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Der Pharmacia Lettre, 2018, 10[2]:73-78
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Aldose Reductase Inhibitory Effect and Identification of Bioactive Compounds in Carica Papaya Extract

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ABSTRACT

Objective and aim: the aim of the research work was to characterize the methanolic extract of *C.papaya* and then carried out the antidiabetic inhibitory potential of the extract against Aldose Reductase enzymes.

Materials and methodology: the *C.papaya* leaves were harvested fresh and air dried for five days at room temperature and blended into powdered form using electric blender. It was subsequently subjected to extraction using analytical grade of methanol solvent. Enzymatic reaction assays were performed using standard recommended protocol with slight modifications and the extract was characterized using Gc-MS. Finally some of the identified compounds were screened for various degrees of drug characteristics using Online OSIRIS property explorer.

Results: the IC₅₀ value (1.22±0.63 µg/mL) of ALR1 was better than the standard vaproic acid of IC₅₀ (57.4±10 µg/mL) and the IC₅₀ (1.22±0.06 µg/mL) of ALR2 of the methanolic extract was better than the sorbinil standard IC₅₀ (3.10±0.20 µg/mL). The promising inhibitory aldose reductase may be due to the compounds present in the methanolic extract and these compounds include; phytol, Oxalic acid, 6-ethyloct-3-yl isobutyl ester, 3-methyl-2-(2-oxopropyl)Furan, Carbonic acid, isobutyl undec-10-enyl ester, D-mannitol, 1 decylsulfonyl and 1H-Imidazole, 1(1-oxooctadecyl), these identified compounds possess different drug characteristics such as, solubility, mutagenic, irritability, H-bond acceptor and H-bond donor.

Conclusion: The promising potent inhibitory activity of *C.papaya* showed that the plant leaves could be further researched into as alternative for resolving cataract eye problem associated with prolonged diabetes mellitus.

Key words: Aldose reductase, Inhibition, Drug characteristics

INTRODUCTION

A class of abnormalities characterized by innate or acquired inability to transport glucose from blood cells to cells is known as diabetes mellitus. Plants have been examined severally for different pharmacological uses such as in the management of diabetes ailment, because of the different secondary metabolites present in the plant extract [1] and used in reducing blood glucose level. There are many diabetes inhibitors among these is aldose reductase inhibitors, which are described as drugs being used for the treatment of cataract eye defect caused as a result of prolonged diabetes disease. The defect occurred as a result of the increase in the sugar level in the human lens and consequently the excess sugar within the lens is reduced to alcohol by the aldose reductase. It has been reported that aldose reductase inhibitors when administered on rats, prevented cataract [2]. There are confirmed reports that aldose reductase having isoforms ALR1 and ALR2 have been attributed to many causes of diabetes ailments that linked to the influx of glucose through the polyol pathway, caused in tissues such as retina, kidney, lens and nerves at high blood glucose level. As a result of this trend, aldose reductase inhibition is attracting the attention of scientists as a source for the treatment of hyperglycemia –induced cardiovascular pathologies [3]. Other long term side effects linked to diabetes as a result of excess free glucose in tissues include cataractogenesis and microangiopathy [4]. Aldose reductase complications have been widely researched [5,6] and there are concerted efforts by scientists to continuously searching for inhibitors by investigating different medicinal plants that may have therapeutic activities.

C.papaya is believed to have been found in Southern Mexico and central America but the plant has spread worldwide [7, 8]. The seed of the plant is edible, can be used for juice fruit and can be cooked as a vegetable [9]. The unripe fruit is said to contain crude papain [10]. The plant leaves have been previously found to have secondary metabolites such as flavonoid, saponin, cardiac glycosides, anthraquinones, reducing sugars, steroids, phenolics, and cardenolides [11].

MATERIAL AND METHODS

All the chemicals needed for the enzyme extraction were of analytical grade. Substrates (D,L-glyceraldehyde and sodium-D-glucuronate) and nicotinamide adenine dinucleotide phosphate (NADPH) as co-factor were purchased from Sigma Aldrich. Eliza microplate reader was used with a UV range of 340nm for the enzymatic reaction.

Plant source

The *C.papaya* leaves were collected in a local farm in Ado Ekiti, Ekiti State, Nigeria on the 10th of April, 2017 and were air dried for five days. The dried leaves were blended to powdered form and stored for analysis.

Research Laboratory

The research work was carried out at the centre for the Advanced Drug Research (CADR), Department of Pharmacy, COMSATS Institute of Information Technology, Abbottabad, Pakistan in the month August, 2017.

