5 cm X 2.5 cm) eluted with MeOH to obtain eight sub fractions D 1-8. D5 (71.3 mg) was loaded on Sephadex LH-20 column (93 x 3 mm) and eluted with MeOH to obtain 11 subfraction D5a-k. D5 g was identified as **compound 4**. D6 was identified as **compound 5** and D7 as **compound 6**. D8 (74.58 mg) was subjected Sephadex LH-20 (107 x 3 mm) and eluted with ethanol to obtain 3 subfractions D8a-c. D8b was identified as **compound 7**.

Fraction E (1.56 g) was subjected to Sephadex LH-20 (106 x 4mm) and eluted with methanol to obtain 7 subfractions E1-7. E4 (152.5 mg) was subjected to silica gel CC (71 x 2 cm) and eluted with Ethylacetae:DCM:MeOH:Hexane (15:8: 1:10) to obtain 11 subfractions E4 a-k. E4g (68.3 mg) was further separated on silica gel column (97 x 1 cm) to obtained 5 subfractions E4gi-vii. E4giii was further identified as **compound 8** via GC-MS.

GC-MS was carried out utilizing an Agilent 7890A gas chromatographic (GC) instrument which was equipped with an Agilent 7693 autosampler (Agilent Technologies, Santa Clara, CA, USA). The GC was connected to an Agilent 5975C mass spectrometer. The capillary column (60 m x 0.25 mm i.d.) utilized was coated with a 100 % Dimethylpolysiloxane (Agilent DB-1MS) film (0.25 µm). Helium at a constant flow rate of 1 mL/min was used as the carrier gas. The sample was analyzed using the following GC oven program: 50 °C held for 1 minute, then heated at a rate of 5 °C/min. to 280 °C and held at 280 °C for 10 minutes. The inlet was programmed at 280 °C in split mode, with a split ratio of 50:1. The transfer tube from the GC to the MS was held at 280 °C.

The Agilent 5975C mass spectrometer was operated with

an electron energy of 70 eV. The source, quadrupole, and transfer line temperatures were 230 ^{o}C, 150 ^{o}C, and 280^{o}C, respectively during the experiment. All mass spectra data were recorded from 40 to 500 m/z after a 7 min solvent delay using MSD ChemStation (E.02.02.1431) acquisition software (Agilent Technologies). Data was further processed using Agilent MassHunter Qualitative Analysis (B.07.00). The NIST database (version 2.3) was utilized for tentative compound identification.[19]

Structure elucidation of the isolated compounds (Fig. 1) was achieved by detailed analysis of their 1D and 2D NMR spectroscopic and mass spectrometric data analysis and further supported by comparison with literature values.

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors that are activated by binding to ligands, and are involved in regulating gene expression. PPAR activation potentials of the isolated compounds were carried out via reporter gene assay to screen ethanolic extracts of the plant and the isolated compounds for activation of PPARα and PPARγ using the method of Yang *et al*., 2013(20). After an incubation for 24 h with the test samples, the cells were lysed and the luciferase activity was measured using a luciferase assay system (Promega, Madison, WI, USA). Fold increase in luciferase activity of sample-treated cells was calculated in comparison to control.

3.0 RESULTS

The results of the qualitative phytochemical analysis of the *A. mexicana* are presented in table 1.

Table 1: Phytochemical analysis of Phytoconstituents present in the leaf extract of *A. mexicana*

Test	Result
Alkaloid	Positive
Anthraquinones	Negative
Flavonoids	Positive
Glycosides	Positive
Deoxy-sugars	Negative
Sterols	Positive
Tannins	Positive
Phenols	Positive
Carbohydrates	Positive
Reducing sugar	Positive
Monsaccharides	Positive
Starch	Negative
Proteins	Negative
Resin	Positive

The isolation procedure yields nine (9) compounds and the chemical data of the compound are compared with literature data to confirm their identity. Assigment tables for compounds 1 to 7 are indicated on tables 2-4 while Figure 3 shows the chromatogram of compound 8 and the corresponding retention time and peak area presented in table 5. Compound 8 was isolated as a fatty acid and the chromatogram and peak area through GC_MS are presented in figure 3 and table 5 respectively.

