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Full Length Research Paper

Growth, phytochemicals and antifungal activity of *Bryophyllum pinnatum* L. subjected to water deficit stress

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The impact of water stress on the growth, concentration of phytochemicals and antifungal potency of *Bryophyllum pinnatum* L. was investigated. Three weeks old seedlings were subjected to 3, 7 and 10 days water deficit regimes and a control (watered daily). Plant height, number of leaves, whole plant dry weight, net assimilation rate, leaf area ratio and relative growth rate were reduced by 6 to 50% as intervals of water deficit increased. Alkaloids, tannins, saponins and flavonoids were present in all plants and all but alkaloids increased with increase in intervals of water deficit. Alkaloid content decreased by 1.3 to 10.5% while the other phytochemicals increased by 12 to 206% in response to water deficit stress. Ethanolic extracts of plants from the four batches showed varying inhibition zones against *Candida albicans*. The inhibition zones decreased with increasing water deficit intervals corresponding with the decrease in alkaloid content. This research has revealed that water stress increases the tannin, saponin and flavonoid contents of *Bryophyllum* but decreases the alkaloid content.

Key words: *Bryophyllum*, water deficit, phytochemicals, inhibition zone.

INTRODUCTION

Bryophyllum pinnatum L., a perennial herb belonging to the Crassulaceae family and commonly known as resurrection plant, miracle leaf, air plant and life plant in English, is found growing widely in tropical Africa, tropical India, China, Australia and South America as a weed (Okwu, 2003). The phytochemical screening of *B. pinnatum* revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins and saponins. The proximate profile shows that the plant is rich in magnesium, calcium, potassium, phosphorus, sodium, iron and zinc. It also contains vitamins: ascorbic acid, riboflavin, thiamine and niacin (Majaz et al., 2011). Three new components: bryophyllol, bryophollone and bryophollenone have also been isolated from the fresh leaves of *B. pinnatum*. *B. pinnatum* possesses significant antimicrobial, anti-inflammatory, analgesic, antihypertensive, wound healing, hepatoprotective and show neuropharmacological and antidiabetic activities (Kamboj and Saluja, 2009). The presence of phenolic compounds indicate that the plant possess anti-microbial activity. The plant is effective in the treatment of typhoid fever, bacterial and fungal infections particularly by *Staphylococcus aureus*, *Escherichia*

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coli, *Bacillus subtilis*, *S. typitic*, *Candida albicans* and *Aspergillus fumigatus* (Akinpelu, 2000). *B*. *pinnatum* has high saponin content which justifies the use of the extracts to stop bleeding and in treating wounds. Saponin has the property of precipitating and coagulating red blood cells. It has tannins that give astringent properties and strong analgesic potency of its alkaloids (Siddarta and Chaudhuri, 2002). It has antihypertensive, antidiabetic activity and it alters the general behavioural pattern (Ojewole, 2002, 2005). Both qualitative and quantitative variations of secondary metabolites have been shown to occur in response to various types of stress (Watermann and Mole, 1989). Water stress has been shown to increase the concentration of secondary metabolites in plant tissues (Mundree et al., 2002). It has also been shown to reduce leaf artemisin content in *Artemisia annua* (Charles et al., 1993). Thus, environmental factors including water deficit can increase or decrease phytochemicals in plants.

Since some of these compounds may have negative impact on health when taken in high doses or may affect the quality of products, it is important to investigate the extent to which environmental factors alter phytochemicals and subsequent bioactivity of plants. In this study, the impact of water deficit stress on the growth, phytochemicals and subsequent antifungal activity of *B*. *pinnatum* was investigated.

MATERIALS AND METHODS

Plant material

B. *pinnatum* L. plants were collected from the Botanic Garden of the University of Lagos and identified in the Herbarium of the Department of Botany. *C*. *albicans* was isolated and identified in the Pharmaceutical Microbiology Unit, Faculty of Pharmacy, University of Lagos. Pure culture of the fungus was obtained and later used for inoculation.

