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Short Communication

ANTI-OXIDANT, ANTI-MICROBIAL AND GLUCOSE DIFFUSION INHIBITION ACTIVITIES OF THE AQUEOUS AND CHLOROFORM EXTRACT OF PHYLLANTHUS URINARIA

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ABSTRACT

Objective: The aim of this research was to analyze the antioxidant, anti-microbial and *in-vitro* glucose diffusion inhibition of the phytochemicals extracted from *Phyllanthus urinaria*.

Methods: The anti-oxidant activity of the two extracts was done using DPPH method. The antimicrobial activity was performed using plate hole diffusion method. The *in vitro* anti-diabetic activity was determined using glucose diffusion inhibition technique.

Results: Both the aqueous and chloroform extract were shown to have declined in antioxidant activity with the increase in concentration. There was a considerable amount of zone of inhibition observed in antimicrobial activity when the assay was performed against *E. coli*. However, no such zone of inhibition was observed against *Bacillus subtilis*. The glucose diffusion inhibition was found to be maximum for chloroform extract.

Conclusion: The present study validates the effective use of *P. urinaria* against the microbial growth and also has anti-oxidant activity. The *in vitro* glucose diffusion inhibition proves the fact that it can act as a potent anti-diabetic agent.

Keywords: DPPH, Anti-oxidant, Anti-microbial, Glucose Diffusion Inhibition, Phytochemicals

Diabetes mellitus is a chronic metabolic disease which has now affected about three percent of the world population [1]. The recent surge in the level of diabetes patients worldwide is primarily due to lifestyle changes, obesity, and genetic disorders. Its long-term persistence may pose serious malfunctioning on several vital organs of the body [2]. Diabetes is generally classified into three categories based on the insulin malfunctioning. Type 1 diabetes is characterized by the fact that the pancreas fails to produce enough insulin to cope up with carbohydrate metabolism rate [3]. It is an autoimmune disorder where the body's own cell kill and destroy beta cells that produce insulin. Type 2 diabetes is a condition that arises when the cells fail to respond to insulin i.e. Insulin resistance. The proposed mechanism for this is that the receptors present on the surface of liver and muscle cells lose their potential to respond to glucose [4]. The third category is a bit unusual that develops in a pregnant woman who has no previous history of diabetes. Insulinoma is a tumorigenic condition that arises from the beta cells that produce insulin. The tumor cells continue to produce insulin in an unregulated manner leading to a drastic decrease in blood glucose level, a condition known as hypoglycemia [5].

The current mode of diabetes treatment includes oral administration of chemical drugs and insulin injection. However clinical trials and epidemiological studies strongly state that the prolonged use of such treatment may not prove to be effective against the long-term complications that arise such as diabetic retinopathy, neuropathy, nephropathy, foot infections, atherosclerosis and other cardiovascular diseases [6]. In view of the serious side effects posed by the chemical drugs, it was rendered mandatory to search for alternative means to cure diabetes. Phytochemicals extracted from medicinal plants are found to be very effective in treating long-term ailment like diabetes [7]. The therapeutic activity of herbal plants depends on some vegetal active principles contained in the plant [8]. Free radicals produced in diabetes patient can destroy cell membrane by oxidizing lipid bilayer. Certain anti-oxidants particularly flavonoids are key sources of free radicals scavenging and chelating deleterious oxidant inducing metal ions in the body [9]. Microbial infections tend to take a longer time to heal in diabetes patients than usual. This can arise due to a condition called hypoxia during which nutrition and oxygen is not transferred effectively to the infected site [10]. Secondary metabolites from plants are of diverse complexity and are found to be very effective against bacterial growth [11]. The blood glucose level in diabetes patients tends to rise enormously due to the cell membrane's inability to retain the glucose molecules. Certain viscous components present in plant extracts have shown to reduce glucose diffusion across the membrane [12]. In this current research, we have primarily focused on the phytochemicals having antioxidant, anti-microbial and glucose diffusion inhibition activity.

Phyllanthus urinaria stems were obtained from the local market in Vellore, Tamil Nadu, India. The stems were dried in shade for 48 h followed by which it was grinded to fine powdered form for further analysis.

The dialysis membrane and DPPH (2, 2-diphenyl-1-picrylhydrazil) were procured from Hi-Media Laboratories, Mumbai, India. All other chemicals and reagents used in the study were of analytical grade and were procured locally.

Two different solvents, chloroform, and water based on their polarity were used to prepare the extract. The aqueous extract was prepared by mixing 50 grams of stem powder with 500 ml of distilled water. On the other hand, organic extract was prepared by mixing 50 grams of stem powder extract with 250 ml of chloroform. Double the amount of water is used in case of aqueous extract because stem powder forms a gelatinous mass when mixed with a lower quantity of water. The mixtures were placed on an orbital shaker (The I L E Company, Chennai, Tamil Nadu, India) for 72 h. The solvents were then filtered through Whatman filter paper, and the filtrates were dried to yield extracts for experimental studies.

