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Comparative Phytochemical and Antimicrobial Study of Ultrasound-Assisted Aqueous Extract from Fresh Plants of Bryophyllum pinnatum with Conventional Extraction Method

A F M Nazmus Sadat[1], Nusrat Zahan[2], Debobrata Sharma[3], Khairunnahar Smriti[4], Fatema Tuz Zohora[5], Sanjida Halim[6], Mohammad Ali[7], Imranul Kabir[8], Sayeda Khadija Tuj Johora[9], Afroza Sultana[10]

[1,2,3,4,5,6,7] Department of Pharmacy, University of Development Alternative (UODA), Dhaka, Bangladesh.
[8,9] Department of Pharmacy, University of Asia Pacific (UAP), Dhaka, Bangladesh.
[10] Department of Nutrition and Food Technology, Jashore University of Science and Technology (JUST), Jashore, Bangladesh.

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Comparative Phytochemical and Antimicrobial Study of Ultrasound-Assisted Aqueous Extract from Fresh Plants of Bryophyllum pinnatum with Conventional Extraction Method

***Abstract*— *Bryophyllum pinnatum* is a potential medicinal plant widely used in different tribes in Bangladesh. The leaves of *B. pinnatum* were used in the present study for the compatibility assessment of a proposed ultrasound-assisted green extraction method with the conventional extraction method. An aqueous Ultrasound-Assisted Extraction (UAE) by using both fresh and dried leaves of *B. pinnatum* was proposed in the present study and successively compared with the traditional methanolic and aqueous (decoction) extraction method. The promising extraction yield was found through the proposed extraction method which was much better than decoction and almost similar to the methanolic extraction method. Phytochemical contents were also observed similar to the methanolic extract. Both methanolic and UAE extracts obtained from *B. pinnatum* leaves showed favorable antimicrobial sensitivity against both grams positive (*Staphylococcus aureus* and *Streptococcus pyogenes*) and gram-negative (Escherichia coli and Salmonella typhi) bacteria. Extracts obtained from the decoction method showed slightly poor antimicrobial activities. The difference in efficiency and efficacy of aqueous UAE extract obtained from fresh and dried leaves was observed insignificant. This indicates there is no necessity to impart the drying stage before extraction normally practiced in the conventional extraction method. Drying of plant material is a common stage of the conventional extraction cycle which ultimately lengthy the process and impose cost due to energy consumption and labor. Ultrasound-assisted extraction may successfully decrease the overall extraction cycle and ultimately reduce the overall cost of extraction. This method also creates opportunities to replace the water instead of with hazardous organic solvent for phytochemical extraction.**

Keywords- Bryophyllum pinnatum, Escherichia Coli, Green Extraction, Pathorkuchi, Salmonella Typhi, Staphylococcus aureus, Streptococcus pyogenes, Ultrasound.

I. INTRODUCTION

Bryophyllum pinnatum (family Crassulaceae) [1,2], is locally known as Pathorkuchi in Bangladesh [2,3]. Several ethnobotanical studies in Bangladesh explored the wide medicinal uses of this plant for different infectious and non-infectious diseases in several tribes. The plant "Figure. 1" is popularly used for the treatment of pneumonia and cough by the Chakma community living in the Rangamati District [1]. The plant is also used for the treatment of blood dysentery by the folk practitioners of Bogra District [4] and Santal Tribe of Chapai Nawabganj District [3]. Popular uses are also observed in Khumi, Marma, and Tripura tribes living in Thanchi Upazila, Bandarban for the treatment of burns, cough, and headaches [5]. Similar applications are also observed in northeast India where the Chakma community of Tripura uses the plant for managing vomiting and food poisoning [6]. Application of this plant is also observed in different health conditions including abscesses, asthma, colds, hypertension, insect stings, skin disorders, etc. [2]. The above studies indicate the anti-microbial, analgesic, anti-inflammatory, hypotensive, and anti-spasmolytic properties of the *B. pinnatum*.

