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EVALUATION OF PHYTOCHEMICAL PROPERTIES AND ANTIOXIDANT ACTIVITIES OF DIFFERENT SOLVENT EXTRACTS OF *PASPALUM CONJUGATUM* LEAVES GROWING IN BANGLADESH

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ABSTRACT: Secondary metabolites found in various plants play an important role in curing various diseases and are used as important raw materials for the production of traditional and modern medicine. One of these plants, *Paspalum conjugatum*, a member of the Poaceae family, has been used for different purposes since prehistoric times. In this research work, we extracted the powdered leaves of *P. conjugatum* by the sequential extraction method using four different solvents (n-hexane, chloroform, ethanol, and water). About eight phytochemical analyses were carried out, out of which four phytochemicals (alkaloid, tannin, flavonoid, and glycoside) were significantly identified in both n-hexane and ethanol extracts. The proximate analysis was carried out for the leaves powder. The moisture content, total ash, acid-insoluble ash, and water-insoluble ash were found to be 3.56, 9.76, 3.22, and 27.08%, respectively. Reducing power assay, total antioxidant activity, total phenolic content, total flavonoids, and reduction of ferric ions were also investigated as a part of the antioxidant test. All extracts showed a significant presence of antioxidants. The ethanol extract showed good antioxidant activity with good phenolic and flavonoid contents compared to other solvents.

INTRODUCTION: A vital component of human life are plants. They are an important supply of clothing, food, shelter, medicine, and other necessities for human habitation. For thousands of years, people have used plants as medicine all across the world, but particularly in developing and disadvantaged nations.

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Bioactive substances with physiological effects on the human body, including as tannins, alkaloids, carbohydrates, triterpenoids, steroids, saponins, glycosides, and flavonoids, are found in medicinal plants [1].

We refer to these plant-based substances as phytochemicals. The phytochemical composition of a plant determines its therapeutic qualities. These phytochemicals enhance the colour, flavour, and perfume of the plant while shielding it from illness. Plants create metabolic compounds to guard against biotic and abiotic stressors, and these chemicals have been turned into medications that people can

use to treat a variety of illnesses [2]. These substances have minimal toxicity and good medicinal efficacy, which makes chemists interested in them. Plants contain a wide range of phytochemical substances that have been found to exhibit negligible toxicity to host cells and potentially distinct modes of action. These substances could be used to make new drugs that inhibit the growth of fungi and bacteria and neutralise reactive oxygen species (ROS). Since oxidative damage to lipids, proteins, and nucleic acids occurs when ROS are produced during cell metabolism, they are extremely hazardous and play a role in the emergence of numerous chronic diseases [3].

While our bodies have an internal antioxidant system, exogenous antioxidants are advised to get rid of extra free radicals. Antioxidants can come from natural or synthetic sources, although natural antioxidants are increasingly being used instead of synthetic ones because of their harmful and cancer-causing properties. The Poaceae family includes *Paspalum conjugatum Berg*., a widespread plant in Bangladesh sometimes referred to as hilo grass or carabao grass. Throughout Bangladesh, this plant is primarily found beside trails and streams, in open waste areas, and in populated regions, including towns. Certain investigations on one of the *Paspalum* genera have reported the presence of proteins, carbohydrates, tannins, phenols, and saponins [4].

According to research, *P. conjugatum* is used medicinally for a variety of conditions, including headache relief, wound healing, antifungal treatments [5]. In keeping with our ongoing investigation into the bioactive screening of medicinal plants in Bangladesh [6, 7, 8], the current study evaluated the phytochemical profile and antioxidant properties of n-hexane, chloroform, ethanol, and aqueous extracts of *P. conjugatum* leaves.

MATERIALS AND METHODS:

Sample Preparation: For this present investigation *P. conjugatum* leaves were collected from Noakhali Science and Technology University, Noakhali, Bangladesh and identified by an expert. They were sun-dried for three weeks and grounded into a coarse powder with the help of a suitable grinder. Fine powders were stored in an airtight container until commencement of the tests.

Proximate Analysis: The proximate analysis of powdered leaves was performed to determine the amount of moisture, ash, acid insoluble ash, and water-soluble ash content as per method describe in Islam *et al.* 2016 [6].

