GROWTH AND ANTIDIABETIC ACTIVITIES OF *PEPEROMIA PELLUCIDA* L. PLANTS GROWN UNDER DIFFERENT WATERING REGIMES

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ABSTRACT
This study was aimed at investigating the potency of Peperomia pellucida plants grown under different watering regimes. Two weeks old seedlings were subjected to daily, 3-days, 5-days and 7-days interval of watering regimes for six weeks. The plant heights, total leaf area, fresh weights and dry weights of plants were measured weekly for six weeks. The rate of growth (all parameters) reduced significantly over time ($p < 0.05$) as watering regime is prolonged from 3 days to 7 days. Plants were harvested six weeks after watering, dried and blend to powder. The total saponins, total tannins, total alkaloids, total flavonoids and total phenols were analysed. The concentration of phenols and saponins decreased as watering interval increases while the concentration of tannins, alkaloids and flavonoids increased. Alkaloids and tannins were absent in plants watered daily. The study shows presence and concentration of these phytochemicals in plants must be related to soil water availability. Alloxan-induced diabetic rats were fed with extracts from plants subjected to the different watering regimes. The body weight of diabetic rats increased significantly ($p < 0.05$) with continued treatment with plant extracts (no significant difference between plant extracts) while the blood glucose level decreased significantly ($p < 0.05$) over time. The blood glucose level of diabetic rats treated with extracts of plants watered at 5-days interval and plants watered at 7-days interval reduced to levels (171 ± 8 mg/ml and 165 ± 11 mg/ml respectively) that were insignificantly different from that diabetic rats treated with standard drug, glibenclamide (152 ± 14mg/ml) by 21 days of treatment. The study suggests that P. pellucida is may be useful as a therapeutic agent in the management of diabetes mellitus and further shows that the potency may be improved by subjecting the plant to water stress.

Keywords: *Peperomia pellucida*, Growth, Antidiabetic, Water, Stress, Blood Glucose

INTRODUCTION
Water is one of the most important environmental factors that regulates plant growth and development. When plants are made to undergo water deficit, it brings about a variety of physiological responses; all metabolic activities in the

plant are affected and often results in severe reduction in productivity of plants (Mundree *et al*., 2002, Umebese *et al.,* 2009). Water deficit has been shown to increase the concentration of secondary metabolites in plants and the variations of secondary metabolites (qualitative and quantitative) are known to happen in response to different types of stress (Watermann and Mole, 1989).

Diabetes mellitus is a major public health problem in the developed as well as developing countries. It is ranked as the seventh cause of death in the world (WHO, 2017). Diabetes is a global epidemic and, according to the International Diabetes Federation (2017), "one of the most challenging health problems in the 21st century." Globally, 12% of the health expenditure is spent on diabetes as an estimated 425 million people suffer from diabetes, a number that is expected to jump to over 592 million by 2035, if nothing is done (IDF, 2017). The common symptoms of diabetes according to the American Diabetes Association (2018) are urinating often, feeling very thirsty, feeling very hungry, extreme fatigue, blurry vision, cuts/bruises that are slow to heal, weight loss (type 1), tingling, pain, or numbness in the hands/feet (type 2). *Peperomia pellucida* L. is one of the plants that are used locally in Nigeria for the treatment of hypertension, diabetes and generally as tonic for healthy well being. Hamzah *et al*., (2012) have used experimental diabetes mellitus to prove that *Peperomia pellucida* has antidiabetic and antioxidant properties.

Peperomia L. is an annual herb growing widely in Asia, South America including tropical and sub-tropical climate as a weed (Bayma *et al*., 2000). *Peperomia pellucida* L. develops during rainy periods and thrives in loose, humid soils (Dos-Santos *et al*., 2001. Mosango, 2008). It is commonly called silver bush, Elder pepper plant, Vietnamese Crab Claw Herb, Pansit-pansitan and rinrin or renren by the Yorubas (Nigeria). It is distributed mainly in Central and South America, Africa, Southeast Asia and Australia. The plant has long been used as a food item as well as a medicinal herb (Loc *et al*., 2010). The ethno-medicinal uses of *P. pellucida* has been reported to include treatment of abdominal pain, abscesses, acne, boils, colic, fatigue, gout, headache, renal disorders, and rheumatic joint pain (Aziba *et al*., 2001; Khan and Omoloso, 2002). The phytochemical screening of *P. pellucida* has revealed the presence of alkaloids, saponins, tannins, flavonoids, steroids, triterpenoids and cardenolides ((Egwuche *et al*., 2011; Abere and Okpalaonyagu, 2015).