Crude extract preparation

The plant leaves of 200g of powdered samples were soaked in 2000ml of methanol of analytical grade for five days and later filtered using filter paper and the extract was concentrated using rotary evaporator at 35°C. The working solution was made by preparing 1mg/ml from the stock of 10mg/ml of 100% dimethyl sulfoxide (DMSO).

Determination of aldose reductase (ALR2 and ALR1) inhibitory activities

UV spectrophotometer was used at 340 nm in order to determine the activity of aldose reductase by measuring the NADPH consumption. Each well of the 96-well plate contained 100 µL of assay mixture containing phosphate buffer 100 mM at pH 6.2 (10µL), with 10µL of 1mg/ml of crude extract followed by addition of 35 µL of enzyme and 20 µL of substrate (D,L-glyceraldehyde for ALR2 and sodium-D-glucuronate for ALR1). The mixture was incubated at 37°C for 5 min and for the

enzymatic reaction to run properly 0.5mM NADPH (20 μ L) as a cofactor was added and reading was taken at 340 nm. The mixture was incubated again at 37 °C for 10 min and reading was taken at the respective UV range in ELIZA plate reader. For ALR2 (10 mM Sorbini) and ALR1 (vaproic acid) of 10 μ L each was used as positive control and 20 μ L buffer solution as negative control respectively. The enzymatic reaction was run in triplicates with a final volume of 100 μ L in each well. Absorbance was noted and results were analysed [12].

Statistical Analysis

The IC₅₀ values were calculated using non linear curve fitting program PRISM 5.0 (Graph pad, San-Diego, California, USA)

GC-MS analysis

GC-MS analysis of the extract was performed using TurboMass GC System, under the following conditions;

capillary column (30 m, 0.25 mm inner diameter, 0.25 μ m film thickness of maximum temperature, 350°C)

Perkin Elmer Clarus 600C MS gas carrier mobile phase ;Helium flow rate of 1.0 ml/min ion source temperatures were 280°C.

the ionizing energy was 70 eV the oven temperature was programmed from 70 °C (hold for 2 min) to 280°C (hold for 10 min) at a rate of 5°C/min. volume of the crude extract injected 1 μ L The data were obtained by collecting the mass spectra within the scan range 50-550 m/z. The identification of chemical compounds in the extracts was based on GC retention time; the mass spectra matched those of standards available at NIST library.

RESULTS

Table 1: showing the aldoses reductase inhibitory effect

Extract	ALR1 IC ₅₀ (μ g/mL)	ALR2 IC ₅₀ (μ g/mL)
Methanolic extract	1.22 \pm 0.63	1.22 \pm 0.06
^a Vaproic acid	57.4 \pm 10	Not tested
^b Sorbinil	Not tested	3.10 \pm 0.20
Note: SEM + standard mean error, aALR1 standard, b ALR2 standard		

Table 2: showing the identified compounds in the chromatogram

Compound name	Molecular formula	Molecular weight	CAS No	Retention time (minutes)
Silane, cyclohexyl dimethoxy methyl	C ₉ H ₂₀ O ₂ Si	188	17865-32-6	32.345
Phenol, 2,4-Bis(1,1-dimethyl ethyl)	C ₁₄ H ₂₂ O	206	96-76-4	18.555
2,Nonadecanone 2,4-dinitrophenyl hydrazine	C ₂₅ H ₄₂ O ₄ N ₄	462	28813-61-8	35.678
Silane, 1,4-phenylene Bis(trimethyl)	C ₁₂ H ₂₂ Si ₂	222	13183-70-5	37.81

Oxalic acid,6-ethyloct-3-yl isobutyl ester	C ₁₆ H ₃₀ O ₄	286	900309-34-1	7.845
3,methyl-2-(2-oxopropyl)Furan	C ₈ H ₁₀ O ₂	138	87773-62-4	29.404
Carbonic acid, isobutyl undec-10-enyl ester	C ₁₆ H ₃₀ O ₃	270	900314-60-8	28.484
Phytol	C ₂₀ H ₄₀ O	296	150-86-7	40.819
1,3-Dioxolane,4-ethyl-5-octyl-2,2-Bis[trifluoromethyl]-trans	C ₁₅ H ₂₄ O ₂ F ₆	350	38274-73-6	45.606
D-mannitol,1 decylsulfonyl	C ₁₆ H ₃₄ O ₇ S	370	900154-76-1	47.047
1H-Imidazole,1(1-oxooctadecyl)	C ₃₇ H ₇₀ O	334	17450-32-7	29.369
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	102608-53-7	28.494