Planting procedure

Fresh leaves were placed in bowls containing water for faster germination of bulbils. After three weeks, 100 plants were transplanted into planting bags containing 2.5 kg of loamy soil. The young plants were separated into four groups of 25 plants and one batch each was subjected to daily watering (control), 3 days water deficit (WD), 7 days WD and 10 days WD regimes and each time 200 ml water was used for watering.

Growth parameters

Growth parameters (plant height, number of leaves, whole plant dry weight, leaf area, leaf area ratio, net assimilation ratio and relative growth rate) were measured in 10 and 14 weeks old plants using methods outlined by Eze (1965) and Noggle and Fritz (1976).

Preparation of plant extract

Plant samples were oven dried at 40°C, powdered and soaked in 300 ml of ethanol for 72 h. The crude ethanol extract was filtered and the filtrate was evaporated into dryness over a water bath (at 45°C) and weighed.

Phytochemical screening

Phytochemical screening was carried out on the extract and on the dried powdered specimens using standard procedures as described by Sofowora (1993), Trease and Evans (1989) and Harborne (1973). The phytochemicals tested were alkaloids, tannins, flavonoids and saponins.

Quantitative determination of the total amount of alkaloids

Alkaloid concentration was determined by the procedure outlined by Harborne (1973). Tannins were determined by the method of Van-Burden and Robinson (1981), flavonoids by Bohm and Kocipai-Abyazan (1994) and saponins by Obadoni and Ochuko (2001).

Determination of the total amount of alkaloids

Five grams (5 g) of the sample was mixed with 220 ml 10% acetic acid in ethanol, covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Harborne, 1973).

Determination of the total amount of tannins

500 mg of the sample was mixed with 50 ml of distilled water and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then, 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 M HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min (Van-Burden and Robinson, 1981).

Determination of the total amount of flavonoids

10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The solution was filtered through whatman filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight (Bohm and Kocipai-Abyazan, 1994).

Determination of the total amount of saponins

20 g of each powdered sample were mixed with 100 ml 20% aqueous ethanol and placed in a hot water bath at 55°C for 4 h with continuous stirring. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added to the aqueous