Four different concentrations (25, 50, 75, 100 µg/ml) of both the extracts were taken and 1 ml of 0.3 milli mol DPPH methanol solution was added. The final volume was made 2.5 ml by adding methanol. The resulting mixture was allowed to react for 30 min at room temperature in the dark. Ascorbic acid was used as the positive control. 1 ml of methanol added to 2.5 ml of extract solution was used as a blank. The spectrophotometer was set to zero using a mixture of 1 ml of 0.3 milli mol DPPH and 2.5 ml of methanol. The absorbance (Abs) was measured at 518 nm after the stipulated 30

min. The percentage antioxidant activity (AA%) was calculated using the formula:

$$AA\% = [100-((Abs_{sample}-Abs_{blank}) \times 100)]/Abs_{blank}$$

25, 50, 75 and 100 mg/ml concentration (conc.) of aqueous and chloroform extract were formulated. The media along with Petri plates were sterilized at 121 °C for 15 min. 25 ml of muller hinton agar was poured on each of the Petri plates. Holes with 6 mm in diameter made of agar puncture method and 0.2 ml of plant extracts were transferred into the four wells. The fifth well was had a solvent of the particular extract, acting as a negative control. The sixth well was had streptomycin, an antimicrobial used as a positive control. The Petri plates were sealed and placed in an incubator overnight followed by which the zones of inhibition were measured.

The aqueous and the chloroform extract (100, 200, 300 and 400 mg/ml) of both the plants were placed in the dialysis membrane along with the glucose solution (0.22milli mol in 0.15 mol sodium chloride). The resulting mixture was tied at both the ends and immersed in a beaker containing 40 ml of 0.15 mol sodium chloride and 10 ml of distilled water. 1 ml of 0.15 mol sodium chloride containing 22 milli mol glucose and 1 ml of distilled water was used as the control. The beakers containing the resulting solutions were then placed on an orbital shaker at room temperature. The extent of diffusion across the dialysis membrane was monitored every half an hour. The absorbance reading was taken at 545 nm.

As depicted in table 1. both, chloroform and aqueous extract are found to have free radical scavenging property. However, the experimental data suggests that with the increase in the concentration of plant extracts the anti-oxidant activity decreases. The aqueous extract has better efficiency when compared to chloroform extract at higher concentration. As the concentration was decreased gradually, the aqueous extract showed a greater decline in its activity, while chloroform extracts were found to be more consistent. This can be correlated to the fact that at higher concentration the bioavailability of the aqueous extract decreases than the chloroform extract due to salvation property of the medium or there might be some inhibitory substance that might get activated after a particular threshold of extract is reached in the medium and start to hinder its activity [13].

Table 1: It depicts the anti-oxidant activity at four different concentrations of chloroform and aqueous extract in terms of percentage free radical scavenging activity

Concentration (µg/ml)	Standard (%)* (Ascorbic acid)	Aqueous (%)*	Chloroform (%)*
25	29.03±0.71	45.59±0.40	42.34±0.30
50	41.82±0.82	32.23±0.20	33.69±0.50
75	56.98±0.67	25.54±0.30	32.99±0.20
100	60.13±0.34	20.50±0.50	32.92±0.60

*mean±SD, n=3.

As shown in table 2. With the increase in the concentration of the plant extracts, their anti-microbial activity also increases. This has been validated by the zone of inhibition formed by the plant extracts in the media containing bacterial culture. Chloroform extract possesses higher anti-microbial activity than aqueous extract. Both the extracts have more potential in hindering microbial growth than streptomycin which was used as the control for this assay. The phytochemicals tend to destroy the cell wall of bacteria and stops the bacterial DNA from replicating thereby are inhibiting its growth [14]. However, they were found to be ineffective against *Bacillus subtilis*-gram-positive bacteria. This could be correlated to the fact that the cell wall of gram-positive bacteria is much thicker and contains teichoic acid which is absent in gram-negative bacteria.

Table 2: It depicts the anti-microbial activity of the chloroform and aqueous extract by measuring the Zone of Inhibition (zoi) of *E. coli* around the wells containing the extract

Concentration (mg/ml)	Aqueous (zoi)*	Chloroform (zoi)*
100	09±0.40	09±0.30
200	11±0.60	10±0.40
300	13±0.20	11±0.60
400	14±0.30	13±0.20
Solvent	00	00
Streptomycin	08±0.30	08±0.30

*mean±SD, n=3.

As depicted in table 3. Both aqueous and chloroform extract showed high potential in inhibiting the movement of glucose molecule across the dialysis membrane when compared to the control used for the assay. To certain extent, chloroform extract was more effective in preventing the diffusion of glucose molecules. This can be attributed to the fact that only chloroform extract contains tannins that are a potent source of lowering plasma glucose level [15].

Table 3: It depicts the extent of glucose diffusion inhibition by chloroform and aqueous extract of *P. urinaria* at 100 mg/ml across the membrane with respect to the regular time interval of 30 min. 1 ml of 0.15 mol sodium chloride containing 22 milli mol glucose and 1 ml of distilled water was used as the standard

Time (min)	Standard (mg/ml)*	Aqueous (mg/ml)*	Chloroform (mg/ml)*
30	12±0.10	13±0.10	05±0.20
60	20±0.20	18±0.30	12±0.10
90	28±0.90	25±0.60	15±0.50
120	32±0.10	29±0.40	23±0.30
150	39±0.50	35±0.30	25±.0.20
180	53±0.40	40±0.20	30±0.60

*mean±SD, n=3.

In accordance with the results obtained it can be concluded that *P. urinaria* can be extensively used as a means for therapeutic measures. Considering the fact that at a lower concentration, extracts from *P. urinaria* have better antioxidant activity than the control ascorbic acid, they can act as a potent source of free radical scavenging agents and prevent the cells from oxidative damage. The phytochemicals extracted from *P. urinaria* can be used as effective reagents to cure microbial infections. The plant extracts showed great potential in inhibiting the extent of glucose diffusion across the dialysis membrane; hence, they can act as a potential barrier in lowering the blood glucose level by inhibiting the movement of glucose molecule across the plasma membrane into the blood vessel.

CONFLICT OF INTERESTS

All authors have no conflict of interest to declare.

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