Botanical nomenclature of Pathorkuchi [2]

Kingdom	: Plantae
Division	: Spermatophyta
Class	: Magnoliopsida
Subclass	: Rosidae
Order	: Rosales
Family	: Crassulaceae
Genus	: Bryophyllum
Species	: *Bryophyllum pinnatum* (lam) Oken

Figure 1. ***Bryophyllum pinnatum Plant***

In the current study, fresh and dried leaves "Figure. 1" of *B. pinnatum* were used for aqueous Ultrasound-Assisted Extraction (UAE) which occupy maximum points of the green extraction principle stated by Chemat et al., [7]. UAE is a potential phytochemical extraction technology operated by simple instruments and methods and the overall process is a relatively low-cost method [8]. In this extraction method, solid and liquid particles are vibrated and accelerated by ultrasonic action which increases the diffusion of solute to the surrounding solvent [9]. This method successfully replaces water as the solvent for the extraction instead of hazardous organic solvent which is much better than the similar decoction method. Extraction from fresh material may create an opportunity to reduce the overall extraction time and cost and probably improve the quality of the intended extract. Drying of

plant material is a common stage of the conventional extraction cycle which ultimately lengthy the process and impose cost due to energy consumption and labor. The above modification successfully complies with the principle of green extraction [7]. The major goal of the study was to compare the aqueous UAE method to the conventional extraction method by using leaves of *B. pinnatum*.

II. MATERIALS AND METHODS

A. Collection and extraction procedures of plant material

The whole plant of *B. pinnatum* was collected in the early morning from the previously labeled sources of Rajshahi University (RU), Bangladesh. A voucher specimen was prepared and deposited in the herbarium of the Institute of Environmental Science (IES). The collected plant was successively washed with running tap water and distilled water. The leaves were separated and equally divided into five parts for conducting five different types of extraction "Table. I". The performance of aqueous ultrasound-assisted extraction was evaluated with the aqueous decoction method and methanolic cold extraction. The UAE and decoction method was applied immediately on parts A & B, whereas "Part-C, D, and E" of fresh leaves were first dried and powder forms were used for UAE, decoction, and methanol cold extraction. Dissolved phytochemicals were separated from the debris by using five layers of polyester clothes and dried at 60oC in a conventional water bath. The dried extracts were then preserved in an airtight bottle with identical labels and preserved in a cold chamber for further use. The efficiency of the extraction process was measured by comparing yield "(1)" [10] and the presence of common phytochemicals in the extract "Table. II". The efficacy of the crude extracts was compared by using antimicrobial sensitivity study. All processes were repeated three times for calculating the level of significance. SPSS 16.0 was used for all types of statistical calculations.

B. Antimicrobial Study

The pharmacological efficacy of the crude extracts was assessed by the disc diffusion method [33-36]. Pathogenic bacteria such as *Staphylococcus aureus* (Gram +ve), *Streptococcus pyogenes* (Gram +ve), Escherichia coli (Gram -ve), and Salmonella typhi (Gram -ve) were enrolled in the present study. The source of all microorganisms was from the Microbiology Laboratory of the Department of Biochemistry and Molecular Biology of Rajshahi University, Bangladesh. 500µg/disc of extracts was prepared by using filter paper and placed on the surface of the inoculated nutrient agar media. After 30 minutes of pre-diffusion, the petri dish was incubated at 370C for 24 hours. The diameter of visible zones of inhibition to the nearest millimeter was measured and the sensitivity pattern was calculated. The above procedure was repeated three times for obtaining the average result.