Preparation of Extracts: Using a range of solvents, including polar and non-polar ones, the Soxhlet extraction process aims to extract the active components from the sample. In this process, the solvent was used to extract the material soluble in a specific range of polarity, and the remaining material was removed using a different solvent.

In Soxhlet, 140 grams of the powdered material were gathered and put through extraction procedures. During the eight-hour extraction process, n-hexane, chloroform, ethanol, and distilled water were added in increasing order of polarity. It was then necessary to filter it with filter paper. The obtained extracts of various solvents were concentrated using a rotary evaporator, then dried at low temperature until completely dry, and then kept for additional research.

Preliminary Phytochemical Screening: The initial phytochemical screening of *P. conjugatum* leaves extracts was conducted by employing the following techniques to identify the presence of active chemical ingredients, including phenolic compounds, proteins, amino acids, saponins, tannins, alkaloids, and reducing sugars.

Alkaloids: Four extracts without solvent, each weighing 50 mg, were mixed with 6 mL of diluted hydrochloric acid and then filtered. The filtered liquids were examined cautiously using a little amount of potassium mercuric iodide (Mayer's reagent), potassium iodide (Wagner's reagent), picric acid (Hager's reagent), and potassium bismuth (Dragendroff's reagent). Cloudiness or formation of solid particles with either of these chemicals was seen as evidence for the presence of alkaloids.

Flavonoid: A solution containing 100 mg of extract was dissolved in 5 mL of water and then filtered. When treated with NaOH, a strong yellow color appeared, which became colorless when

dilute HCl was added (Alkaline test). Additionally, a yellow precipitate occurred when 10% lead acetate solution was added to the extract (Lead acetate test). Both investigations verified the existence of flavonoid.

Tannin: The 100 mg sample was dissolved in 5 mL of water and then filtered. The filtered liquid was tested with ferric chloride and potassium dichromate. The dark greenish substance in the first test and the yellow substance in the second test proved the presence of tannin.

Reducing Sugars: The samples (100 mg) were diluted separately in 5 mL of distilled water and then filtered. The filtered solutions were used to test for the presence of carbohydrates. The filtrate was treated with Benedict's reagent, and the appearance of an orange-red precipitate following heating on a water bath confirmed the presence of reducing sugars. The filtrate was treated with weak hydrochloric acid, then balanced with alkali and heated with Fehling's A and B solutions. A reddish or brick-colored solid was produced, indicating the existence of a type of sugar that can reduce other substances.

Saponins: The samples (containing alcohol and water) were mixed with 20 mL of distilled water individually and then agitated for 15 minutes in a measuring cylinder (Froth's test). A foam layer of approximately 1 cm was seen, indicating the presence of saponin.

Proteins and Amino Acids: A solution was made by dissolving 100 mg of extract in 5 mL of water and then filtering it. The filtered liquid was treated with 4-5 drops of strong nitric acid. The development of a yellow tint showed the existence of proteins and amino acids.

Glycosides: 50 mg of extract was broken down with strong hydrochloric acid for 2 hours in a water bath, filtered, and the resulting solution was analyzed using a legal test and the Liebermann Burchard test. In the first test, the color shifted from pink to red, while in the second test, the presence of steroidal or triterpenoid saponin glycosides was confirmed by the formation of brown or pink-colored rings at the junction, respectively.

Antioxidant Activity: The antioxidant properties of plant extracts can be assessed using many approved methods. The antioxidant activity of several extracts of *P. conjugatum* leaves was evaluated using measures such as power reduction, total antioxidant capacity, and reduction of ferric ion assay.