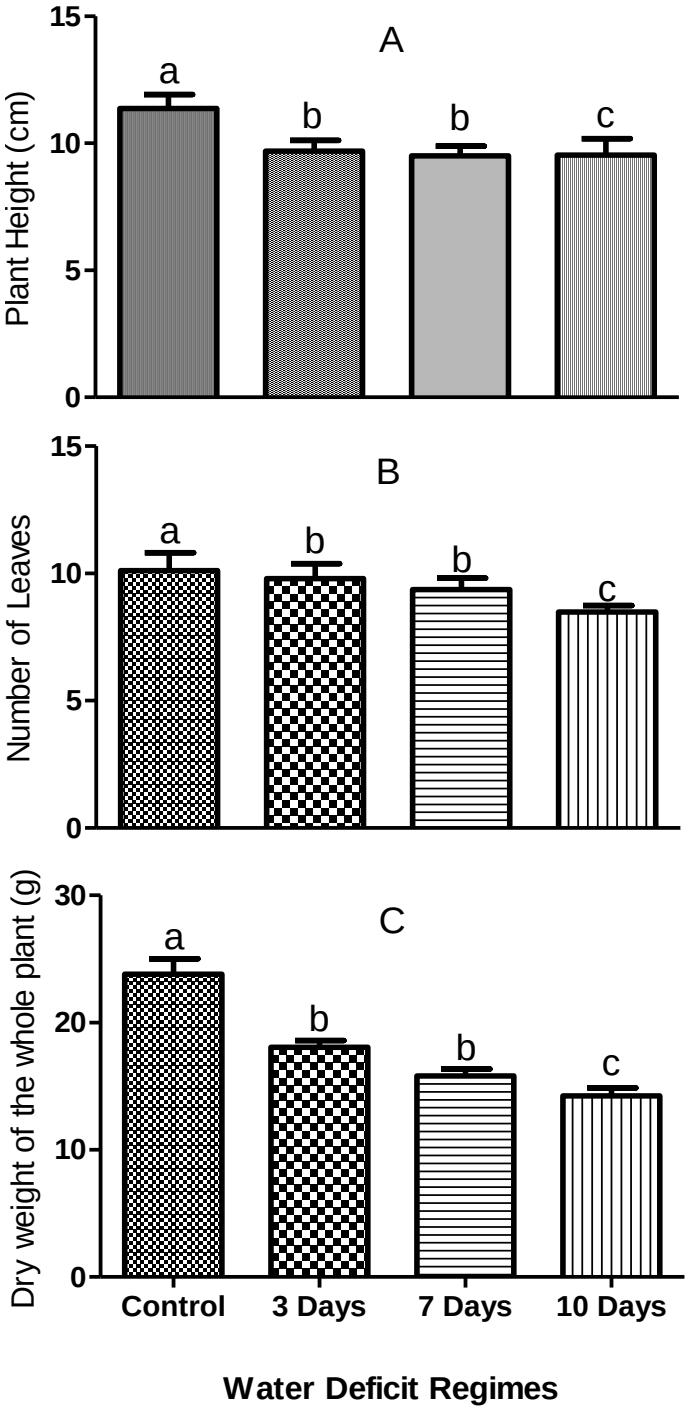

Figure 1. Mean height (A), number of leaves (B) and whole plant dry weight (C) of *Bryophyllum* plants subjected to different water deficit regimes (bars with similar letters for each parameter are not significantly different at p< 0.05 using Duncan's multiple range test.

extract. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight (Obadoni and Ochuko, 2001).

Innoculation and incubation

25 ml Saboraund dextrose agar (SDA) was measured aseptically and 1 ml of the calibrated organism was mixed with the medium and poured into a sterile Petri dish and was mixed and allowed to solidity. When the plates set, wells were bored using a cork borer of size 10 mm. Then, 0.2 ml of each extract and standard were introduced into the wells with the use of 1 ml sterile syringe. It was covered and kept in the incubator for 48 h at room temperature while the presence of measurable zone of inhibition was observed. Means of three replicates were recorded with the standard errors. Test of significance between treatments was done using analysis of variance (ANOVA) and Duncan's Multiple Range Test.

RESULTS AND DISUSSION

B. pinnatum L. subjected to 3, 7 and 10 days water deficit had marked reduction in growth. Plant height, number of leaves, whole plant dry weight, leaf area ratio, net assimilation rate and relative growth rate were reduced by 6 to 50% with greater reductions as intervals of water deficit increased (Figures 1 and 2). This corroborates an earlier study by Umebese et al. (2009) that water stress treatments lead to reduction in water potential in amaranth and tomato plants resulting in marked decreases in growth. The reduction in growth during water deficit stress has been attributed to the formation of reactive oxygen species (ROS). ROS which include oxygen ions, free radicals and peroxides, form as a natural by-product of the normal metabolism of oxygen and have important role in cell signaling. However, during environmental stress such as drought, ROS levels increase dramatically resulting in oxidative damage to proteins, lipids and DNA (Devasagayam et al., 2004). The reduction in growth caused by water deficit stress may have resulted from oxidative damage. The phytochemical screening and quantitative estimation of the phytochemicals of *Bryophyllum* showed that alkaloids, tannins, flavonoids and saponins were present in both stressed and unstressed plants (Table 1). These four phytochemicals are among those reported by Majaz et al. (2011) to be present in *Bryophyllum*; others being phenolic compounds, bryophyllol, bryophollone, bryophollenone and bufadienolides.