$$\%\ Yield = \frac{(W2\ x\ 100)}{W1} \qquad (1)$$

Here, W1 = weight of the plant materials for extraction

W2= weight of the crude extract after drying

TABLE I. EXTRACTION PROCEDURE OF *BRYOPHYLLUM PINNATUM* LEAVES

Method of extraction	Type of leaves	Extraction Method	Method
A	Fresh*	Ultrasound	Juice of fresh leaves was placed in the ultrasonic bath (model: Power-Sonic 405) and allowed 30 minutes of ultrasonic treatments at 40^{oC} bath temperature before the separation of the extract [11-13].
B	Fresh*	Decoction	The juice of fresh leaves was boiled for 5 minutes before the separation of the extract [14-15].
C	Dried**	Ultrasound	Fine powder of dried leaves was mixed with distilled water in a ratio of 1:5 and placed in an ultrasonic bath for 30 minutes at 40^{oC} bath temperature before the separation of the extract [11-13].
D	Dried**	Decoction	Fine powder of dried leaves was mixed with distilled water in a 1:5 ratio and boiled for 5 minutes before extraction [14-15].
E	Dried**	Methanol cold extraction	Fine powder of dried leaves was mixed with five parts of methanol and permitted for cold extraction for up to 72 hours with intermittent shaking [16-19].

* Juice of fresh plants (100 gm) was prepared by a blender diluted to 500ml by distilled water.
** Powder was prepared after a week-long drying of the leaves.

TABLE II. QUALITATIVE TEST FOR SOME IMPORTANT PHYTOCHEMICALS

Phytochemicals	Confirmation test	Pharmacological importance
Alkaloids	Alkaloids were identified by Dragendoff's test (Orange-red precipitate was obtained by heating the mother solution with 2% of H_2SO_4 and adding a few drops of Dragendoff's reagent) [20-21] or Mayer's Test (turbidity or yellow precipitate was observed by heating the mother solution with HCl (2%) and Mayer's reagent) [20, 22]	Alkaloids are potentially active biological compounds including anesthetics, CNS stimulants, narcotics, and poisons [23,24]
Anthraquinones	The presence of anthraquinones was conformed after changing the color (generally pinkish) of the mother solution treated with benzene or chloroform and 10% (v/v) ammonia solution [21, 25]	Anthraquinones have potential anticancer, anti-inflammatory, diuretic, antiarthritic, antifungal, antibacterial, and antimalarial activities [26].
Flavonoids	Indication of flavonoids was confirmed by the formation of yellow color (which may disappear on standing) after adding dilute ammonia solution and Conc. H_2SO_4 in mother solution [25]. The presence of flavonoids was also confirmed by the formation of yellow color after adding a few drops of 1% aluminum solution to the mother solution [25].	Its antioxidant property provides protection against diseases like cancer, aging, atherosclerosis, and inflammation [23, 27]
Glycosides	Brown or blue color is formed in the interface of the mother	Glycosides have including antioxidant,

	solution after mixing with glacial acetic acid, ferric chloride solution (5%), and Conc. H_2SO_4 [22, 25]	anti-inflammatory, antihypertensive, and antidiabetic activities [28]
Saponins	The formation of stable foam after vigorous shaking of the mother solution indicates the presence of saponins [22]	Saponins are prominent to provide anticancer, antioxidant, hyperglycemia, hypolipidemic, hypercholesterolemia, anti-inflammatory, weight loss,s, etc. effects [27, 29-30]
Steroids / Terpenoids	Conc. H_2SO_4 creates a reddish-brown color in the interface of the mother solution and chloroform (This is known as the Salkowski test) [25]	Steroids possess cardiotonic. insecticidal and antimicrobial properties [23, 27]
Tannins	Tannin was indicated by the formation of dark green color after adding 1% $FeCl_3$ solution to the mother solution [31-32]	These are used for the treatment of diseases like leucorrhoea, rhinorrhoea, and diarrhea [27, 31]