Reducing Power Assay: An essential measure of the ability to reduce phenolic compounds is the activity of donating electrons, often assessed through the reduction of Fe (III)[9]. When potassium ferricyanide (Fe^{3+}) is mixed with extracts that have reduction potential, it forms potassium ferrocyanide (Fe^{2+}). This compound subsequently reacts with ferric chloride to create a ferric ferrous complex that has its highest absorbance at 700 nm. To make the reaction solution, the extracts (0.25 mL) at different concentrations (31.25 to 500 µg/mL) were combined with 0.25 mL phosphate buffer (0.2 M, pH 6.6) and 0.25 mL of potassium ferricyanide solution (1%). Following that, the combination was kept at a temperature of 50 °C for a duration of 20 minutes. The reaction was halted by adding 0.25 mL of 10% trichloroacetic acid, and the tube was cooled for five minutes using running water. The combination that was obtained was spun for 10 minutes at 3000 revolutions per minute. After taking off a little amount of 1 mL from the upper layer of each solution, 0.25 mL of distilled water and 0.2 mL of a solution containing 0.1% ferric chloride were added. The optical density was recorded at a wavelength of 700 nm.

Total Antioxidant Capacity: The overall antioxidant capacity of various solvent extracts was measured using the phosphomolybdate technique, with ascorbic acid serving as the standard. A portion of 0.1 mL of the sample solution was combined with 1 mL of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate). The tubes were sealed and incubated in a water bath at 35 °C for 90 min. Once the samples had reached the temperature of the room, the absorbance of the mixture was measured at 695 nm compared to a blank. A standard blank held 1 mL of the reagent solution and the necessary amount of the solvent and was kept under identical conditions.

Reduction of Ferric Ions: A mixture was prepared by combining 1 mL of o-phenanthroline (5 mg in 10 mL of methanol), 2 mL of ferric chloride (0.2 mM), and 2 mL of different concentrations of the extracts. The mixture was then incubated at room temperature for 10 minutes, after which the absorbance was measured at 510 nm. Ascorbic acid and gallic acid were utilized as reference standards.

Total Phenolic Content: The content of phenolic compounds was measured using the modified Folin-Ciocalteu spectrophotometric method outlined in earlier publication [8]. The ethanolic extract was examined for its total phenolic content, as it contains alkaloids, tannins, flavonoids, and glycosides. Initially, the combination including ethanolic extract/standard, Folin-Ciocalteu reagent, and sodium carbonate was mixed vigorously for 15 seconds and left undisturbed for 30 minutes at 40 °C to develop color. After cooling to the temperature of the surrounding room, the absorbance was measured at a wavelength of 765 nm compared to a solution without any substance. A calibration curve was created using gallic acid. Using the recorded absorbance values, the phenolics concentration was determined (in µg/mL) using the calibration line. The phenolics content in the extracts was then represented as milligrams of gallic acid equivalent per gram of extract (mg of GAE/g of extract).

Total Flavonoids Content: The flavonoid levels of extracts were assessed using the Dowd technique[10]. 0.5 milliliters (1 gram extract in 10 milliliters ethanol) of extract solution in methanol was combined with 0.1 milliliters of 10% (weight/volume) $AlCl_3$ solution, 0.1 milliliters (1 molar) potassium acetate, and 2.8 milliliters distilled water. The combination was let to sit for 30 minutes at room temperature and tested for absorbance at 415 nm compared to the blank. A calibration curve for quercetin (31.25 to 250 µg/mL) was created to calculate the total phenolic content. The outcome was stated as quercetin equivalents (mg of QE/g of dry extract).

RESULTS AND DISCUSSIONS:

Proximate Analysis: The moisture, total ash, acid insoluble ash, and water-soluble ash content of the leaves of *P. conjugatum* were evaluated. The levels of moisture, total ash, acid-insoluble ash, and water-soluble ash were measured and determined to be 3.56%, 9.76%, 3.22%, and 27.08% correspondingly. The low moisture level of items created from dehydrated leaves helps enhance their durability, decrease the weight for shipping, and cut the cost of transportation. The elevated natural moisture level in leaves must be rapidly decreased to a residual moisture level that is considered acceptable, in order to prevent any enzymatic reaction and oxidation. Dried leaves are typically said to contain nutritional levels that are three to four times higher than those seen in fresh leaves [11]. The ash level of 9.75% suggests that the leaves has a relatively high amount of mineral components, such as sodium, potassium, magnesium, calcium, and inorganic radicals like phosphates, carbonates, and silicates. Measuring the amount of ash that dissolves in acid and water is significant because it provides information about the quality and purity of a raw medicinal substance [8].