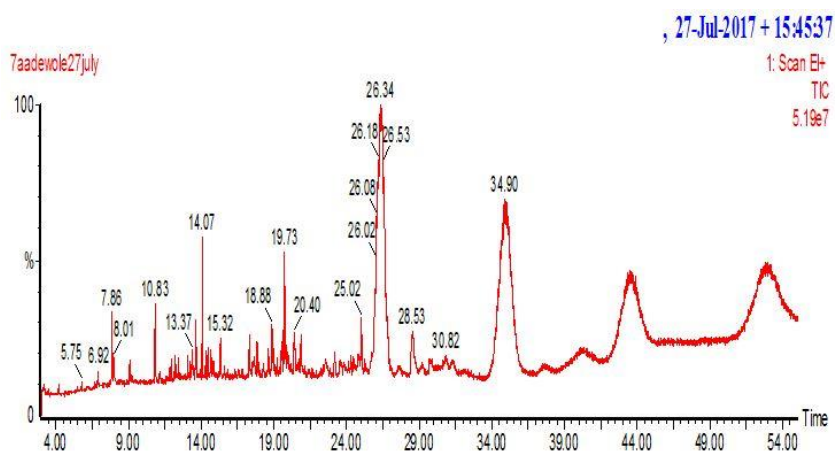
Figure 1: Chromatogram of *C. papaya*

Table 3: showing the drug characteristics of identified compounds

Compound	Drug likeness	cLogS	cLogP	Polar surface area	H-bond Acceptor	H-bond Donor
				(Å ²)		
^a Silane	-1	-0.53	0	NaN	0	0
^b Phenol	-2.2721	-1.32	1.3139	0.16455	1	1
^c Oxalic acid,	-6.1289	0.066	-1.5754	0.84733	4	2
^d Furan	-2.0899	-1.274	0.7943	0.22415	1	0
^e Carbonic acid	-2.521	-0.646	-0.5238	0.89283	3	2
^f Phytol	-3.7661	-4.633	7.4212	0.046626	1	1
^g Imidazole	0.44659	-0.431	-0.1802	0.40526	2	1

^aSilane has the same functional group with Silane, cyclohexyl dimethoxy methyl

^bPhenol shares the same functional group with Phenol, 2,4-Bis(1,1-dimethyl ethyl

^cOxalic acid, has the same functional group with Oxalic acid, 6-ethyloct-3-yl isobutyl ester

^dFuran shares the same functional group with 3, methyl-2-(2-oxopropyl)Furan

^eCarbonic acid shares the same functional group with Carbonic acid, isobutyl undec-10-enyl ester

^fPhytol

^gImidazole shares the same functional group with 1H-Imidazole, 1(1-oxooctadecyl)

Discussion

The IC₅₀ value (1.22±0.63 µg/mL) of ALR1 was better than the standard vaproic acid of IC₅₀ (57.4±10 µg/mL) and the IC₅₀ (1.22±0.06 µg/mL) of ALR2 of the methanolic extract was better than the sorbinil standard IC₅₀ (3.10±0.20 µg/mL). The therapeutic effect of the methanolic extract may be due to the compounds present such as Phytol, Oxalic acid, 6-ethyloct-3-yl isobutyl ester, 3, methyl-2-(2-oxopropyl)Furan, Carbonic acid, isobutyl undec-10-enyl ester, D-mannitol, 1 decylsulfonyl and 1H-Imidazole, 1(1-oxooctadecyl). Heterocyclic compounds have been found to play a lot of significant roles in the metabolisms of living cells and quite a large number possesses either five or six membered rings having different pharmacological properties. Among this is imidazole rings with varying therapeutic potentials such as antiinflammatory, anticancer, antibacterial, antifungal, anti-tubercular, anti-diabetic and antiviral products [13]. The presence of different compounds in the extract has shown that the plant leaves possess high potential aldose reductase inhibition as earlier demonstrated by the herbal practitioners in Lagos, Nigeria where the plant was adopted as antidiabetic herbal tool [14].

Osiris Drug Properties

Some of the identified compounds were found to have various drug characteristics when screened using OSIRIS Online server explorer [15] and this include; drug likeness, cLogS, cLogP, mutagenic, tumorigenic, irritability, H-bond acceptor and H-bond Donor. The promising inhibitory of the plant against aldose reductase, aldehyde reductase, and the identified bioactive compounds could be taken as a tool for further insight into the usefulness of the plant.

CONCLUSION

The promising potent inhibitory activity of *C.papaya* is an indication that the plant leaves could be further researched into as a potential remedy for resolving cataract eye problem associated with prolonged diabetes mellitus.

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