According to Cowon (1999), flavonoids, carotene and tannins are among several plant products utilized as antimicrobial agents, along with quinines, coumarines and terpenoids. Egunjobi (1969) reported that medicinal plants with high concentration of tannins, flavonoids and saponins are of greater medicinal uses and can be used as livestock and poultry feed. The water deficit treatments had resulting impact on the concentrations of phytochemicals of *Bryophyllum* plants (Figure 3). As the water deficit intervals increased from 3 to 10 days, the concentra-

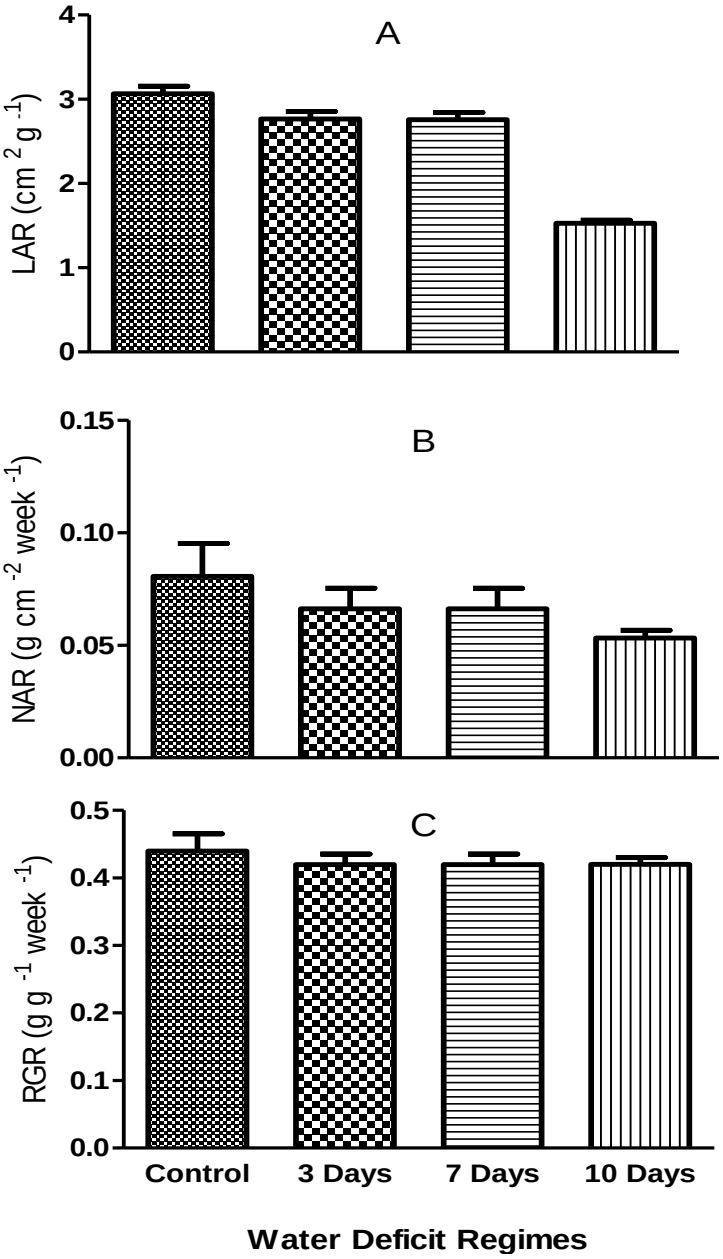

Figure 2. Leaf area ratio (A), net assimilation rate (B) and relative growth rate (C) of *Bryophyllum* plants subjected to different water deficit regimes.

Table 1. Qualitative analysis of the phytochemicals of *Bryophyllum* plants subjected to water deficit (WD).

Phytochemical	Control	3 days WD	7 days WD	10 days WD
Alkaloids	++	++	++	+
Tannins	+	++	+++	+++
Saponins	+	+	++	++
Flavonoids	+	+	+	++

+, Slightly present; ++, moderately present; +++, highly present.

