

Anti-Diabetic Activity of *Dracaen cinnabari Balf.f* Extracts from Resin in Socotra Island-Yemen

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Abstract

In the developing countries, the medical plants play an important role in treatment diabetes mellitus. This significant comes due to their cost effectiveness. Diabetes mellitus, a metabolic disorder is becoming a serious threat to mankind health. The herbal drugs with anti-diabetic activity are till now to be commercially formulated as modern medicines, even though they have been acclaimed for their therapeutic properties in the traditional systems of medicine. In this paper, we have reported a study based on anti-diabetic properties of Dragon *cinnabari* resin. The resin of plant material was collected, shade dried and extracted with different solvents using soxhlet extraction procedure. *In vitro* anti diabetic activity is assayed with standard glucose uptake procedure against MCF-7 cell line. From the analysis it was found that, glucose uptake inducing activity of ethyl acetate extract was found to be higher than Metformin, whereas other extracts did not display much anti diabetic activity against MCF-7 breast cancer cell line. Based on the glucose uptake studies against MCF-7 cell line, the ethyl acetate extract could be used as potential source for anti-diabetic drugs.

Keywords: Anti diabetic activity; Dragon *cinnabari* Blaf.f; Glucose uptake; Plant extract

Introduction

There are various medicines derived from plant extracts are being used in the treatment of a wide variety of clinical diseases, though relatively little knowledge about their mechanisms of action is known [1]. Many herbal preparations are being prescribed widely for the treatment of diabetic conditions. There is a need for research and developmental work in herbal medicine because apart from the social and economic benefits, it has become a persistent aspect of present day healthcare in developing countries [2,3]. Dragon's blood tree is a non-specific name for dark red resinous exudations from different plant species endemic to various regions around globe that belongs to four genera *Dracaena* Spp [4]. (Agavaceae), *Croton* Spp. (Euphorbiaceae), *Daemonorops* Spp. (Palmaceae) and *Pterocarpus* Spp. (Fabaceae) have a long history of being used as a traditional medicine the world over. Medicinal use of dragon's blood dates back to the ancient Greeks, Romans, Chinese and Arabs [4-7]. However, *Dracaena cinnabari Balf. f. (D. cinnabari)* belongs to Agavaceae family, which is commonly known as Damm Al- akhwain in Yemen (Figure 1). It is endemic to the Socotra Island, Yemen. *D. cinnabari* resin has traditionally been used to treat diarrhea, wounds, fevers, ulcers, hemorrhage, control bleeding, fractures, and burns [5,7]. The Dragon's blood tree has a wide application in biological area as antimicrobial, antioxidant, anti-diarrhea and cytotoxicity effect [7-11]. Diabetes mellitus is dispersion in an alarming way throughout the world and three fourth of the world populations and careful as a major cause of high economic loss which can in turn impede the development of nations. Moreover, uncontrolled diabetes leads to many chronic complications such as blindness, heart disease, and renal failure [12]. Therefore, treating diabetes mellitus with plant derived compounds which are accessible and do not require laborious pharmaceutical synthesis appears highly attractive [4,12]. Although, Dragon's blood tree is extensively used, little research has been done to know about its true source, quality control, bioactive compounds and clinical applications. Therefore, it is of a great interest to carry out a screening of these plant parts in order to validate their use in folk medicine and to reveal the active principle by isolation and

characterization of their constituents. The systematic screening of them may result in the discovery of novel active compounds [8,9,12]. However, no work has been reported on the anti-diabetes property of this plant. Keeping in view, the present study has been undertaken to investigate anti-diabetic activity of the different extracts of *Dracaen cinnabari Balf.f* against MCF-7 cells lines.

Materials and Methods

Materials

Plant extracts, 2-NBDG, (2-(N-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl) amino)-2-deoxyglucose)), a fluorescent deoxy glucose analogue, is used as a probe for the detection of glucose taken up by cultured cells. It is transported by both SGLTs and GLUTs and has allowed the study of the effects of drugs targeting glucose uptake and glycolysis. NBDG fluorescence typically displays excitation/emission maxima of ~465/540 nm and can be visualized using optical filters designed for fluorescein, plant extracts, 2-NBDG (Invitrogen: Cat no. 13195), MCF-7 cell lines (from NCCS Pune), DMEM- High Glucose (High Media), Fetal Bovine Serum (High media), Penicillin-streptomycin (High media) 6-7 well plates (Corning), Serological pipettes (Corning), Pipettes (Benchtop), Pipette tips (Thermo), Bio safety cabinet (Biobase), CO₂ incubator (Healforce), Inverted microscope (Biolinx), Flow cytometer (BD FACS callibur), Heamocytometer.