Peperomia pellucida is known to thrive in wet seasons; however some populations manage to survive in dry season in places that get moist due to

intermittent flow of water along the path. It will be interesting to see if *P. pellucida* plants found in dry seasons would be as medicinally active as those flourishing in wet seasons. The present study aims at investigating the effect of water regimes on the growth, accumulation of phytochemicals and antidiabetic activities of extracts of whole plants of *P. pellucida*.

MATERIALS AND METHODS

PLANT COLLECTION AND TREATMENTS

Plants were collected from the Botanical Garden of the University of Lagos and identified in the Herbarium of the Department of Botany by the curator, Mr Tola Oyebanji. The seeds were planted in nursery bowls and watered daily for faster germination. About 200 two-week old seedlings were transplanted into small bowls containing 2.5kg of loamy soil. The young plants were separated into four groups and were subjected to different watering regimes as follows: daily watering (control); 3-days interval watering (3 days); 5-days interval watering (5 days) and 7-days interval watering (7 days).

ASSESSMENT OF GROWTH

Growth analyses of plants subjected to different watering regimes were carried out at one week interval. Plants were randomly uprooted from each treatment section (five plants per section per week). The height of each plant was measured from the soil level to apex of the leaves with a meter rule. Plants were carefully uprooted and the soil was rinsed off the roots. Plants were mopped dry using tissue and immediately the fresh weights of the plants were taken using an analytical weighing balance (Mettler Toledo Model AB 204). The leaf area was measured by tracing the leaves on each plant on a graph paper and subsequently counting the number of squares occupied by each leaf. The plants were placed in envelopes and dried at 80 °C in an oven for 3 days. The dried plants were thereafter weighed using the weighing balance.

PREPARATION OF PLANT SAMPLES

Plants were harvested after six (6) weeks of watering at the stipulated rates. The plants harvested were air-dried (away from sunlight) for three weeks, blended into fine uniform powder. Thereafter, some of the powdered samples were taken for phytochemical analyses based on their different water treatment regime, and the rest were stored in different airtight opaque containers at room temperature until use.

QUANTIFICATION OF PHYTOCHEMICALS

The amount of saponins, tannins, alkaloids, flavonoids and phenols in each extract were determined according to methods described by Obadoni and Ochuko (2001), Harborne(1973), Khanahmadi *et al.* (2010), Nile and Khobragade (2010) and Grubesic *et al.* (2005) respectively.

ANTIDIABETIC STUDY

Selection and Acclimatization of Animals

Male wistar strains of albino rats weighing about 100 - 120g were obtained from the Animal House, Nigerian Institute of Medical Research, Yaba, Lagos (authenticated the research institute). The animals were housed in large spacious cages and they were fed on standard rat pellet diet and water was provided *ad libitum*. The animals were well acclimatized to the standard environmental condition of temperature (22 ± 5 °C) and humidity (55 ±5 %) and 12 hrs light: dark cycles throughout the experimental period. Experimental protocols complied with the "Principle of Laboratory Animal Care" (NIH publication No 85-23) guidelines.

Experimental design

A total of 35 rats about the same body weight were used. The rats were randomly distributed into seven groups of five rats each:

Group 1 (Normal)	–	non-induced rats, served as Normal control
Group 2 (Diabetic)	-	alloxan induced diabetic rats, served as Diabetic control
Group 3 (Glibenclamide)	-	alloxan induced diabetic rats, received glibenclamide (standard drug)
Group 4 (Daily)	-	alloxan induced diabetic rats, received extracts of plants watered daily
Group 5 (3 days)	-	alloxan induced diabetic rats, received extracts of plants watered at 3-days interval
Group 6 (5 days)	-	alloxan induced diabetic rats, received extracts of plants watered at 5-days interval
Group 7 (7 days)	-	alloxan induced diabetic rats, received extracts of plants watered at 7-days interval.