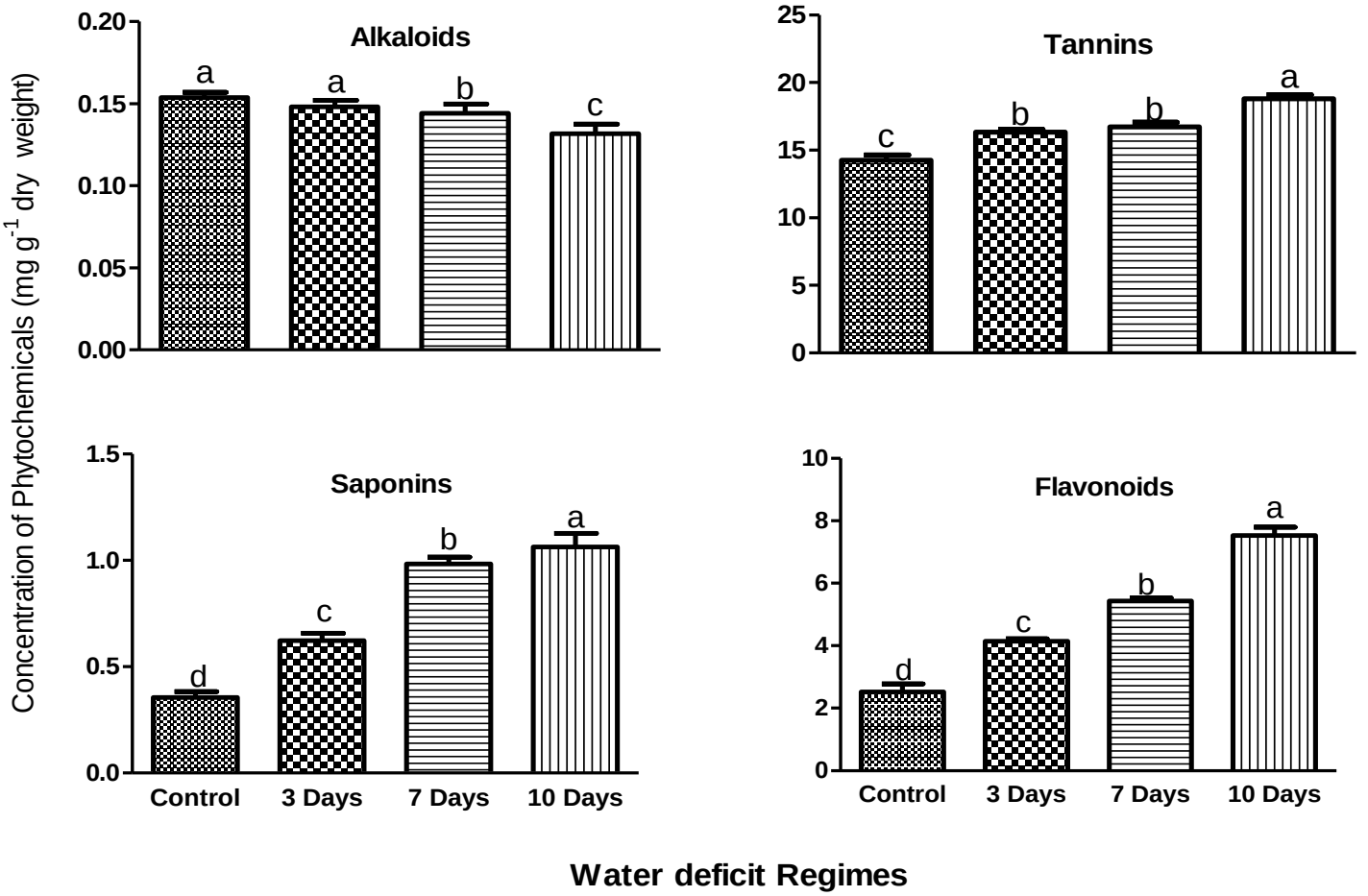

Figure 3. Concentrations of alkaloids, tannins, saponins and flavonoids of *Bryophyllum* plants subjected to different water deficit regimes (bars with similar letters for each phytochemical are not significantly different at p< 0.05 using Duncan's multiple range test.