Preliminary Phytochemical Screening: Phytochemicals are chemical substances that occur naturally in plants and have a biological effect on the human body. There are over a thousand recognized phytochemicals. The analysis of the ethanolic extract showed the existence of alkaloids, tannins, flavonoids, and glycosides **Table 1**. The n-hexane extract indicated the existence of alkaloids, flavonoids, tannins. The chloroform extract indicated the existence of alkaloids, glycosides, and flavonoids. The water extract indicated the existence of alkaloids and flavonoids. The existence of many chemical groups suggests that *P. conjugatum* has diverse positive impacts on health

TABLE 1: QUALITATIVE CHEMICAL ANALYSIS OF DIFFERENT SOLVENT EXTRACTS OF *P. CONJUGATUM* LEAVES

Phytochemicals	Name of the tests	Extracts			
		n-Hexane	Chloroform	Ethanol	Water
Alkaloid	Mayer's test	+	+	+	+
	Wagner's test	-	+	+	-
	Hager's test	-	+	+	-
	Dragendroff" test	+	-	+	-

Carbohydrates/reducing sugar	Fehling's test	-	-	-	-
	Benedict's test	-	-	-	-
Glycosides	Liebermann-Burchard test	-	+	+	-
	Legal test	-	+	+	-
Tannins	$FeCl_3$ test	-	-	+	-
	$K_2Cr_2O_7$ test	+	-	-	-
Protein and amino acid	Xanthoproteictets	-	-	-	-
Flavonoid	Lead acetate test	+	+	+	+
Saponin	Froth test	-	-	-	-

(+) = Present and (-) = Absent.

Antioxidant Activity Test:

Reducing Power Assay: Fig. 1 displays the dose response curves for the reducing powers of all extracts (31.25-500 µg/mL) from *P. conjugatum*. The test solution's yellow color shifts to different colors of green and blue, depending on the reducing power of antioxidant chemicals. The existence of radicals (namely, antioxidants) leads to the transformation of the Fe^{3+}/ ferricyanide complex utilized in this procedure into the ferrous form.BHT was utilized as a reference antioxidant for comparison. The power reduction of several extracts of *P. conjugatum* was seen to rise as the concentration increased. The highest absorbance values were recorded at a concentration of 500 µg/mL for n-hexane, chloroform, ethanol, and water, with values of 2.280, 3.068, 3.311, and 1.171, respectively. A higher absorption indicates a greater reducing power. The highest absorbance, 3.311, was observed for the ethanol extract of *P. conjugatum* at a concentration of 500 µg/mL. **Fig. 1** shows that the ethanol and chloroform extracts reduced the ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) more effectively (3.311 and 3.068) as compared to the n-hexane and water extracts (2.280 and 1.171) respectively at 500 µg/mL concentration. This result is in agreement with others [12]. In the present study, the ethanol and chloroform extracts of *P. conjugatum* leaves exhibited good reducing potential.

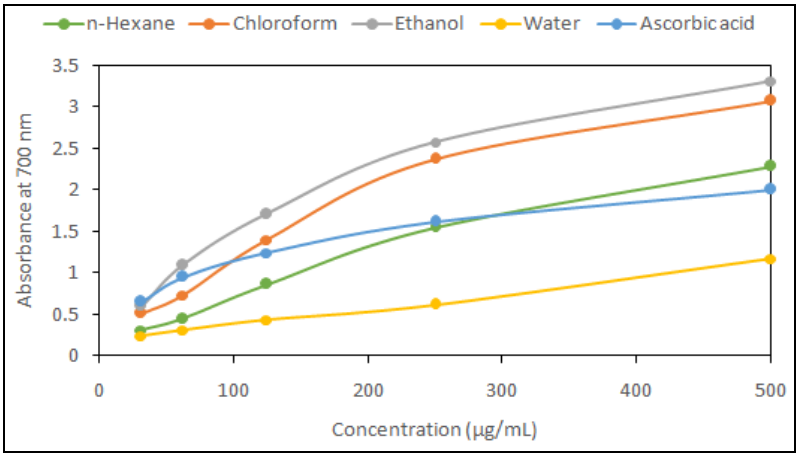

FIG. 1: REDUCING POWER OF DIFFERENT EXTRACTS OF *P. CONJUGATUM* WITH STANDARD ANTIOXIDANT

Total Antioxidant Capacity: The total antioxidant capacity of extracts from *P. conjugatum* leaves was shown to rise as the concentration of the extract increased **Fig. 2**. Total antioxidant capacity of different extracts was determined using the standard curve equation ($y = 0.0012x - 0.015$, $R^2 = 0.9988$) obtained from the standard ascorbic acid.