(i) Medium control (medium without cells).

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Figure 1: Dragon's blood tree (*D. cinnabari Balf. f*) in Socotra Island, Yemen.

- (ii) Negative control (medium with cells but without the experimental drug/compound).
- (iii) Positive control (medium with cells treated with a known drug, Metformin; 5mM).

Methods

Collection of plant material: Dragon's blood tree (*D. cinnabari*) resin was collected from Socotra Island (Yemen) On May 2014. It has a matchless and strange appearance, described as upturned, densely-packed crown having the shape of an umbrella (Figure 1).

Preparation of extracts: About 800 gm of dry powder of the resin of *Dracaena cinnabar* was taken in a soxhlet apparatus and subjected for sequential extraction of solvents from non-polar to polar end (hexane, benzene, diethyl ether, dichloromethane, chloroform, ethyl acetate, acetone, ethanol, methanol and water) the extract samples were kept at 4°C for further assays. All the extracts were subjected to glucose uptake assay.

Cell culture and *in vitro*: MCF-7 cells were cultured in DMEM HG (Himedia) in T-75 tissue culture flask (Corning) until they reached 70% confluency. The cells were then harvested and used for the evaluation of anti-diabetic activity of the plant extracts by glucose uptake assay [13,14].

Glucose uptake assay

D-Glucose acts as the main fuel for maximum cells in mammals and is principally important for the heart and brain, which use glucose as their sole source of energy. Carbohydrates are only absorbed in the form of the monosaccharide through glucose transporter (Glucose uptake cell-based assay kit: Cayman Chemical Catalog no. 600470).

Cultured cells in a 6-well plate at a density of 1×10^5 cells/2 ml and incubated them in a CO₂ incubator overnight at 37°C. After 24 hours, the spent medium is aspirated and the cells are treated with experimental compounds, control them in 2 ml glucose-free culture medium containing 100 μM 2- NBDG and incubated the cells for 2 hours. At the end of the treatment, the medium is removed from all the wells and PBS is given wash. The PBS is removed and 200 μl trypsin is added. The wells are Incubated at 37°C for 3-4 minutes, 2 ml culture medium is added and harvested the cells directly into 12 × 75 mm tubes. The tubes are centrifuged for five minutes at 300 g × 25°C. Carefully the supernatant is an aspirated. Then, the cells are suspended in 0.5-1 ml of PBS. Mixed well to ensure separation of individual cells. The cells analyzed with a flow cytometer. The cells must be analyzed immediately. The cells taken up 2-NBDG displayed fluorescence with excitation and emission at 465 nm and 540 nm, respectively, they can be measured in the FL1 channel which is used to detect FITC [13].

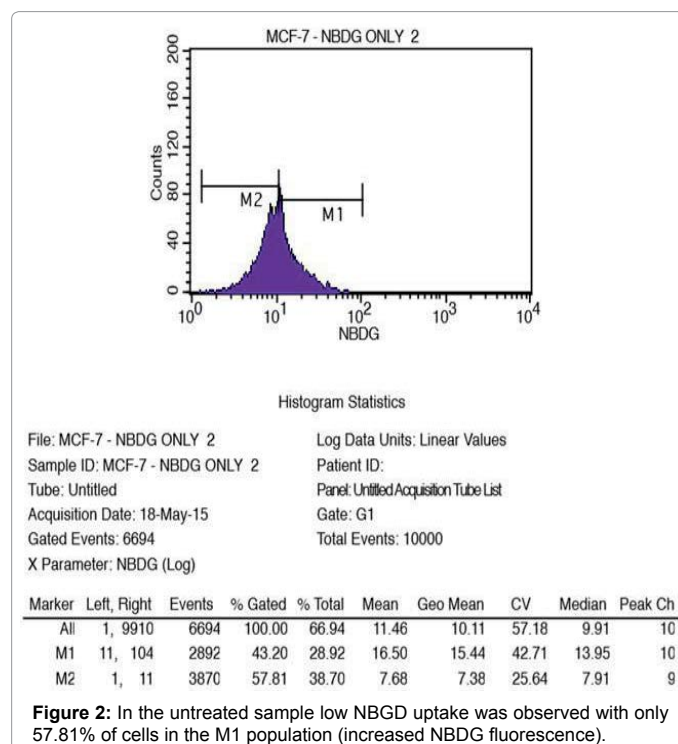
Results

The exaction process starting from non-polar to polar are summarized in Table 1. The results obtained from the glucose uptake assay are as follows:

1. Untreated (NBDG only) (Figure 2)
2. Positive control- Metformin 2.5mM (Figure 3)
3. Ethyl acetate extracts (Figure 4)
4. Hexane extracts (Figure 5)
5. Chloroform extracts (Figure 6)
6. Dichloromethane (DCM) extracts (Figure 7)
7. Acetone extracts (Figure 8)
8. Benzene extracts (Figure 9)
9. Ethanol extracts (Figure 10)
10. Methanol extracts (Figure 11)
11. Ether extracts (Figure 12)

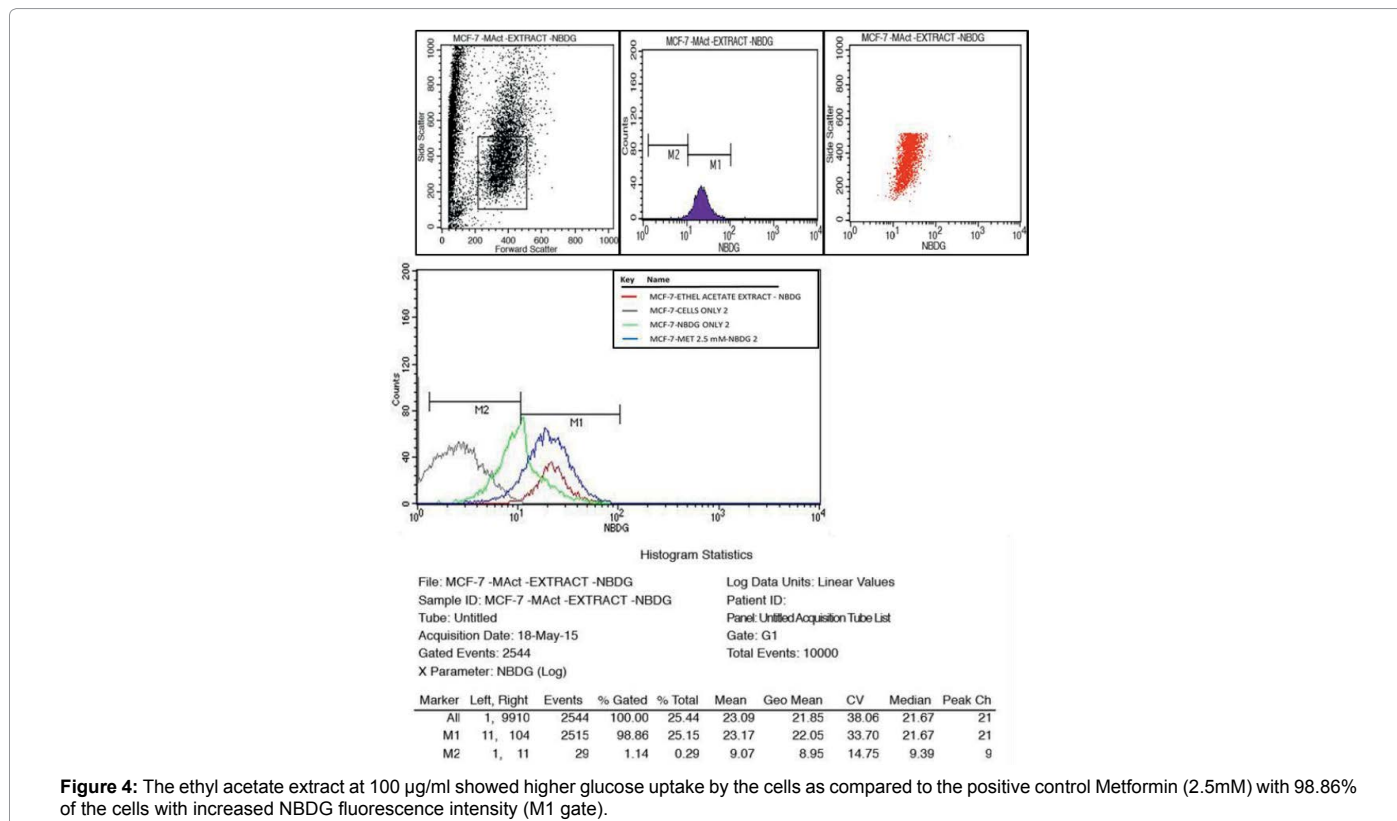
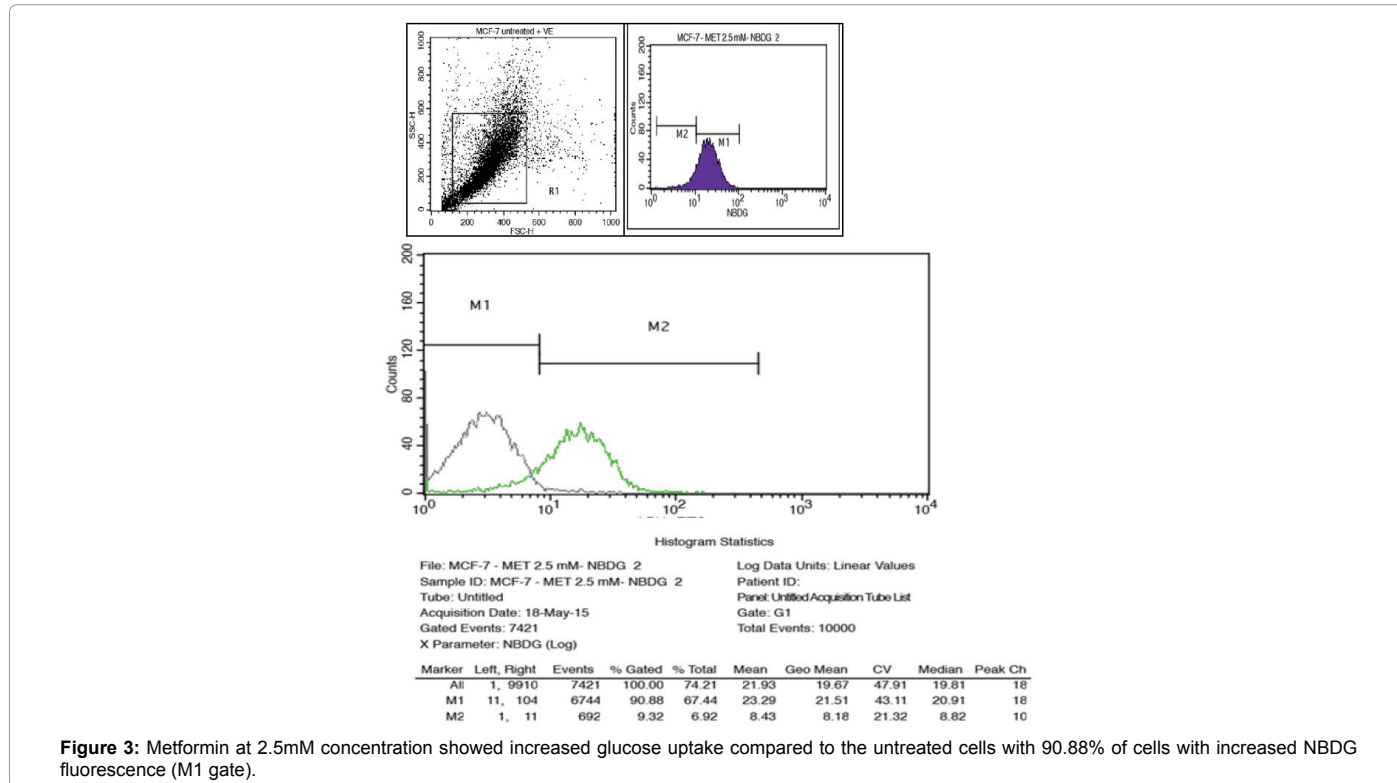
Discussion