III. RESULTS AND DISCUSSION

The aqueous Ultrasound-Assisted Extraction (UAE) method is a comparatively notable and proven green extraction method probably the first time applied to *B. pinnatum* for extraction. More than 20% extraction yield was observed from both fresh and dried leaves through aqueous UAE, which was significantly higher than the conventional methanol (14%) "Table. III". Poor extraction was observed in the decoction method applied on fresh leaves (% yield, 8.66±1.46) and dried leaves ((% yield, 7.24±1.31), the result was similar to the previous study of aqueous cold maceration (% yield = 7%) of leaves of *B. pinnatum* [37]. Similar phytochemical contents were observed in both aqueous UAE extracts from fresh and dried leaves of *B. pinnatum* with the methanol extract, which was much better than the decoction method "Table. IV". The Above results indicated that ordinary water may be a good solvent if ultrasound treatment is applied to the plant material during extraction. Moreover, fresh and dried leaves did not show any significant difference in the aqueous UAE method. Extracting from fresh leaves or other parts may successfully reduce the time and energy-consuming drying stage before conventional extraction. The crude extracts obtained from UAE and methanol eventually showed favorable antibacterial activities on *S. aureus*, *S. pyogenes*, *E. coli*, and *S. typhi* "Figure.2". Aqueous UAE both from fresh and dried leaves showed almost similar antimicrobial sensitivity compared to the methanol extract "Table. V", whereas the simple decoction method showed negligible activities. Previously it was observed that the hot water extracts had no antimicrobial activity against *S. aureus* and *E. coli* [38]. However, another study showed promising antimicrobial activities of aqueous extract on *S. aureus*, B. subtilis, *E. coli*, P. aeruginosa, S. Flexner, etc., and suggests the traditional uses for the treatment of wound infection, sore, ear infection, abscesses and dysentery [39]. Similar antibiotic activities were also observed in the present study and conform to the previous pharmacological activities of the *B. pinnatum* leaves. The study also showed that ultrasound-treated aqueous leaves extract showed almost similar pharmacological activities to the conventional, time-consuming, hazardous, and expensive methanol extract. Indeed, the intended ultrasound-assisted extraction method was proved inexpensive, rapid, safe, and easily operated technique with simple equipment and an aqueous solvent, which may be a good alternative to conventional extraction methods for large-scale phytochemical extraction in industrial purposes.

TABLE III. VARIATION OF YIELD OBSERVED IN DIFFERENT EXTRACTION METHODS

Method of Extraction	Starting materials (gm)	Weight (gm) after drying (Mean±S.D)	% Yield
A*	100	-	21.13±1.70
B*	100	-	$8.66±1.46^{x}$
C*	100	33.67±2.08	$20.58±2.64^{x}$
D*	100	$34.33±2.52^{a}$	$7.24±1.31^{x,y}$
E*	100	$34.33±2.08^{a}$	$14.72±1.93^{x,y}$

* Method of extraction as per Table-1. Mean calculated by considering successive 3 studies.
After drying, the weight variation of leaves was insignificant ($p \geq 0.05$) compared to "Method -C".
[x] Yield variation was significantly ($p \leq 0.05$) different compared to "Method -A".
[y] Yield variation was significantly ($p \leq 0.05$) different compared to "Method -C".

TABLE IV. PHYTOCHEMICAL SCREENING STUDY OF CRUDE EXTRACTS

Phytochemical Tests	Crude extract				
	A*	B*	C*	D*	E*
1. Alkaloid	+	+	+	+	+
2. Anthraquinones	-	-	-	-	-
3. Flavonoid	+	-	+	-	+
4. Glycoside	+	+	+	+	+
5. Saponin	+	+	+	+	+
6. Steroid/ Terpenoid	+	-	+	-	+
7. Tannin	+	-	+	-	+

Here, (+) presence, (-) absence, and (*) Method of extraction as per Table-1.

TABLE V. ANTIMICROBIAL SENSITIVITY STUDY OF CRUDE EXTRACTS OF *B. PINNATUM* LEAVES

Method of extraction*	Zone of inhibition in mm (Mean ±SD)			
	S. aureus	*S. pyogenes*	*E. coli*	*S. Typhi*
A	19.33±3.57	20.22±3.70	18.00±3.16	19.44±3.47
B	$11.89±2.37^{a}$	$13.22±2.44^{a}$	$10.78±1.86^{a}$	$11.33±2.00^{a}$
C	20.44±3.17	21.67±3.24	19.89±3.13	18.67±3.32
D	$10.89±2.09^{a,b}$	$11.89±2.47^{a,b}$	$11.89±2.26^{a,b}$	$13.56±1.88^{a,b}$
E	20.56±3.36	20.33±4.360	20.00±3.46	21.44±3.84