The n-hexane extract showed the highest amount of total antioxidant capacity 3033mg AAE/g of dry extract. The total antioxidant capacities for chloroform, ethanol, and water extracts are 1750, 1551, and 153mg AAE/g of dry extract respectively.

Reduction of Fferric Ions: Reduction of ferric ions by *o*-phenantholine colour method for different extract of *P. conjugatum* was found to increase with the increasing concentration.

All four samples revealed the existence of an antioxidant. The highest absorbance of 2.914 was observed for the n-hexane extract at a concentration

of 500 µg/mL. Phenolic compounds with ortho-substituents were discovered to be more active compared to phenol without any substitutions. Therefore, these chemicals might have pro-oxidant effects by reacting with iron [13]. Iron (II) quickly interacts with 1,10-o-phenanthroline to produce a red colored complex that is highly stable. This compound exhibits significant absorption in the visible range at a wavelength of 510 nm. The produced chemicals react with Fe^{3+} to decrease and transform it into Fe^{2+}. The level of color represents the ability of the compounds to undergo reduction. The alterations in the absorbance generated at 510 nm have been employed as an indicator of the effectiveness of ferric ions in reduction. Gallic acid has been utilized as the reference medication in this procedure. **Fig. 3** displays the comparative examination of various extracts from *P. conjugatum* leaves. The extracts obtained from n-hexane, chloroform, and ethanol exhibited significant antioxidant activity. Surprisingly, the values (2.914, 2.334, and 2.197) are considerably more than the conventional antioxidants (0.324) that were evaluated at a concentration of 500 µg/mL. In addition, the water extracts (0.857) also showed quite impressive antioxidant activity at a concentration of 500 µg/Ml.

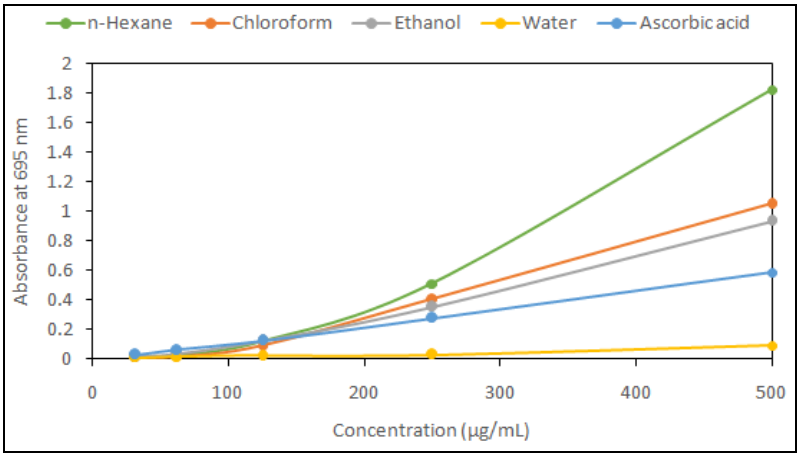

FIG. 2: TOTAL ANTIOXIDANT CAPACITY OF DIFFERENT SOLVENT EXTRACT OF *P. CONJUGATOM* LEAVES AND ASCORBIC ACID.

