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Study of Hypoglycemic Properties of Fresh Leaves of *Bryophyllum pinnatum* Extracted in Aqueous Medium by Using Ultrasound

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***Abstract*—** *Bryophyllum pinnatum is a medicinal plant widely used for several purposes in different tribes in Bangladesh. From ethnobotanical study it was observed that several ethnic groups used these leaves for the treatment of diabetes. The present study was designed to scrutinize the anti-diabetic properties of the leaves of B. pinnatum. The study was also emphasized on developing a green extraction method. In this study ultrasound assisted extraction in aqueous medium from fresh plant's parts was applied and compared with two conventional methods such as aqueous decoction and the ethanol cold extraction method. Glucocorticoid hormone dependent diabetes was created by the intraperitoneal injections administration of the dexamethasone on the Swiss albino mice used as diabetic animal models. Hypoglycemic potency of the crude extracts of B. pinnatum leaves was compared to the standard antidiabetic drug glibenclamide. However, healthy control and diabetic control groups were also used to minimize the biasness. After two weeks of treatment, a significant change was observed in hypoglycemic status in the plasma. The effects of crude extracts on body weight was also examined, however, no significant correlation was observed.*

***Keywords*—** *Bryophyllum pinnatum, Green extraction, Ultrasound.*

I. Introduction

Bryophyllum pinnatum is locally known as Pathorkuchi in Bangladesh and used as a popular medicinal plant. The family of *B. pinnatum* is Crassulaceae [1,2] and the plant is generally classified as a weed [3]. The plant is around 1-1.5 m in height and usually branched. The leaves are glabrous (with 3–5 deeply crenulated, fleshy leaflets) with obtusely four angled stems [3]. The lower leaves are simple, whereas the upper ones 3-7 foliate and are long-petiole [4]. Leaves are furnished with rooting vegetative buds and capable of growing new plants shortly after falling to the grounds. Ethnobotanical studies conducted on different tribes lives in Bangladesh such as, Chakma [1], Khumi [5], Santal [6], Marma [5], Tripura [5] identify the traditional uses of this plant for the treatment of pneumonia, cough, burn, headache, vomiting, food poisoning etc. Several publications reported its anti-diabetic antihypertensive, antileishmanial, antimicrobial, analgesic, anticancer properties [4]. Presence of different pharmacologically active compounds such as astragalin, bryophollone, bryophollenone, kaempferol, phenanthrene, quercetin, rutin, etc. make this plant suitable for different pharmacological research against known clinical symptoms [7-8].

In the present study hypoglycemic properties of *B. pinnatum* were studied. Extraction was conducted by using a green extraction method named "aqueous Ultrasound Assisted Extraction (UAE)" and the justification of the method was described and validated the process compared to the conventional method [9]. Application of the concept of ultrasound in the field of phytochemical extraction was introduced at the end of the last century and the feasibility studies were conducted on different plant's material in the present century. The above method is now recognized as a green extraction method as it does not require any complex instruments and hazardous organic solvents, and may be capable of reducing the overall extraction time, energy and cost [10].

II. Materials and Methods

Preparation of Crude Extracts

Fresh leaves from the upper part of *Bryophyllum pinnatum* were collected from the Botanical Pesticide Garden of the Institute of Environmental Science (IES) of Rajshahi University (RU), Bangladesh. The harvested leaves were allowed sufficient time to dry the gum. Leaves were washed properly by the running tap water followed by distilled water and the clean leaves were placed under air for drying the surface water and equally divided into three parts. Crude extracts were prepared through (A) ultrasound treatment method, (B) decoction method and (C) ethanol cold extraction method.

Processing of Part-A leaves: Within 6 hours 100 gm of leaves were taken in a conventional juice blender machine by addition distilled water q.s. to 500 ml [11]. The whole juice was passed through a 20 mesh size net for getting fine particles and transferred to a 500 ml conical flask and placed in an ultrasonic bath (Model: Power sonic 405: Microprocessor controlled Bench Top Ultrasonic Cleaner). Ultrasound treatments were done at 40^0C bath temperature and the mixture was then filtered by three layers of cloth and dried at 55^0C temperature in a water bath.

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Processing of Part-B leaves: Juice of 100 gm leaves in 500 ml distilled water was prepared and transferred to a 1000ml beaker and boiled 10 minutes before filtration by three layers of cloth and dried at 55^0C temperature in a water bath.