Table 2: Chemical shift for compound 1-3

	Compound 1 (D-Ribofuranosyl) -1H-1,2,4-triazone)			Compound 2a ((Z)-2-(2' ,4' -Dihydroxy-2' ,6' ,6' -trimethylcyclohexylic en e) acetic acid (2))		Compound 3 (E)-4-(r-1' ,t-2' ,c-4' -trihydroxy-2' ,6' ,6' -trimethylcyclohexyl)but-3 en 2 one,	
Position	δH (J in Hz)	δC	Position	δH (J in Hz)	δC	δH (J in Hz)	δC
1	-	-	1		182.3	-	148.8
2	-	-	2		86.5 (27.3, OCH3)	-	198.2
3	4.76 (1H, s)	153. (				-	131.1
4	-	-				-	77.1
5	5.01 (1H, s)	142. :	1'			-	45.1
6			2'			1 . 16, s, 2' Me;	25.6
1'	-	123	3'			-	26.6
2'	6.92 (s)	117	4'			-	27.4
3'	-	146	5'	5.68, s		1.62, m, (H 5')	27.8
4'	-	149	6'		35.8	0.85, s, 6' Me; 1 .27, s, 6' Me	50.2
5'	6.82 (s)	116				-	-
6'					171.1	-	45.2

Table 3: Chemical shift for compound 4

	Compound 4 (Rhein)	
Position	δH (J in Hz)	δC
1	11.74 s	160.9
2	6.91 d (0.8)	113.1
3	-	151.2
4	7.10 d (0.8)	116.7
5		146.6
6	6.79 d (8.8)	116.7
7	7.19 d (8.8)	124.8
8	11.36 s	156.2
9	-	
10	-	117.6
11	-	127.1
12	-	116.8
13	-	145.2
14	-	123.5

Figure 3: Chromatogram of Compound 8 through GC-MS

Table 4: Chemical shift for compound 5-7

	Compound 5 (Tamarexiten)		Compound 6 (41-methoxyl Quercetin 3-β-D-glucoside)		Compound 7 (Tamarixetin 3-O-β-D-robinoside)	
Position	δH (J in Hz)	δC	δH (J in Hz)	δC	δH (J in Hz)	δC
1	-	-	-	-	-	-
2	-	146.96	-	163.7	-	157.4
3	-	132.00	-	143.1	-	135.5
4	-	175.05	-	181.9	-	179.1
5 (OH)	12.45 (1H, s)	161.24	7.2 (d)	161.9	11.90	163.1
6	6.17 (1H, d, J = 1.76 Hz)	98.69	6.4 (meta coupled-d)	98.6	6.77	100.1
7, (OH)	10.76 (1H, s)	164.52	10.98 (s)	163.9	9.97	166.3
8	6.45 (1H, d, J = 1.76 Hz)	94.09	6.9 (meta coupled-d)	94.1	4.67	94.9
9	-	156.65	-	157.0	-	
10	-	103.36	-	103.5	-	105.6
1^1	-	122.39	-	121.2	-	124.4
2^1	7.73 (1H, d, J = 2.35 Hz)	112.25	7.13 (1H,d)	113.0	7.40 (d)	117.3
3^1 (-OCH3)	3.82 (3H, s)	56.26	3.23 (OH, s)	145.5	6.98, dd (1.2, 9.0)	147.9
4^1 (-OH)	9.40 (1H, s)	147.80	8.98 (3H, s)	149.3	7.33 (d)	150.9
5^1	6.92 (1H, d, J = 8.80 Hz)	116.06	7.05 (1H)	116.5	7.49 (d)	111.8
6^1	7.67 (1H, dd, J = 8.80-2.35 Hz)	122.24	6.97 (1H, dd)	119.3	7.30 (d)	122.8
1^{11}	3.65 (m)	103.01			3.48 (m)	74.84
2^{11}	3.57 (m)	72.34			3.48 (m)	78.12
3^{11}	3.82 (m)	72.06			3.32 (m)	71.12
4^{11}	3.92 (m)	73.76			3.32 (m)	78.12
5^{11}	3.83 (m)	72.54			3.71 (m)	63.11
6^{11}	3.70	17.27			3.85, 3.84 (m)	56.08, 50.47
6a					5.37 (m)	103.92
6b					2.20 (m)	72.21
6c					3.29 (m)	72.43
6d					3.28 (m)	73.21
6e					3.27 (m)	73.20
6f					CH3 0.93 (s)	17.20