Diabetes mellitus is spreading in a very fast way throughout the world. Blindness, heart disease, and renal failure are some chronic complications results of the uncontrolled diabetes. In a matter of fact, it seems attractive to treat with plant that doesn't require more pharmaceutical synthesis. Glucose uptake inducing activity of ethyl acetate extract was found to be higher than Metformin with 98.86% which indicates that this extract can be used as a source of anti-diabetic drugs. Which is followed by benzene, ethanol and chloroform extract with 88.57%, 70%, 55.09% respectively and hexane extracts have shown moderate with 23% while diethyl ether, methanol, dichloromethane and acetone have not shown activity.



Solvent	Hexane	Benzene	Diethyl ether	Di-chloromethane	Chloroform	Ethyl acetate	Acetone	Ethanol	Methanol	Water	Residue
Resin (gm)	20	33.5	60	14.8	88.8	120.5	85	55	90	20	200

Table 1: Shows the yield of phytochemicals in the solvent extracts of *D. cinnabari* resin.



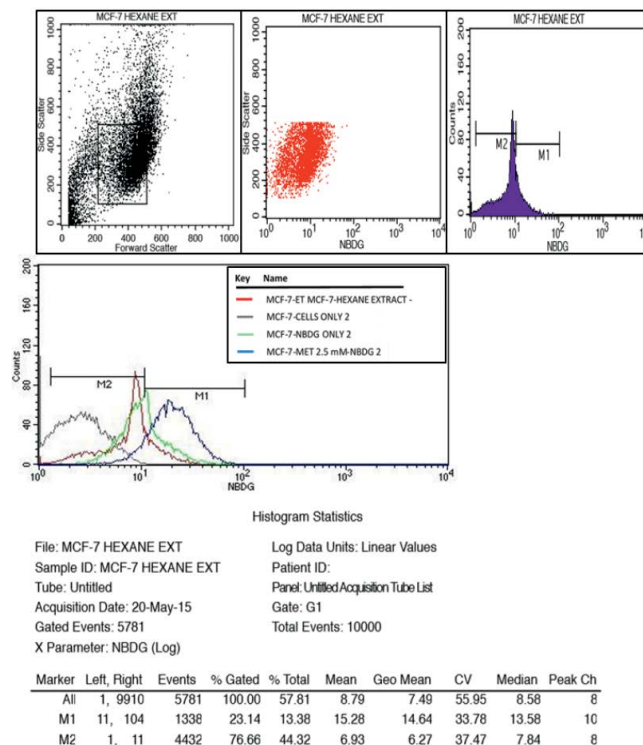


Figure 5: As compared to the positive control Metformin (2.5mM), The hexane extract at 100 µg/ml showed low glucose uptake by the cells with 23.14% of the cells with increased NBDG fluorescence intensity (M1 gate).

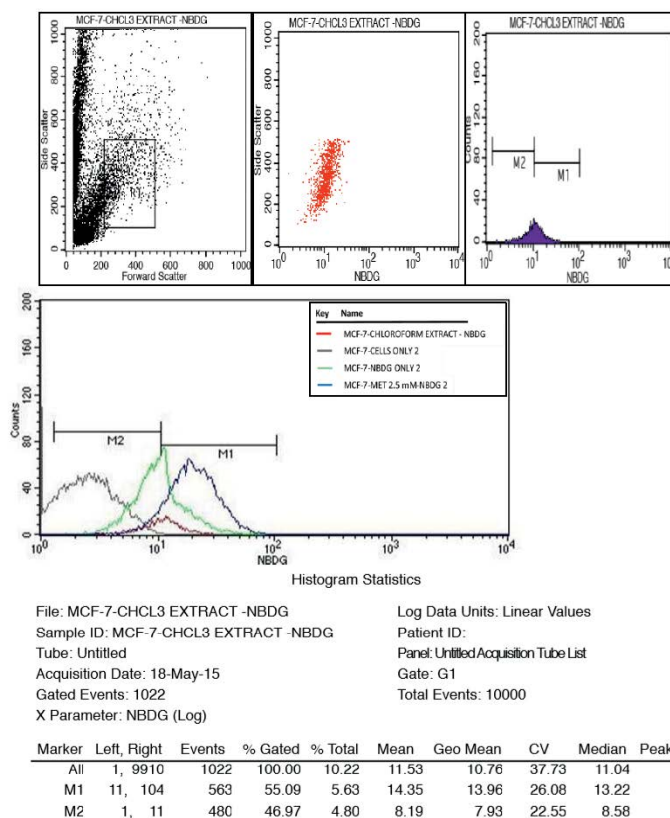


Figure 6: The chloroform extract at 100 µg/ml showed medium glucose uptake by the cells as compared to the positive control Metformin (2.5mM) with 55.09% of the cells with increased NBDG fluorescence intensity (M1 gate).