Rats in Group 1 were administered with physiological saline at 10 mL/kg body weight and served as Normal control. Diabetes was induced in rats in the other groups before administering with either plant extract or antidiabetic drug.

Induction of Diabetes mellitus

Diabetes was induced in rats following the method of Adeyi *et al*. (2012). Alloxan monohydrate was first weighed individually for each animal according to their body weight and then solubilized with 0.12 ml saline just prior to injection. Diabetes was induced in some wistar rats by injecting it at a dose of 120 mg/kg body weight intraperitonially after overnight fasting for 12hr. After 1 hour of alloxan administration, the animals were given feed *ad libitum* to overcome the early hypoglycemic phase and kept under observation. Seventy-two hours later, blood was extracted from the tail vein for glucose analysis. Blood glucose level was checked by using one-touch glucometer (On Call Plus). Rats that showed fasting blood glucose (FBG) above 200mg/dl as well as with polydipsia and polyuria were considered to be diabetic and were selected for antidiabetic studies. The diabetic rats were separated from the non-induced rats (Group 1) and divided into six groups of 5 rats each.

Preparation of reference antidiabetic drug

The reference antidiabetic drug, glibenclamide (Brand: Daonil, Swiss Pharma, Nigeria) was purchased from Tabade Pharmaceuticals, a local Pharmaceutical store in Akoka, Lagos. The drug was dissolved freshly in normal saline and appropriate volumes were given to the animals depending on their weight. The animals were given 600 µg/kg body weight of the active ingredient (Hamzah *et al*. (2012).

Preparation of plant extract

The method of Hamzah *et al.* (2012) in treating alloxan induced diabetic rats with *P. pellucida* plants was modified in this study. 150 g dried, powdered leaves of *P. pellucida* (an average of the amount used by Hamzah *et al.,* 2012) was dissolved in appropriate distilled water for 3 days and the filtrate dried in oven at 35 °C. The dried extract was freshly dissolved in saline water and used in feeding alloxan induced diabetic rats as required.

All doses (saline water, standard drug and plant extracts) were started 72 hours after alloxan injection and the treatments were given once daily for 21 days (3 weeks). All rats were allowed free access to drinking water and rat feed. The animals were observed daily for any signs of morbidity and mortality.

Body Weight Measurement

The body weight of each rat in all the groups was measured four times during the course of study i.e. before alloxan administration (initial values, 0 day), and on

the 7^{th}, 14^{th} and 21^{st} days of treatment, using a weighing scale (Vijayanand and Wesely, 2011).

Determination of Blood Glucose Level

Blood samples were obtained by tail vein puncture of rats. Blood glucose (mg/ml) was by single touch glucometer (On Call) measured at 7-days interval after diabetes induction and expressed in milligrams per deciliter (mg/dL) and recorded (Vijayakumar *et al*., 2006).

STATISTICAL ANALYSIS

Data obtained from the plants were expressed as means of five replicates and comparison was between plants subjected to different watering regimes. Test of significance between treatments was done using analysis of variance (ANOVA) at 5% level of significance ($p = 0.05$) and means were compared using Duncan Multiple Range Test.

RESULTS

Figure (1) shows the mean value of growth parameters (plant height, number of leaves, total leaf area, fresh weight and dry weight) of plants subjected to the different watering regimes. The data obtained followed same pattern in terms of difference: plants watered daily had the highest value followed by plants watered at 3-days interval, then plants watered at 5-days interval while plants watered at 7-days interval had the least growth values throughout the period of analysis (6 weeks of treatment). Plants watered at 5-days interval and those watered at 7-days interval showed no significant difference ($p>0.05$) in total leaf area and fresh weight from 4 weeks of treatment, however the difference in fresh weight was significant ($p<0.05$) by 6 weeks of treatment.