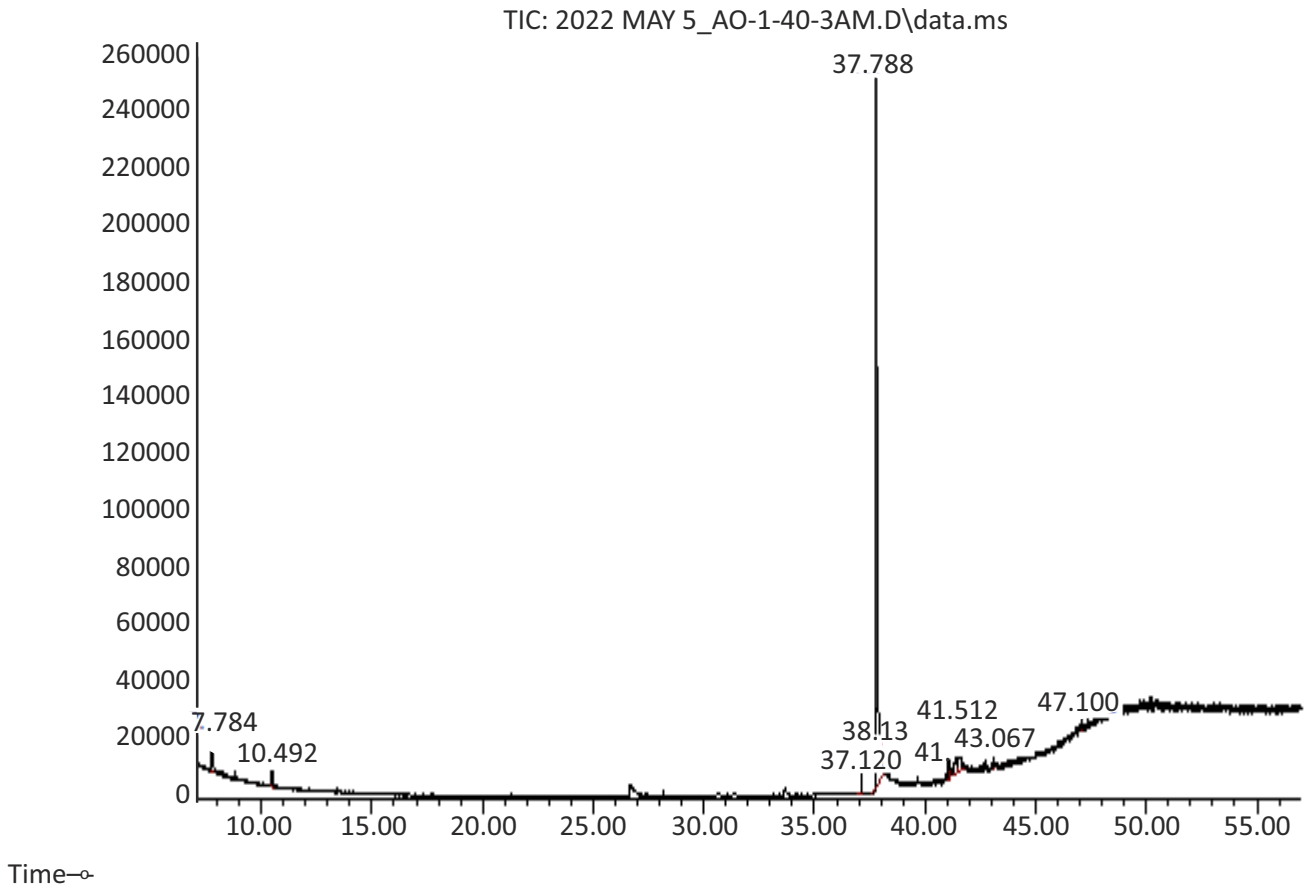