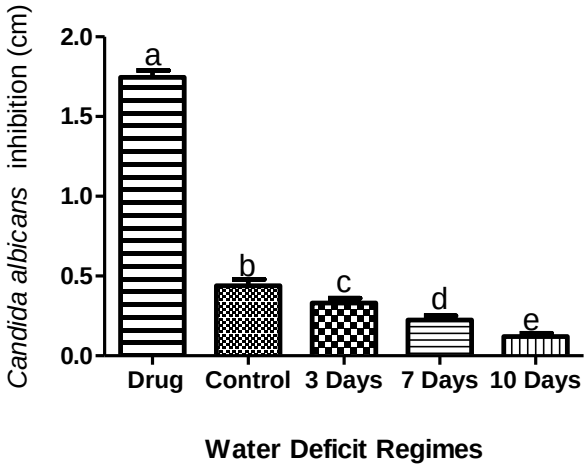

Figure 4. Mean inhibition lengths of *Candida albicans* by extracts of *Bryophyllum* plants subjected to different water deficit regimes (bars with similar letters for each phytochemical are not significantly different at p< 0.05 using Duncan's multiple range test.

trations of tannins increased by 12.9 to 29.5%, flavonoids increased by 22.1 to 45.1%. The increase in saponin content was remarkable; by 60 to 206%, respectively. The alkaloid content was reduced by water stress by 1.3 to 10.5%. Mundree et al. (2002) had earlier shown that water deficit increases the concentration of metabolites. Water stress leads to increases in cyanogenic glycosides, glucosinolates, terpenoids, alkaloids and tannins (David, 1998). However, the observed decrease in alkaloid concentration as a result of water deficit is supported by Belesky (1989) that water deficit regime decreases pyrrolizidine alkaloid yield in endophytes. It appears that not all plant secondary metabolites respond in similar ways to environmental stress. Many phytochemicals participate in the detoxification of active oxygen and are important for plant responses to biotic or environmental stress (Caldwell et al., 2005). Jain et al. (2008) and Tatsimo (2012) reported the antifungal property of *Bryophyllum* against the following fungi: *C*. *albicans*, *C. glabrata*, *C. tropicalis* and *C. pseodotropicalis.* The ethanolic extracts of the treated

plants showed varying lengths of inhibition zones of *C. albicans*; decreasing with increase in water deficit interval (Figure 4). This implies that the more the water deficit stress *Bryophyllum* is subjected to, the less is its potency against the growth of fungus.

The decrease in inhibition of *C. albicans* corresponded with the decrease in alkaloid content. Since concentrations of tannins, saponins and flavonoids increased with increasing water deficit intervals while alkaloids decreased corresponding with reduced inhibition of fungal growth, alkaloids may form the active antifungal property of *B. pinnatum*. Inhibition growth of *C. albicans* was remarkably high with the control antifungal drug (clotrimazole) compared with the plant extracts. This may imply that the concentration of extracts should be increased to obtain close antifungal activity.

Conclusion

Water deficit stress caused marked reduction in growth and increased the concentrations of tannins, flavonoids and saponins while the alkaloid content was reduced. The inhibition zones decreased with increasing water deficit intervals corresponding with the decrease in alkaloid content. Since the concentration of extracts used in this study showed low inhibition zones, higher concentrations of extracts of *Bryophyllum* may be required in the treatment of diseases caused by *Candida* spp.

REFERENCES

Akinpelu DA (2000). Antimicrobial Activity of *Bryophyllum pinnatum* Leaves. Fitoterapia 71(2):193-194.

Belesky CB (1989). Influence of Endophyte and water regime upon tall fescue accessions II pyrrolizidine and Ergopeptine alkaloids. Oxford J. 64(3):343-349.

Boham BA, Kocipal-Abyzan R (1994). Flavonoids and condensed tannins from leaves of *Hawaiian vaccinium vaticulatum and V. calycinium.* Pacific Sci. 48**:**458-463.

Caldwell CR, Britz SJ, Mirecki RM (2005). Effect of temperature, elevated carbon dioxide and drought during seed development on the isoflavone content of soybean (*Glycine max* L.) grown in controlled environments. J. Agric. Food Chem. 53:1125-1129.