Processing of Part-C leaves: 100 gm leaves were allowed for shade drying and prepared fine powder by blender. The powder leaves were mixed in ethanol at the ratio 1: 5 for 72 hours. The mixture was then filtered by three layers of cloth and dried at 55^0C temperature in a rotary evaporator.

All the above dried crude extracts were collected in the glass vial and preserved in the refrigerator before conducting hypoglycemic study on animal models.

Enrolment of animal in the Study:

Two weeks old *Swiss albino* mice were collected from the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR'B) and they were kept in a plastic cages under laboratory condition of pleasant temperature and humidity with 12 hours light/dark cycle. They were supplied standard diet and allowed free access to the water [12]. After six weeks nursing and adjustment with the condition, healthy mice (average weight 25±3 gm) for control group were separated including four mice of either sex (Table-1) and maintain the ideal condition. Rest mice were administered intraperitoneal injections of dexamethasone (to induce glucocorticoid hormone dependent diabetes) at 10 mg/kg/day basis for around 7 days [13]. Diabetic conditions and intensity were measured by Oral Glucose Tolerance Test (OGTT) as per WHO guideline [14-15]. A 2 hours plasma glucose value during an OGTT of ≥11.1 mmol/l (200 mg/dl) considered as diabetic condition [14-15] (ADA 2014; TUEPG 2015). Diabetic mice were segregated into 5 groups (Table-1) including four mice of either sex in each on the basis of their average weight (25±3 gm) and average OGTT level (250±50 mg/dl). All groups were treated as per Table-1 and allowed the ideal condition, food & water similar to the healthy control. Fasting Plasma Glucose (FPG) test was performed periodically for detecting the hypoglycemic nature of the crude extract on animal model. Fasting is defined as no calorie intake for the last eight hours and fasting plasma glucose (FPG) levels ≥7 mmol/l (126 mg/dl) is considered as diabetic condition [14-15]. During the study maximum 15 hours fasting were allowed. The test drugs were administered orally by gavaging method after 8 hours fasting of taking morning feed. Blood samples were drawn 2-hours after administration of drug by punching the tail-tip of the mice. Determination of the blood glucose level was done by the glucose-oxidase principle using the ONE TOUCH basic instrument and results were reported as mmol/L (which was manually converted to mg/dl). One sample t-test with specific cutoff point was used to test the group variance and Paired t-tests among the groups were conducted to test the variation of the group. All statistical tests were conducted by the SPSS software to compare the variation at 5% level of significance.

Table 1: Enrolment of animal and study design

Treatment group	Treatment Dose (Once daily)	Enrolment of mice (M+F)	Study of diabetic profile
Group I: Healthy control	Distill water	2 + 2	1. OGTT (for enrollment of diabetic mice in the study) 2. FPG at '0' hour (before treatment) 3. FPG at 1st day 4. FPG at 7th day 5. FPG at 14th day
Group II: Diabetic control	Distill water	2 + 2	
Group III: Glibenclamide	600 µg/kg-bw	2 + 2	
Group IV: Aqueous extract of *B. pinnatum leaves by ultrasound*	600 mg/kg-bw	2 + 2	
Group V: Aqueous extract of *B. pinnatum leaves by decoction*	600 mg/kg-bw	2 + 2	
Group VI: Ethanolic extract of *B. pinnatum leaves*	600 mg/kg-bw	2 + 2	