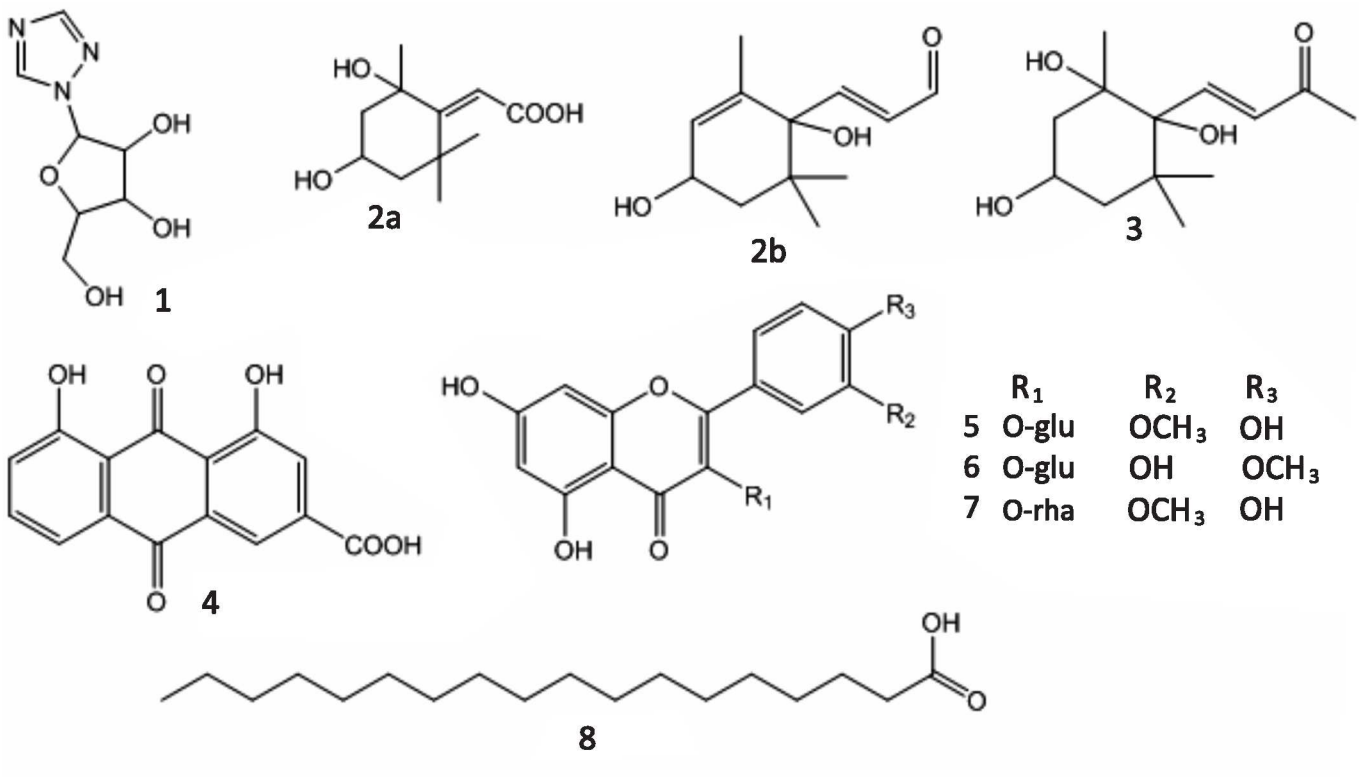