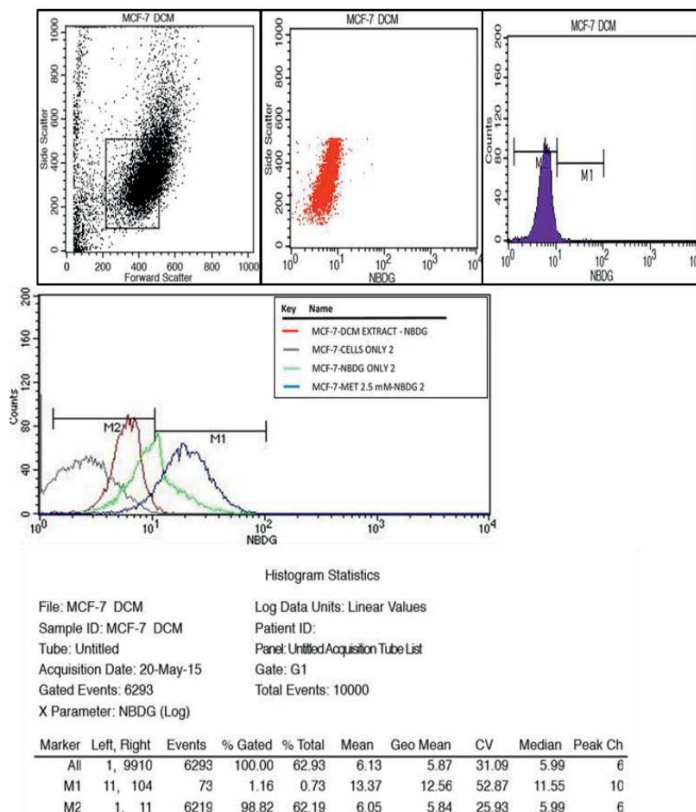


Figure 7: The DCM extract at 100 µg/ml didn't showed glucose uptake by the cells as compared to the positive control Metformin (2.5mM) with 1.19% of the cells with increased NBDG fluorescence intensity (M1 gate).

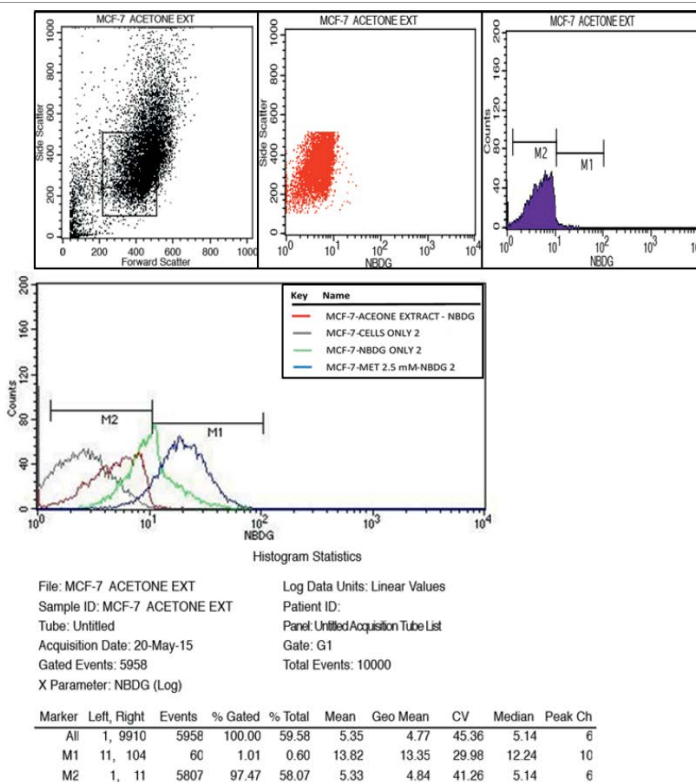
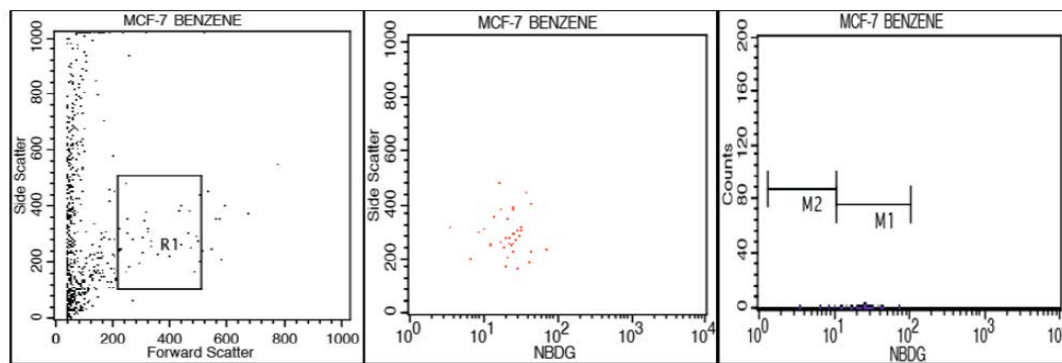


Figure 8: The acetone extract at 100 µg/ml didn't show glucose uptake by the cells as compared to the positive control Metformin (2.5mM) with 1.01% of the cells with increased NBDG fluorescence intensity (M1 gate).