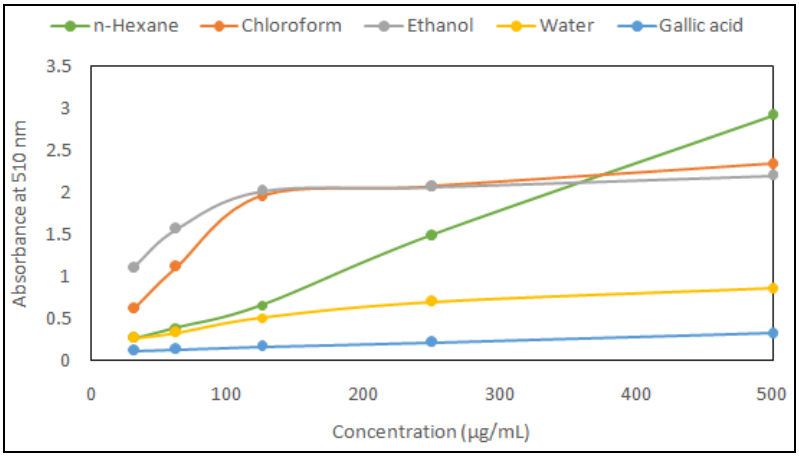

FIG. 3: COMPARATIVE ANALYSIS OF FERRIC IONS REDUCTION OF DIFFERENT EXTRACT WITH STANDARD GALLIC ACID

Total Phenolic Content: Phenols are highly significant plant components because of their potential to scavenge due to their hydroxyl groups. Based on the latest data, a strong correlation was discovered between total phenols and antioxidant activity in several plant species [8]. During the initial analysis of plant chemicals, the ethanol extracts indicated the existence of a majority of the plant chemicals (alkaloids, tannins, flavonoids, glycosides). That is why this extract was analyzed for the total phenolic content. The total phenolic content of the ethanol extract of *P. conjugatum*

leaves was determined using the standard curve equation ($y = 0.005x + 0.164$, $R^2 = 0.9935$) obtained from the standard gallic acid. In this equation, y represents the absorbance at 765 nm and x represents the total phenolic content in µg/mL of the extract. The concentration of total phenolics in the extract was found to be 466 µg/mL for a 500 µg/mL solution, which corresponds to 932 mg GAE/g of dry extract. The remaining three extracts (n-hexane, chloroform, and water) did not indicate the presence of phenol in the initial phytochemical analysis. Nevertheless, it is understood that antioxidants that are not phenolic could also have a role in the antioxidant activity of an extract.

Total Flavonoid Content: The total flavonoid concentrations of *P. conjugatum* leaves were determined using the colorimetric method of aluminium chloride. The extracts tested included n-hexane, chloroform, ethanol, and aqueous extracts. The total flavonoid content of the extracts was calculated using the standard curve ($y = 0.0127x + 0.1011$, $R^2 = 0.9954$) obtained from the standard quercetin, where y represents the absorbance at 415 nm and x represents the total flavonoid content in micrograms per milliliter of the extract. The ethanol extracts contained the highest quantity of flavonoid concentration (176.4 mg QE/g of dry extract). The n-Hexane and chloroform extracts have a substantial quantity (57.3 and 63.8 mg QE/g of dry extract) of flavonoids, but the water extract contains a smaller amount (25.0 mg QE/g of dry extract) compared to the ethanol extract.

CONCLUSION: Through a relatively straightforward and affordable procedure, the study effort revealed the existence of several bioactive phytochemical components that demonstrated the therapeutic significance of *P. conjugatum* leaves. n-Hexane, chloroform, ethanol, and water extract were used to analyze the phytochemical composition of *P. conjugatum*. The results indicated the presence of numerous significant phytochemicals, including alkaloids, carbohydrates, saponins, tannins, flavonoids, proteins, and glycosides. The antioxidant activity of the extracts was assessed since any product's antioxidant qualities may be linked to the prevention of a wide range of illnesses, including diabetes, dysentery, senility, ulcers, scabies, bronchitis, and cancer. The results of the reduction of ferric ion, total antioxidant capacity, total phenolic, and total flavonoid were shown to be well supported by the extracts of *P. conjugatum.* Therefore, it can be said that *P. conjugatum* leaves cultivated in Bangladesh may be a rich source of antioxidants. The plant has the potential to yield chemically and physiologically fascinating therapeutic candidates, and it may be further investigated against a variety of ailments to determine its undiscovered effectiveness. To get a deeper understanding of antioxidants' capacity to manage illnesses that significantly affect human health, more research is required to examine their mode of action.

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