Figure 3: Chromatogram of Compound 8 through GC-MS

Table 5: Retention time and Peak area of compound 8

Compound number	RT (min)	Area	Area %
1	7.786	395198	3.04
2	10.492	153540	1.18
3	37.127	136796	1.05
4	37.789	10350739	79.73
5	38.133	115354	0.89
6	41.067	324203	2.50
7	41.347	371311	2.86
8	41.512	930874	7.17
9	43.068	115443	0.89
10	47.098	88225	0.68
		12981683	

Figure 4 shows the structures of the isolated compound whose data where presented above



1: 1-(β-D-Ribofuranosyl)-1H-1,2,4-Triazone).
2a: (Z)-2-(2,4-dihydroxy-2,6,6-trimethylcyclohexylidene)acetic acid
2b: cis-3,6-dihydroxy-a-ionone
3: (E)-4-(r-1',t-2',c-4'-trihydroxy-2',6',6'-trimethylcyclohexyl)but-3-3en-2-one
4: Rhein
5: Tamarixetin
6: 4¹-methoxyl-Quercetin 3-β-D-glucoside
7: Tamarixetin-3-O-β-D-robinoside
8: Palmitic acid

Figure 4: Structures of isolated compounds from *A. mexicana*

The isolated compounds were subjected to PPAR activation and presented in table 6-8

Table 6: Extracts and standard showing PPARα activation

	Fold increase in PPAR alpha		
Sample ID	**100 µg/ml**	**50 µg/ml**	**25 µg/ml**
Ethanol extract	2.01±0.01	2.15±0.07	1.79±0.04
Drug control	**25 µM**	**12.5 µM**	**6.25 µM**
ciprofibrate	3.85±0.125	2.88±0.148	2.20±0.039

Table 7: Extracts and standard showing PPARγ activation

	Fold increase in PPAR Gamma		
Sample ID	**100 µg/ml**	**50 µg/ml**	**25 µg/ml**
Ethanol extract	1.76±0.03	1.74±0.15	1.61±0.04
Drug control	**25 µM**	**12.5 µM**	**6.25 µM**
rosiglitazone	2.31±0.05	2.17±0.120	2.07±0.270

Table 8: Isolated compound and standard showing PPARα activation

	Fold increase in PPAR alpha		
Sample ID	**50 µM**	**25 µM**	**12.5 µM**
Compound 1 (1-(β-D-Ribofuranosyl)-1H-1,2,4-Triazone	0.88±0.27	0.77±0.14	0.99±0.27
Compound 4 (Rhein)	1.32±0.17	1.12±0.34	0.79±0.13
Compound 5 (Tamarixetin)	1.34±0.12	1.12±0.07	0.99±0.11
Compound 6 (4^1-methoxyl-Quercetin 3-β-D-glucoside	1.43±0.08	1.24±0.21	1.02±0.13
Compound 7 (Tamarixetin-3-O-β-D-robinoside)	0.87±0.24	0.80±0.15	1.27±0.11
Ciprofibrate		3.85+0.12	

	Fold increase in PPAR gamma		
Sample ID	**50 µM**	**25 µM**	**12.5 µM**
Compound 1 (1-(β-D-Ribofuranosyl)-1H-1,2,4-Triazone	0.68±0.12	0.81±0.11	0.93±0.13
Compound 4 (Rhein)	0.79±0.12	0.80±0.12	0.79±0.10
Compound 5 (Tamarixetin)	1.25±0.02	0.99±0.01	0.99±0.01
Compound 6 (4^1-methoxyl-Quercetin 3-β-D-glucoside	1.26±0.12	1.29±0.12	1.03±0.06
Compound 7 (Tamarixetin-3-O-β-D-robinoside)	0.74±0.10	0.92±0.15	1.09±0.08
Rosiglitazone		2.31+0.5	