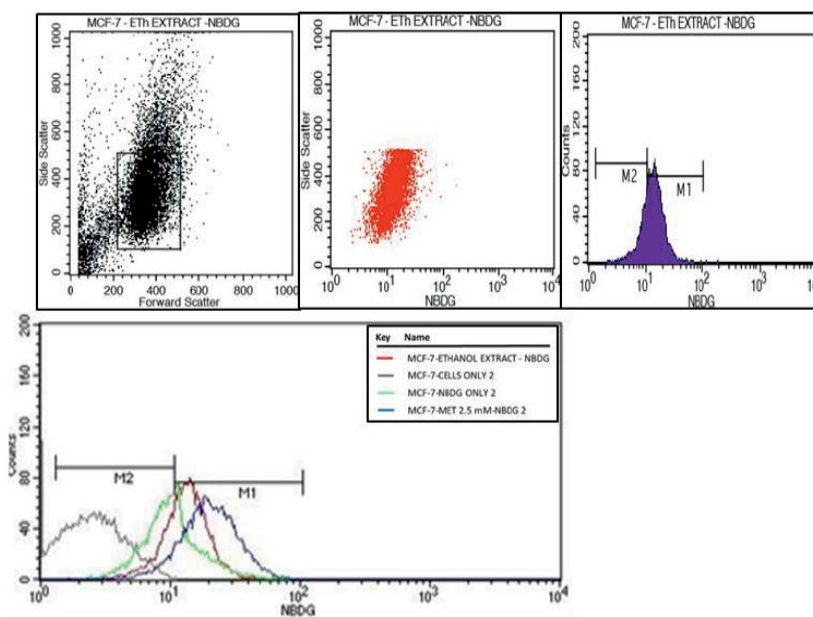
Histogram Statistics

File: MCF-7 BENZENE
 Sample ID: MCF-7 BENZENE
 Tube: Untitled
 Acquisition Date: 20-May-15
 Gated Events: 35
 X Parameter: NBDG (Log)

Log Data Units: Linear Values
 Patient ID:
 Panel: Untitled Acquisition Tube List
 Gate: G1
 Total Events: 630

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	35	100.00	5.56	24.32	21.23	51.19	23.93	24
M1	11, 104	31	88.57	4.92	26.53	24.63	43.09	24.80	24
M2	1, 11	4	11.43	0.63	7.19	6.70	39.29	7.50	3

Figure 9: The Benzene extract at 100 µg/ml showed good glucose uptake by the cells but also killed most of the cells as compared to the positive control Metformin (2.5mM) with 1.01% of the cells with increased NBDG fluorescence intensity (M1 gate).



Histogram Statistics

File: MCF-7 - Eth EXTRACT -NBDG
 Sample ID: MCF-7 - Eth EXTRACT -NBDG
 Tube: Untitled
 Acquisition Date: 18-May-15
 Gated Events: 6813
 X Parameter: NBDG (Log)

Log Data Units: Linear Values
 Patient ID:
 Panel: Untitled Acquisition Tube List
 Gate: G1
 Total Events: 10000

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	6813	100.00	68.13	14.01	13.03	42.49	13.22	14
M1	11, 104	5053	74.17	50.53	15.93	15.35	31.09	14.72	14
M2	1, 11	1816	26.65	18.16	8.41	8.19	20.82	8.82	10

Figure 10: The ethyl acetate extract at 100 µg/ml showed high glucose uptake by the cells as compared to the positive control Metformin (2.5mM) with 74.17% of the cells with increased NBDG fluorescence intensity (M1 gate).