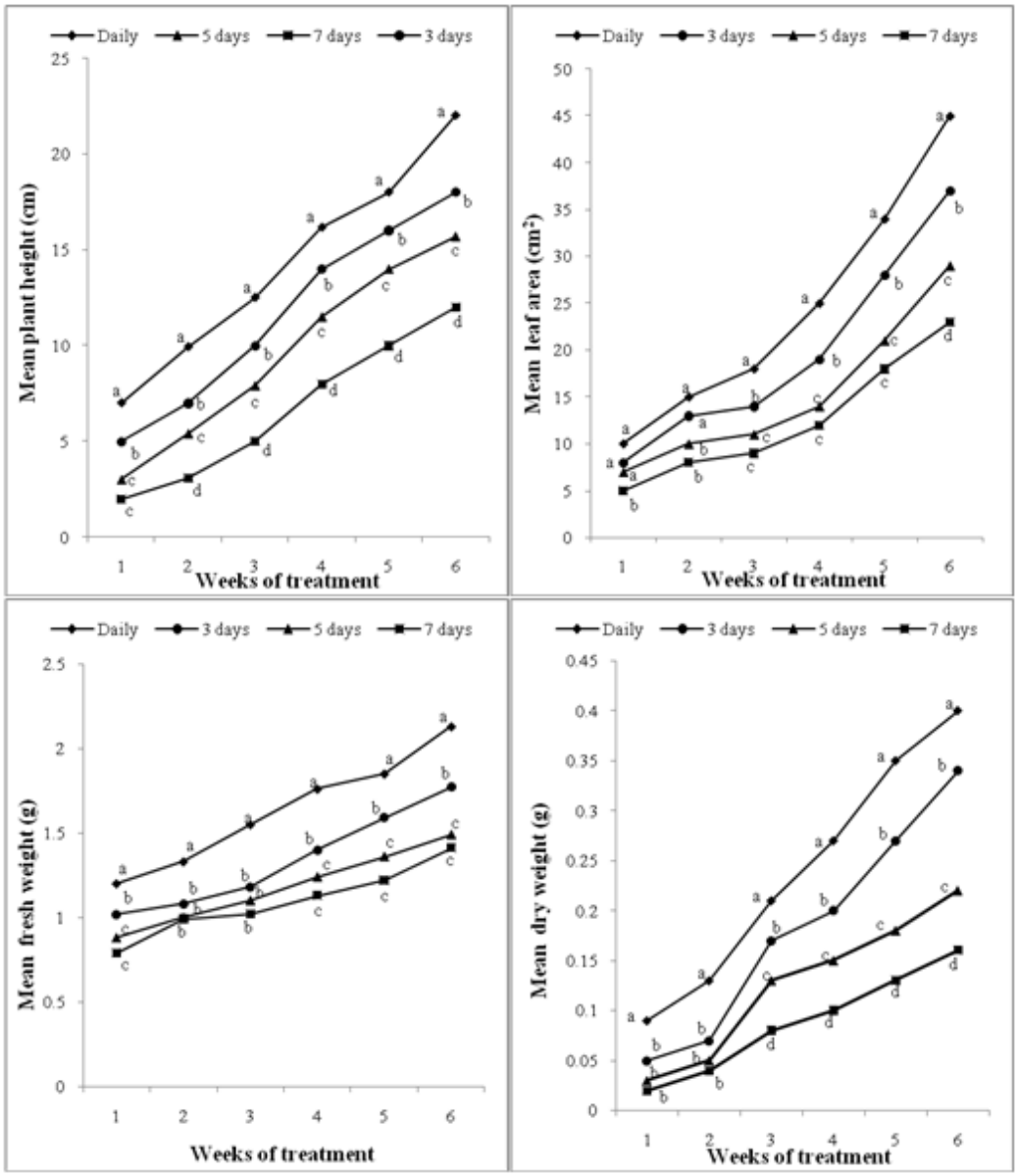

Fig. (1): Mean values of growth parameters (plant height, leaf area, fresh weight, dry weight) of plants grown under different watering regime (daily, 3-days interval, 5-days interval and 7-days interval) over six weeks of treatment (Value points with same letters at same week of treatment are not significantly different at p> 0.05)