4.0 DISCUSSION

The detailed phytochemical report of the leaves of *A. mexicana* is presented in this study. The leaves of *Argemone mexicana* are found to be a rich source of bioactive phytochemicals, including alkaloids, flavonoids, and tannins in this study. These compounds exhibit a wide range of pharmacological activities, such as antimicrobial, antioxidant, anti-inflammatory, anticancer, and antidiabetic effects.[21] The phytochemical profile of the plant supports its traditional use in treating infections, inflammatory conditions, wounds, and other ailments.

Structure elucidation of the isolated compounds was achieved by analyses of their ^{1}H and ^{13}C NMR data. Nine compounds were isolated from the ethanol extract of the leaves of *Argemone mexicana* namely; **1**-(β-d-ribofuranosyl)-1H-1,2,4-triazone **(1)**, (Z)-2-(2,4-dihydroxy-2,6,6-trimethylcyclohexylidene) acetic acid) **(2a)**, cis-3,6-dihydroxy-a-ionone **(2b)**, (E)-4-(r-1',t-2',c-4'-trihydroxy-2',6',6'- trimethylcyclohexyl)but-3-en-2-one **(3)**, Rhein **(4)**,Tamarixetin **(5)**, 4^{1}-methoxylquercetin 3-β-D-glucoside **(6)**, Tamarexetin 3-β-D-ribonoside **(7)** and Palmitic acid **(8)** (Figure 4). Structures of isolated compounds were elucidated by 1D and 2D NMR spectroscopic methods and comparison with previously reported data. The determination of nine compounds of diverse classes including furan derivatives, fatty acid, two megastigmens, one anthraquinone, three flavonoids and one ionone, showed the high phytochemical diversity of genus Argemone.

Compound 1 was obtained as a yellowish oil with molecular formula deduced as $C_7H_{11}N_3O_4$ via the spectroscopic data generated and in comparism with literature data, compound 1 was confirmed as D-(ribofuranosyl)-1H-1,2,4-triazone[22] (Table 2). Compound 2a was obtained as a colorless oil. The molecular formula was deduced as $C_{11}H_{18}O_4$ by ESI-MS ([M + H]+ m/z 215.1, [M + Na]+ m/z 237.1). The molecular formula was further confirmed by the NMR data including DEPT and ^{13}C NMR spectra, which showed 11 carbon signals. The ^{1}H NMR spectrum exhibited one olefinic proton at 5.68 (1H, s) and one oxymethine proton at 4.32 (1H, quint, J = 3.3 Hz). The proton resonances at 1.77 (3H, s), 1.45 (3H, s), and 1.26 (3H, s) were attributed to the methyl group. In the ^{13}C NMR spectrum, the downfield signal at 182.3 was assigned to unsaturated carbonyl carbon (C-8), and the signals resonating at 171.7 and 113.0 were due to the olefinic carbons. The signals at C 86.5 and 66.6 could be assigned to oxyquaternary and oxymethine carbons, respectively. The HMBC cross-peaks from H-3 to C-4 (66.6), Me (27.0) and from H-5 to C-6 (35.8), Me (26.6) indicated the existence of the cyclohexyl group. The substitution of an olefinic acid group at C-1 of 1 was proved by the HMBC correlation from H-7 (5.68, s) to C-1 (182.3), C-2 (86.5), C-6 (35.8), and C-8 (171.7). With all these spectral data taken into account, compound 2a was named (Z)-2-(2,4-dihydroxy-2,6,6-trimethylcyclohexylidene) acetic acid[23] (Table 2). Compound 2b occurs as a mixture with compound 2a. in comparison with literature data, compound 2b was confirmed as cis-3,6-dihydroxy-a-ionone.[24]