III. Results and Discussion

The leaves of *Bryophyllum pinnatum* are occasionally used as diabetic remedy in different tribes in Bangladesh. In the present study the hypoglycemic properties of *B. pinnatum* leaves were examined on diabetic animals. The prime objective of this study was to assess the hypoglycemic properties of aqueous ultrasound assisted extract (Aq. UAE) of *B. pinnatum* leaves and subsequently compare the efficacy to the crude extract obtained from conventional extraction methods. Hypoglycemic properties of crude extract *B. pinnatum* leaves prepared by Aq. UAE, decoction method and ethanol cold extract method, were examined on glucocorticoid hormone induced diabetic animals by considering fasting plasma glucose (FPG) test procedure. Initially the OGTT test was performed to confirm the diabetes of the dexamethasone induced mice and classified them in the treatment groups on the basis of their average weight and average plasma glucose level. The results presented in the Table-2 indicated that all the treatment groups (G2, G3, G4, G5 & G6) were suffering almost similar diabetic condition ($P>0.05$ indicated by 'n') (Chart 1) and had plasma glucose level significantly higher ($p<0.05$) than the healthy control mice group. Similar conditions are also observed from the FPG data obtained immediately before administration of the first dose of treatment. After administration of the first dose of glibenclamide (standard anti-diabetic drug), a drastic change was observed. Whereas, very insignificant change compared to the standard drug was observed after the first dose of *B. pinnatum* leaves extract obtained by three different extraction methods. However, after regular administration of the Aq.UAE and ethanol crude extract of *B. pinnatum* leaves showed significant reduction of plasma glucose level at 7th and 14th days which was almost similar to the standard antidiabetic drug glibenclamide. On the 14th day, it was observed that the intensity of the diabetic symptom was lowered in all the groups, which indicates the diminish effects of the dexamethasone on the animal. Previous studies also support the antidiabetic properties of aqueous extract of study *B. pinnatum* on

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streptozotocin induced diabetes rat model [16]. A significant (Table 2) change in the body weight was also observed during the study among all the treatment groups except the diabetic control. Though the difference of weight gain during treatment was not significant compared to the diabetic control, a clear change is observed in the Chart 2. The result indicates that the extract of *B. pinnatum* may have some weight gaining properties which are normally lost in diabetic patients.

Table 2: Effect of drug or extracts on artificial diabetic mice

Experiment Group	Plasma Glucose Level (mmol/L) (Mean ± STD for n = 4)				
	OGTT at "2" Hour	FPG at "0" Hour	FPG at "1st" day	FPG at "7th" day	FPG at "14th" day
G1 (HC)	172.4±24.1	88.65±18.08	92.3±21.5	94.1±21.3	82.8±10.6
G2 (DC)	271.3±37.5[a]	171.5±30.4[a]	169.7±25.6[a]	162.5±17.9[a]	127.8±17.9[a]
G3	279±35.2[a,n]	174.2±32.6[a,n]	113.4±32.7[b,m]	105.8±16.5[b,m]	85.5±15.5[b,m]
G4	272.8±32.1[a,n,y]	172.8±30.3[a,n,y]	148.5±26.7[b,n,y]	126.9±27.8[b,n,y]	90.5±24.9[b,m,y]
G5	269.4±37.6[a,n,y]	170.1±28.3[a,n,y]	160.65±25.3[a,n,x]	145.8±21.9[b,n,y]	111.6±23.2[b,n,x]
G6	277.8±23.4[a,n,y]	171.7±34.9[a,n,y]	144.5±25.1[a,n,y]	125.1±23.5[b,n,y]	92.7±32.9[b,n,y]

Here, OGTT at "2" Hour ≥200 mg/dl indicate diabetic condition [14-15]
FPG level ≥ 126 mg/dl indicate diabetic situation [14]
[a] The difference compared to the healthy control (G1) was significant ($p<0.05$)
[b] The difference compared to the healthy control (G1) was not significant ($p>0.05$)
[m] The difference compared to the diabetic control (G2) was significant ($p<0.05$)
[n] The difference compared to the diabetic control (G2) was not significant ($p>0.05$)
[x] The difference compared to the standard drug (G3) was significant ($p<0.05$)
[y] The difference compared to the standard drug (G3) was not significant ($p>0.05$)