Fig. (2) shows the concentration of the phytochemicals in plants subjected to the different watering regimes. The concentration of phenols and saponins in plants were in the order: daily > 3days > 5days > 7days; and the differences were significant at $p<0.05$. Alkaloids and tannins were absent in plants watered daily. The concentration of tannins was in the order: 7days > 5 days ≥3 days, differences were not significant ($p>0.05$) in plants watered at 3-days interval and those watered at 5-days interval. Plants watered daily had the least amount of flavonoids. The concentration of alkaloids and flavonoids were in the order: 7days ≥5 days > 3 days, differences were not significant ($p>0.05$) in plants watered at 7-days interval and those watered at 5-days interval.

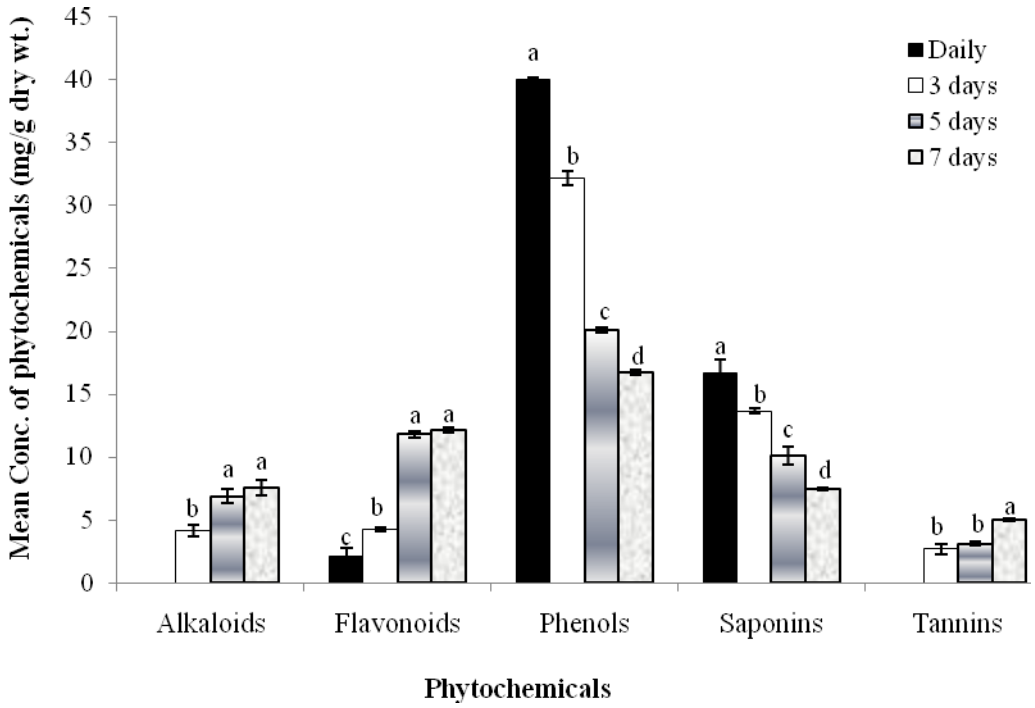

Fig. (2): Concentration of phytochemicals (with standard error bars) in *Peperomia pellucida* plants subjected to different water regimes (Bars of similar compound with same letters on top, are not significantly different at p > 0.05

Fig. (3) shows the body weight of experimental rats over a period of 21 days. The body weight of normal rats and diabetic rats treated with glibenclamide increased significantly over the 21 days of examination, with no significant difference ($p > 0.05$) between the weights of the rats. The body weight of untreated diabetic rats

decreased significantly throughout the period of examination. The body weight of diabetic rats treated with the plant extracts continued to decrease for the first 14 days of treatment, with no significant difference ($p > 0.05$) between treatments. However, body weight of diabetic rats treated with extracts from plants increased by 21 days of treatment, while body weight of untreated diabetic rats continued to decrease. The body weight of diabetic rats treated with plant extracts were significantly higher ($p < 0.05$) than those of untreated diabetic rats throughout the experimental period.