Compound 3 was obtained as a white mass with ^{1}H n.m.r.(400 MHz) 0.85, s, 6'-Me; 1.16, s, 2'-Me; 1.27, s, 6'-Me; 1.62, m, (H 5')~ and 3x OH; 1.78, dd, 2~ 13.1, 3~ 11+2Hz, H3'; 1.88, ddd, 2~ 13.1, 3~ 4.6, 1.5Hz, H3'; 2.31, s, COMe; 4.17, m, In comparism with literature data, compound 3 was confirmed as (E)-4-(r-1',t-2',c-4'-trihydroxy-2',6',6'- trimethylcyclohexyl) but-3-en-2-one[25,26] (Table 2).

Compound 4 was obtained as a yellowish crystal with molecular formular $C_{15}H_8O_6$. Spectra data comfirm compound 4 as Rhein[27] (Table 3). Compounds 5, 6 and 7 isolated in this study had similar NMR spectra with minor differences representing the different compounds attached. Compound 5 was obtained as pale yellow crystals with a characteristics hydroxyl peak at δ 12.30 indicative of the interaction between the carbonyl and hydroxyl group at position 4 and 5 of a flavonoid skeleton. The ^{1}H NMR shows double-doublet at positions δ 6.92-7.34 in agreement with the two aromatic protons of the A benzene ring (positions 6 and 7). The anomeric carbon was observed at δ 5.33 and the hydroxyl group on the adjourning benzene was seen at δ 6.30 and 6.20 respectively, attributable to the B benzene ring. The doublets of doublets signal at δ 2.76 and 2.88, and the multiplet at 4.20 are characteristic of the CH_2-CH-CH-O group of a flavanol skeleton. A characteristics sugar with the hydroxyl on the sugar were observed at δ 3.31-3.76 representing glucose were observed. Carbon NMR shows 22 carbon atoms and in comparison with literature data, compound 5 was confirmed as Tamarixetin.[28] Compounds 6 and 7 had a similar spectra but a distinct methoxyl singlet peak at δ 3.14 for compound 6 and a characteristics methyl of the rhamnose observed as a singlet at δ 0.9 for compound 7. In comparison with literature data, compound 6 was identified as 41-methoxyl-quercetin 3-β-D-glucoside[29] while compound 7 was confirmed as Tamarixetin-3-O-β-D-robinoside[30]

(Table 4).

Compound 8 shows a peak characteristics of fatty acid and was subjected to GC-MS analysis. In comparism with spectra library, compound with retention time of 37.789 minutes and percentage peak area of 79.73 was identified as Palmitic acid.

The extract of the plant showed higher activity for PPAR alpha than PPAR gamma. PPARγ is the primary target for the thiazolidinediones (TZDs) class of anti-diabetic drugs. TZDs (such as ciglitazone and rosiglitazone) improve insulin resistance which is the key symptom involved in the pathogenesis of T2DM. Increased expression of PPARα enhances insulin signaling and insulin-responsive glucose uptake,[31] PPARα is an important target for the treatment of insulin resistance, which is a hallmark of T2DM. PPARα activators show hypolipidemic effects by enhancing fat degradation in peripheral tissues and thus, lowering the level of circulating and cellular lipids.[30] Clinical studies indicate that the PPARα selective fibrates, such as fenofibrate, reduce triglyceride and LDL cholesterol and raise HDL cholesterol in dyslipidemia patients.[32]

The extracts of the plants were found to activate PPARα with a fold induction of more than 2.0 over the vehicle control, 2.01 ± 0.01, 2.15 ± 0.07 and 1.79 ± 0.04 at 100 µg/ml, 50 µg/ml and 25 µg/ml respectively (table 6). Under similar assay conditions, positive controls Ciprofibrate, a well-known PPAR alpha agonist used as standard in this assay shows a substantial fold increase (3.85 ± 0.125), 2.88 ± 0.148 and 2.20 ± 0.039 ($p < 0.001$) fold of the control at a dose of 25, 12.5 and 6.25 µM, respectively.