Charles DJ, Simon JE, Shock CC, Feibert EB, Smith RM (1993). Effect of water stress and post-harvest handling on artemisinin content in leaves of *Artemisia annua* L. In: Janick J, Simon JE (eds.), New crops. Wiley, New York.

Cowan MM (1999). Plant Products as antimicrobial agents. Clinical Microbiol. Rev. 12:564-582.

David SS (1998). Plant Secondary Metabolism. Springer USA.

Devasagayam TPA, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD (2004). Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects. J. Assoc. Phys. India 52: 796.

Egunjobi JK (1969). Some common weeds of West Africa. *Bull. Resource Division.* Ministry of Agriculture. National Resources western State, Ibadan, Nigeria.

Eze JMO (1965). Studies on Growth Regulation, Salt uptake and Translocation Ph.D. Thesis. University of Durham, England.

Harborne JB (1973). Phytochemical Methods, London. Chapman and Hall, Ltd.

Jain VC, Shah DP, Patel NM (2008). Microscopical and Preliminary Phytochemical Studies on Leaves of *Bryophyllum pinnatum*. J. Pharm. Res. 1(2):130-232.

Kamboj A, Saluja AK (2009). *Bryophyllum pinnatum* (Lam.) Kurz: Phytochemical and pharmacological profile: A review. Phcog. Rev. 3(6):364-374.

Majaz QA, Tatiya AU, Khurshid M, Nazim S, Siraj S (2011).The Miracle Plant (*Kalanchoe pinnata*): A Phytochemical and Pharmacological Review. Inter. J. Res. Ayur. Pharm. 2(5):1478-1482.

Mundree S, Baker B, Mowla, S, Govender K, Maredza S, Willingen A, Muyanga S, Farrant J, Thomson J (2002). Physiological and molecular insights into drought tolerance. Afr. J. Biotech. 1**:**28-33.

Noggle GR, Fritz GJ (1976). Introductory Plant Physiology. Prentice-Hall Publishers. New Jersey.

Obadoni BO, Ochuko PO (2001). Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta states of Nigeria. Global J. Pure and Appl. Sci. 7 (3):455-459.

Okwu DE (2003). The potentials of *Ocimum gratissimum, Pengluria extensa* and *Tetrapleura tetraptera* as spice and flavouring agents. Nig. Agric. J. 34:143-148.

OjewoleJA(2002).Antihypertensivepropertiesof*Bryophyllumpinnatum* (Clam; Oken) leaf extracts. Am. J. Hypert. 15(4):143-148.

Ojewole JA (2005). Anti-nociceptive, Anti-inflammatory and Anti-diabetic Effects of *Bryophyllum pinnatum* (Crassulaceae) Leaf Aqueous Extract. J. Ethnopharm. 99(1):9-13.

Siddarta PAK, Chaudhuri N (2002). Studies on the anti-ulcer activity of *Bryophyllum pinnatum* leaf extract in experimental animals. J. Ethnopharm. 30:97-102.

Sofowora A (1993). Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria.

Tatsimo SJN, Tamokou JD, Havyarimana L, Csupor D, Forgo P, Judit H, Kuliate J, Tane P (2012). Antimicrobial and antioxidant activity of Kaempferol rhamnoside derivatives from *Bryophyllum pinnatum*. BMC Res. Notes 5:158-186.

Trease GE, Evans WC (1989). Pharmacognsy. 11th edition. Brailliar Tiridel Can. Macmillian Publishers.

Umebese CE, Olatimilehin TO, Ogunsusi TA (2009). Salicyclic Acid protects Nitrate reductase activity, growth and proline in Amaranth and Tomato Plants during water deficit. Am. J. Agric. Biol. Sci. 4(3):224-229.

Van-Burden TP, Robinson WC (1981). Formation of complexes between protein and Tannin acid. In: Medicinal plants and Traditional medicine in Africa. A Sofowora ed. Spectrum Books Ltd, Ibadan, Nigeria. p. 289.

Watermann PG, Mole S (1989). Extrinsic factors influencing production of secondary metabolites in plants. CRC press. USA.