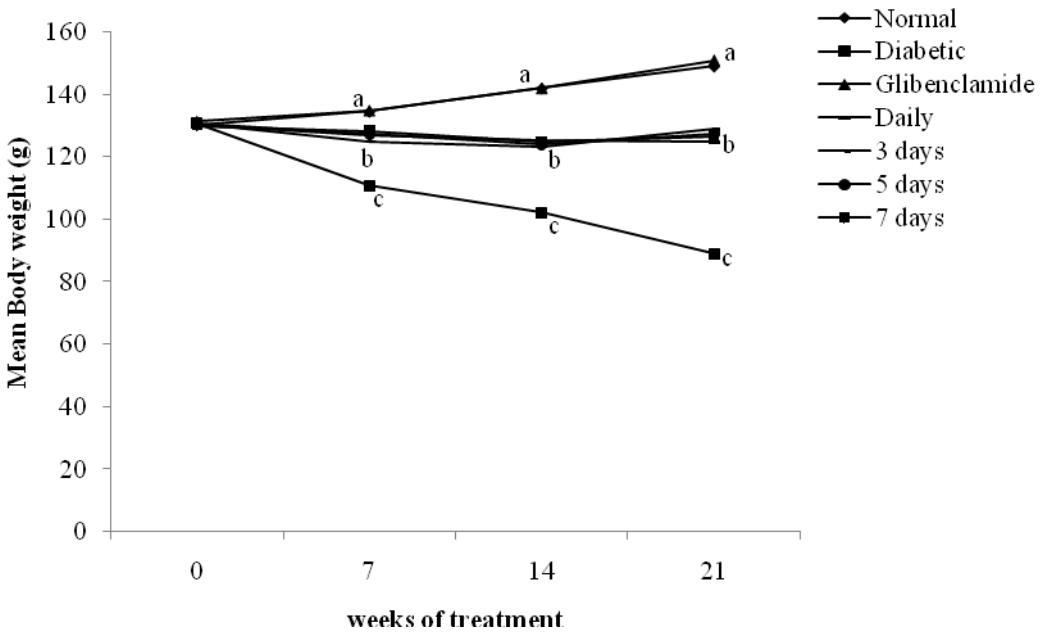

Fig. (3): Mean Body Weight of Normal rats, Diabetic rats treated with Glibenclamide and Diabetic rats treated with extracts of *Peperomia pellucida* subjected to four different water regimes (Value points with same letters at same week of treatment are not significantly different at p > 0.05)

Fig. (4) shows the blood glucose level of experimental rats over a period of 21 days. The blood glucose level of diabetic rats treated with extracts decreased over the experimental period in similar way as those of diabetic rats treated with glibenclamide. There was no significant difference in the blood glucose level of diabetic rats treated with glibenclamide, extracts of plants watered at 5-days interval and extracts of plants watered at 7-days interval, by 21 days of treatment; their values were significantly higher ($p < 0.05$) than those of diabetic rats treated with extracts of plants watered daily and extracts of plants watered at 3-days interval. There was no significant difference in the blood glucose level of

diabetic rats treated with extracts of plants watered daily and extracts of plants watered at 3-days interval, by 21 days of treatment. The blood glucose level of untreated diabetic rats remained significantly higher than those of treated diabetic and Normal rats throughout the period of experiments.