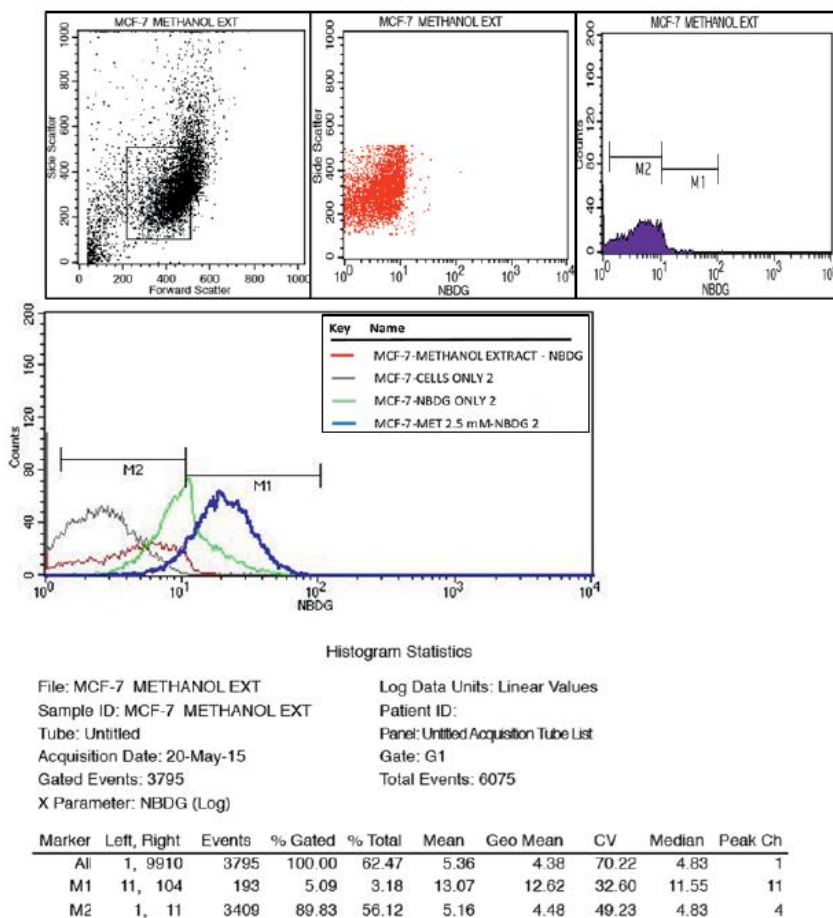


Figure 11: The methanol extract at 100 µg/ml showed good glucose uptake by the cells but also killed most of the cells as compared to the positive control Metformin (2.5mM) with 5.09% of the cells with increased NBDG fluorescence intensity M1 population (M1 gate).

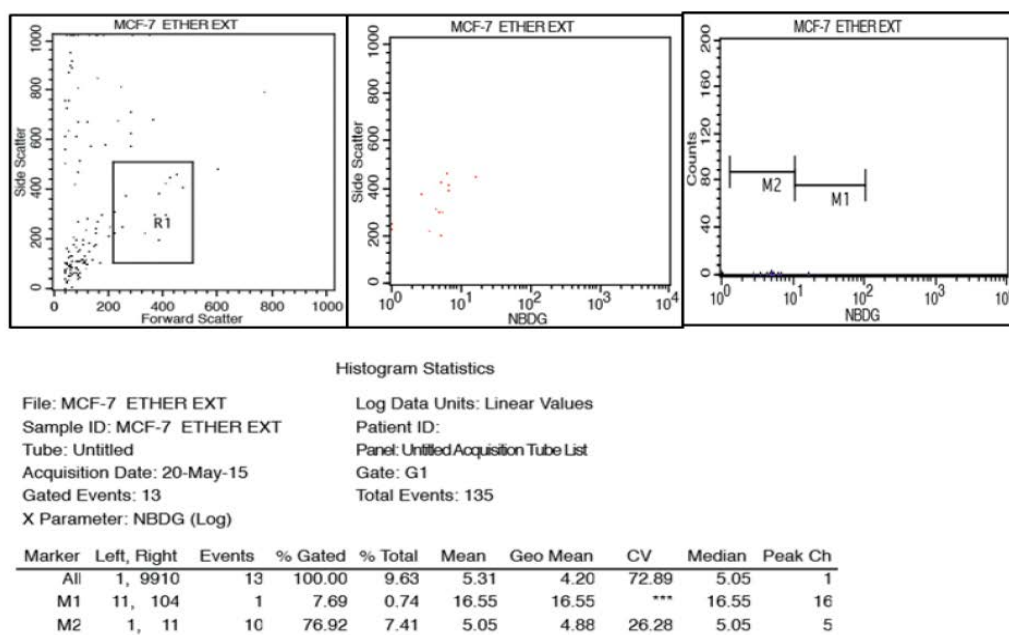


Figure 12: The Ether extract at 100 µg/ml showed no glucose uptake by the cells and also killed most of the cells as compared to the positive control Metformin (2.5mM) with 7.69% of the cells with increased NBDG fluorescence intensity (M1 gate).

Conclusion

In this study, we have reported the antidiabetic properties of Dragon *cinnabari* resin. The resin of plant material was extracted with different solvents and evaluated for them *in vitro* antidiabetic activity with standard Glucose uptake procedure against MCF-7 cell line. From the results, the ethyl acetate extract was found to be higher glucose uptake inducing activity than Metformin, whereas other extracts did not display much anti-diabetic activity against MCF-7 cell line. Based on the glucose uptake studies against MCF-7 cell line, the ethyl acetate extract could be used as potential source for anti-diabetic drugs.

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