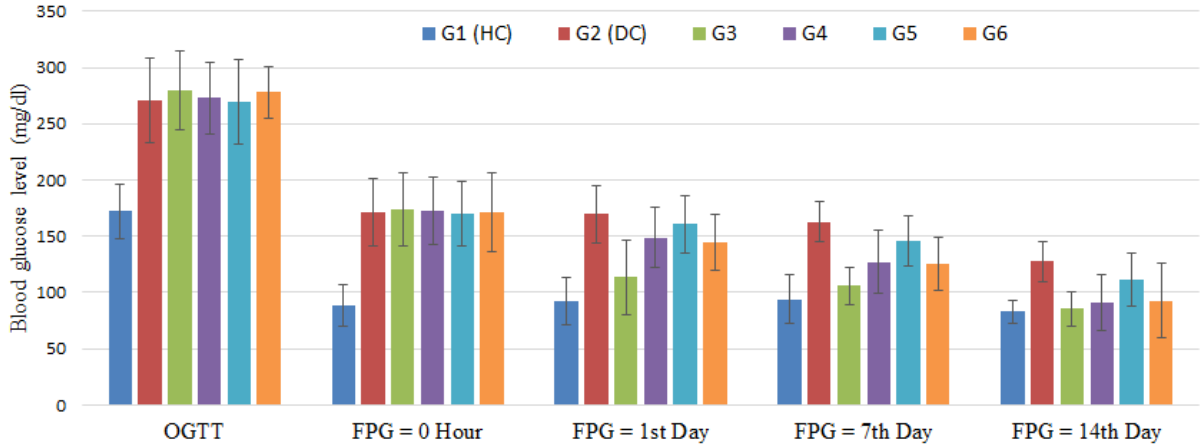

Chart 1: Effects of crude extracts of *B. pinnatum* on glucocorticoid induced diabetic mice

Here, OGTT was performed to conform to the diabetic condition of the mice before enrolment in the study.
FPG at 0 level was performed to measure the intensity of diabetes before treatment.
FPG at Day 1, 7 & 14 were performed to measure the intensity of diabetes after treatment by single dose and multiple dose.

Table 3: Effect of Crude Extracts on body weight of experimented Mice after 14 days treatment

Treatment group	Body Weight Mean ± STD, for n = 4 (M+F)	
	Weight Before Treatment	Weight After Treatment
G1 (HC)	24.75±3.30	27.5±3.42[b]
G2 (DC)	24±2.94[a]	25.5±2.89
G3	24.25±2.75[a]	28.75±2.50[b,c]
G4	23.75±2.87[a]	26.5±2.65[b,c]
G5	24±2.58[a]	25.75±2.87[b,c]
G6	23.75±3.30[a]	26.75±2.5[b,c]

[a]The difference of average WBT is not significant ($p>0.05$) compared to the expected body weight (25 gm) analyzed by one sample t test. indicate that the weight variation among the test groups was minimum.
[b]The difference of WAT is significant ($p<0.05$) compared to the WBT analyzed by paired t test
[c] The difference of WAT is not significant ($p>0.05$) compared to the diabetic control analyzed by paired t test

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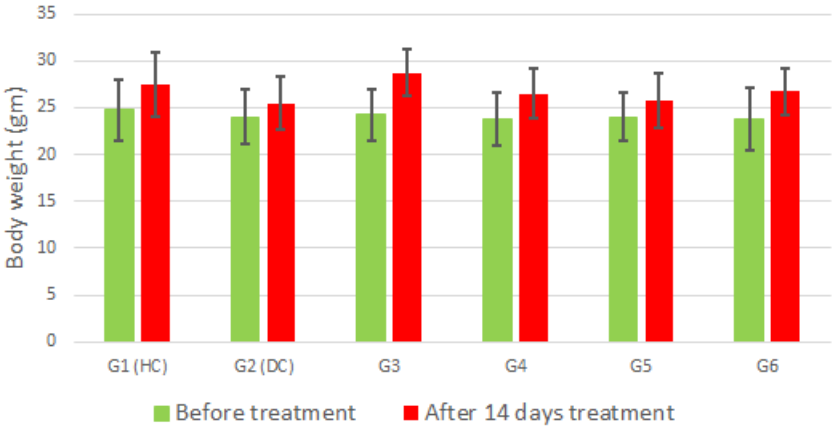

Chart 2: Comparison of body weight after 14 days treatment

IV. Conclusion

Crude extracts obtained by using three methods from *B. pinnatum* were observed as potential hypoglycemic on diabetic animal models. The extract also showed the prevention of weight loosing effects on diabetic animals. The intended green extraction method implemented here by using the ultrasound treatment on the plant materials in aqueous medium was proved almost similar to the ethanolic cold extraction method and much better than the decoction method. The above results showed that ultrasound treatment did not make any potential changes on the extracted compounds to provide hypoglycemic properties on animal models. So, as a green extraction method the ultrasound assisted extraction may be recommended for larger scale production in the industry. From the above study, it was observed that the leaves of *B. pinnatum* may be a promising source of natural drugs for the treatment of diabetes. In that case, people may be encouraged to drink *B. pinnatum* dried leaves powder, as like green tea.

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