For the PPARγ assay, a lower induction was observed, 1.76 ± 0.03, 1.74 ± 0.15 and 1.61 ± 0.04 at the same doses of 100 µg/ml, 50 µg/ml and 25 µg/ml respectively compared with Rosiglitazone, a potent PPAR gamma agonist, showing a fold increase of 2.31 ± 0.05, 2.17 ± 0.120, and 2.07 ± 0.270 at doses of 25 µM, 12.5 µM, and 6.25 µM respectively, serving as the positive control.

The isolated compounds showed a different pattern in the activation of PPARα and PPARγ. Compound 1 (1-(β-D-ribofuranosyl)-1H-1,2,4-triazone) show lower PPARα activity and the least activity among the tested compounds for PPARγ, with a maximum fold increase of only 0.93 at 12.5 µM, indicating minimal agonistic effect. Compound 4 (Rhein) shows moderate PPARα activity, with the highest fold increase at 50 µM (1.32), which decreases to 0.79 at 12.5 µM. This suggests some potential for PPAR alpha agonism but less potency at lower concentrations. It display lower PPARα activity, with fold increases close to 1. Compound 5 (Tamarixetin) shows strong PPARγ activity, peaking at 50 µM (1.34) and decreasing slightly with concentration, although it maintains activity down to 12.5 µM (0.99). It shows a noticeable increase in PPARα activity at 50 µM (1.25), though it remains stable at lower concentrations (0.99 at both 25 µM and 12.5 µM), indicating moderate agonistic potential. Compound 6 (41-methoxyl-quercetin 3-β-D-glucoside) demonstrates the highest PPAR alpha activity among the tested compounds, with a consistent increase across concentrations, peaking at 50 µM (1.43) and showing moderate activity at lower concentrations (1.24 at 25 µM, 1.02 at 12.5 µM). This suggests it could be a potential PPARα agonist. In the PPARγ assay, it has the highest PPAR gamma activity among the tested compounds, particularly at 50 µM (1.26) and 25 µM (1.29), showing a promising and consistent agonistic effect across concentrations.

Compound 7 (Tamarixetin-3-O-β-D-robinoside) shows a decrease at higher concentrations, with the highest fold increase (1.27) only at 12.5 µM and activity increases slightly in PPARγ as the concentration decreases, suggesting a unique response pattern compared to the other compounds. Compound 2 and 3 occurs as a mixture and thus, were not subjected to PPAR assay (Table 4).

The PPAR activity of the ethanol extract shows promising effect of A. mexicana activation on PPAR with fold increase greater than 2.0, but a lesser effect on PPARγ. This might be due to the synergistic effect of the constituents as the pattern is different for the isolated compounds. Compound 6 (41-methoxyl- quercetin 3-β-D-glucoside) appears to be the most promising candidate for both PPAR alpha and PPAR gamma agonism due to its consistently high activity across concentrations. Compound 5 (Tamarixetin) also shows moderate activity, particularly for PPAR alpha. However, Compound 4 (Rhein), Compound 7 (Tamarixetin-3-O-β-D-robinoside), and Compound 1 exhibit lower activities, suggesting they may not be as effective as PPAR agonists or could require higher concentrations for significant effects.

CONCLUSION

A total of nine compounds were reported from the ethanol extract of Argemone mexicana, in this study. These are three flavonoids, palmitic acid, Rhein, a triazole

and three substituted cyclohexane which are the first report of such compounds from the plant. Structural elucidation of the isolated compound was carried out in comparison with literature data. The PPAR activities of the ethanol extract and isolated compounds was established in this study.

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