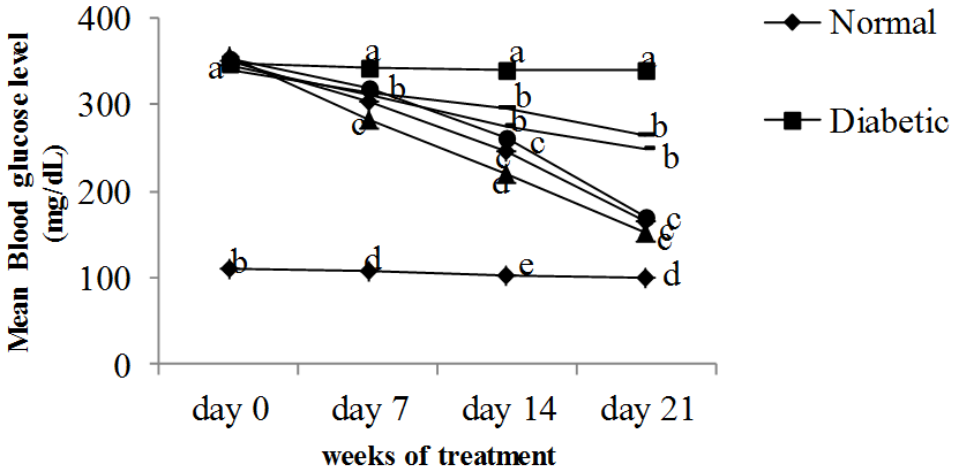

Fig. (4): Mean blood glucose level (mg/dL) of Normal rats, Diabetic rats treated with Glibenclamide and Diabetic rats treated with extracts of *Peperomia pellucida* subjected to four different water regimes (Value points with same letters at same week of treatment are not significantly different at p > 0.05)

DISCUSSION

This study shows that *Peperomia pellucida* grew more with increase in frequency of watering; plant growth reduced as watering regime is prolonged. This is expected as water stress have been shown to cause reduction in plant height, number of leaves, fresh and dry weight, leaf area, leaf area ratio, net assimilation rate and relative growth rate in previous studies (Mohamed and Abdu, 2004; Razmjoo *et al*., 2008, Shah *et al*., 2010; Umebese and Falana, 2013).

Phenols and saponins were shown to reduce with watering regime while alkaloids, flavonoids and tannins increased with water stress. Alkaloids were completely absent under daily watering condition. Previous works on the phytochemical content of *P. pellucida* are inconsistent as they reported absence of tannins, saponins and alkaloids (Edewor-Kuponiyi, 2012), presence of alkaloids, saponins, tannins, flavonoids (Mensah *et al*., 2013), absence of flavonoids (Omotayo and Borokini, 2012). These reports were based on the analyses of plants from the wild in which frequency of water availability was not recorded.

This tells a lot about the need for authors not to draw conclusions about the presence or absence of a phytochemical in a plant species without stating the growth conditions of plants used for their studies.

Extracts of *P. pellucida* increased the body weight of alloxan induced diabetic rats while reducing their blood glucose level. Antidiabetic and antioxidant properties of *P. pellucida* have been observed in alloxan induced diabetic rats fed with feed supplemented with the plant powder (Hamzah *et al*., 2012). This effect according to Hamzah *et al*. (2012) may be due to the presence of tannin, saponin, flavonoid and other constituents in the plant, which could act synergistically or independently in enhancing the activity of glycolytic and antioxidant enzymes. Flavonoids are known for their antioxidant activity, thereby protecting the body against cancer and other degenerative diseases such as arthritis and type II diabetes mellitus (Lee and Shibumoto, 2002). Saponins have hypocholesterolemic effect and this has been attributed to their intra-luminal physicochemical interaction which reduces the uptake of certain nutrients including glucose and cholesterol (Ali-Smith, 2009). Tannins are well known for their antioxidant and antimicrobial properties as well as for giving relief, stimulating skin regeneration, as well as anti-inflammatory and diuretic effects (Okwu and Okwu, 2004). Saponins and flavonoids were found in plants from all the watering regimes but tannins was absent in plants that were watered daily. Also, the concentration of flavonoids and tannins increased with water stress in contrast to the concentration of saponins which decreased with water stress. However, this study shows that while the antidiabetic activity of *P. pellucida* plants in terms of ability to increase in body weight appear not to be affected by the watering condition of the plants, antidiabetic activity in terms of ability to reduce blood glucose level appeared to be affected by watering regime.

It has been reported that *P. pellucida* plants are known to flourish during rainy periods (Dos-Santos *et al*., 2001); therefore, it is not surprising that in this study, the plants grew better under more regular watering conditions. It, however, appears that reducing the rate of water supply does not affect the antidiabetic properties of the plants but rather may even improve it. The study suggests that *P. pellucida* may be able to act as a therapeutic agent in the management of diabetes mellitus and further shows that the potency may be improved by subjecting the plant to water stress.

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