** Method of extraction as per Table-1. Mean calculated by considering successive 3 studies.*
[a] Difference in antimicrobial activities compared to "Method -A" was significant ($p \leq 0.05$)
[b] Difference of antimicrobial activities compared to "Method -C" was significant ($p \leq 0.05$)

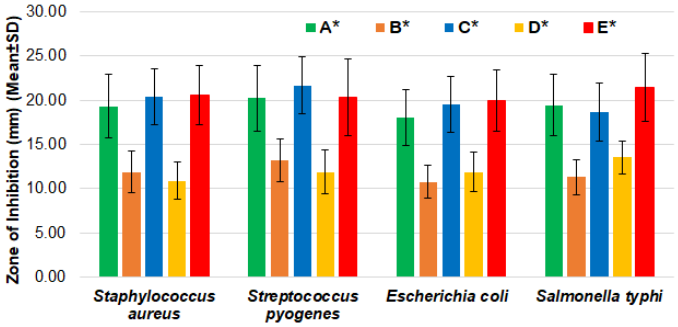

Figure 2. ***Graphical Representation of Antimicrobial Sensitivity Study of B. pinnatum Leaves***

IV. CONCLUSION

Bryophyllum pinnatum is a phytochemically rich plant and has ethnobotanical evidence of traditional uses for different medications. Sometimes extraction difficulties create obstacles to using phytochemicals for the medication. Aqueous UAE from leaves of *B. pinnatum* may be a new addition in the field of phytochemical extraction without using hazardous organic solvents and avoiding the time-consuming drying stage. Similar antimicrobial efficacy indicates the absence of potential loss of active compounds in the crude extract during ultrasound treatment. The antimicrobial efficacy against gram (+ve) and gram (-ve) bacteria resemble the broad-spectrum properties of the crude extracts of the *B. pinnatum* leaves.

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AUTHORS PROFILE

Dr. A F M Nazmus Sadat is an Associate Professor in UODA, and Consultant of ISO, GMP, EHS, 5S, TQM, Kaizen, Bioequivalence Study, etc. (QHSE Solution BD). He is MS in Pharmaceutical Technology and B.Pharm (Hons.). He has completed his Ph.D. in Environmental Science from IES (RU).

Ms. Most. Nusrat Zahan is Medical Affairs Servier for Bangladesh Operations. She has completed her B.Pharm (Hons.) and currently pursuing M.Pharm from UODA.

Mr. Debobrata Sharma has completed his B.Pharm (Hons.) and is currently pursuing M.Pharm from UODA.

Ms. Khairunnahar Smriti has completed her B.Pharm (Hons.) and is currently pursuing M.Pharm from UODA.

Ms. Fatema Tuz Zohora has completed her B.Pharm (Hons.) and is currently pursuing M.Pharm from UODA.

Ms. Sanjida Halim has completed her B.Pharm (Hons.) and is currently pursuing M.Pharm from UODA.

Mr. Mohammad Ali is QA Executive at Eskayef Pharmaceuticals Ltd. He has completed his B.Pharm (Hons.) and is currently pursuing M.Pharm from UODA.

Mr. Imranul Kabir is Senior Assistant Manager in QA at Eskayef Pharmaceuticals Ltd. He is currently pursuing B.Pharm (Hons.) from UAP.

Ms. Sayeda Khadija Tuj Johora is Senior Executive in R&D at Aristopharma Ltd. She is currently pursuing B.Pharm (Hons.) from UAP.

Mrs. Afroza Sultana is a Lecturer in the Department of Pharmacy at KYAU. She has completed her M.Pharm and B.Pharm (Hons.) and currently she is a Ph.D. Fellow in the Department of Nutrition and Food Technology at JUST.