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Micropropagation Photoautotrophic *Kalanchoe pinnata* in Water and Humus with use of Natural Light, and Determination of Total Flavonoids: A Review

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ABSTRACT

Micropropagation is a vegetative propagation method widely studied in many different plant species, beinga mode in tissue culture, the one that has found widespread and proven practical applications. Among the advantages of its use is the possibility of obtaining various plants from an initial explant, regardless of the season, besides the reduction of time and area required for the species propagation. The development of photoautotrophic micropropagation systems with natural light usage, emerge as potential possibilities to increase the micropropagation efficiency and help reduce costs, making it commercially viable for active principles and phytotherapeutics production. Thus, the flavonoid content contained in *Kalanchoe pinnata* plant extract, can base the pharmacological activity of it when used by population, for the cure and prevention of various types of chronic noncommunicable diseases (NCDs).

Keywords: *Kalanchoe Pinnata*, Micropropagation, Photoautotrophic, Flavonoids, Cost Reduction

I. INTRODUCTION

Photoautotrophic Micropropagation

Micropropagation is a vegetative propagation me-thod widely studied in many different plant species, being the mode in tissue culture, the more widespread and has found proved practical applications [50]. Among the advantages of its use is the possibility of obtaining various plants from an initial explant, regardless of the season, besides the reduction of time and area required for the species propagation. Moreover, due to best sanitary conditions by cultivation of meristems previously treated by thermotherapy to eliminate diseases, there is the reproduction of the mother-plant genotype usually with fidelity during multiplication, which allows the propagation of species difficult to propagate by other methods.

The development of photoautotrophic micropro-pagation systems (production of micropropagules without addition of sucrose in the culture medium and under ambient conditions favoring photo-synthesis) [47], with natural light usage, emerge as potential possibilities to increase micropropagation efficiency and help reduce costs, making it commercially viable.

Studies have shown that light can determine the growth direction of plants (phototropism). It is able to determine chloroplasts [21, 22] and plant organs (photomorphogenesis) [21] differentiation, espe-cially the leaf, the most susceptible organ to environ-mental changes [23, 24], and in other substances production, such as flavonoids [21]. This fact raises the possibility of studying various light sources effects at different wavelengths, focusing on plants in order to verify and modulate the amount of synthe-sized substances.

The light also strongly influences hormones biosynthesis. Vegetal hormones are molecules responsible for the seedlings development, present in low concentrations [26]. These substances act in all morphogenetic processes, including those induced by light. Studies have shown that gibberellin levels in events like disestiolation, germination and tuberization are directly linked to light, regulated by phytochrome or cryptochromes. The indole acetic acid (IAA) and cytokinins levels in disestiolation are also regulated by phytochrome. The light is able to regulate abscisic acid (ABA), ethylene and brassinosteroids levels in a phytochromes and cryptochromes dependent process [32]. The interaction between light, photoreceptors and plant hormones has a strong influence on plant life, even regulating tissues and organs development, that is, morphogenesis.

Studies have shown that the relationship between light and flavonoid biosynthesis is also closely linked. Flavonoids provide coloring for plants ranging from lilacto blue. The ability of these molecules to absorb wavelength in UV range gives to these substances a protection function against damage caused by this type of radiation [36, 37] and microorganisms or fungi [36].

Other authors have shown that different spectral ranges differently influence the development of plant organs, leading to changes in several anatomical characteristics such as leaf thickness, leaf area, stem diameter, stomata and trichomes density [53, 54, 55]. Besides morphological and anatomical features, light may influence secondary metabolites production such as flavonoids. The flavonoid biosynthesis occurs by secondary and mixed pathways (shikimate and acetate pathways), being regulated by blue and UV light in a process mediated by cryptochromes. In this process occurs the expression of the enzyme phenylalanine ammonia lyase, essential for the first step of fenilpropanoídica and chalcone synthase pathways, the first flavonoid biosynthesis pathway [38]. Due to these molecules protective action against microorganisms in plants, it makes them a target to pharmacological studies using plants.

The perception of color and the amount of substances that plants produce, as a rule, are related to spectral quality of light they receive. The solar radiation that reaches the Earth covers a spectral range extending from 290 nm (ultraviolet) to 4000 nm (infrared) [21]. Many of these wavelengths are absorbed by ozone in the atmosphere, by the atmospheric oxygen and CO2. On average, 45% of the radiation coming from the sun are in the range from 380 nm to 710 nm [21]. It is in this spectral range that is the visible light, composed of seven spectral ranges, the seven rainbow colors.

In this range, there are also the wavelengths used by plants for photosynthesis (photosynthetically active radiation, FRG) [21]. However, light is not only utilized by the plant as a source of energy, it also controls the plant growth and development through signals. Plants are able to monitor the intensity, quality, direction and duration of light. As already mentioned, the solar radiation is decisive in many physiological processes such as stem elongation, germination, stomatal conductance, chlorophyll synthesis.

Due to its characteristics of sessile beings and their autotrophic nature, dependent on light energy, plants have developed environmental recognition mechanisms realizing the variations in the quantity and quality of light they receive, according to the vegetation cover in a particular location which can be closed or opened, light is gradually assimilated by layers of superimposed sheets.

Thus, the canopy energy is greater than the lower layers, meaning that the light reaching the soil is richer in wavelengths than that reaching the canopy, that is, it tends to contain more light in the red range. In addition, a few feet below the topsoil, only reaches lengths corresponding to the extreme red [21]. This effect is called radiation attenuation [21]. Different species require different amounts of light to its perfect development. Hence it is so important that plants recognize the different quantities and qualities of light where they are located.

In this manner, plants can regulate their development in order to seek light. This recognition process is through special molecules called photoreceptors, able to recognize different wavelengths. The processes regulated by light are categorically divided into two main classes: phytochrome mediated, red light receptors and cryptochromes mediated, blue light / UV-A receptors [26]. The phytochrome are the most characterized photoreceptors and form a family of

five proteins (phytochrome A, B, C, D and E) of approximately 125 kDa [27].

Each phytochrome has two forms, in particular spectrally reversible: one that absorbs red light (Pr - biologically inactive) and one that absorbs extreme red wavelength (Per - biologically active). Pr, when absorbs light in the 620-680 nm range is converted to the Per, this by absorbing radiation in the 700-800 nm range becomes Pr. The balance between the two forms, given by red and extreme red proportion in the environment light, will cause physiological responses in the plant [28, 21, 27].
The greater the amount of far red, the plant is more shaded. The amount of red and far red that the plant receives also indicates the seasons (long or short days).

This information determines the plants aspects such as germination, flowering, maintenance or not plumular hook, chlorophyll synthesis and circadian rhythm. Although in most cases, responses regulated by phytochrome are linked to red light, some studies show dependent responses of blue and UV-A light [29,30].

The cryptochromes are a family of blue / UV-A light photoreceptors extremely important during disestiolation of plants grown in the dark [31]. They are involved in the inhibition of stem growth processes, cotyledon expansion and chlorophyll synthesis. There have been shown that cryptochromes act in coordination with phytochromes in several processes and its action is temperature dependent [31]. However, phytochrome and cryptochromes, along with other photoreceptors, are not the only substances that act in photomorphogenic events. Other substances act on plant tissue, translating in them environmental light conditions. These substances are plant hormones.

It is possible to observe that among the environmental factors that most influence the plant development is the lighting. Studies have indicated that in addition to the amount of light received, the spectral quality of it is also important as it can induce morphoanatomic answers, for example, it can cause different red and extreme red ratios altering the structure of the mesophyll [24] or the stomata density optional components. The standard medium used universally for micropropagation of medicinal [33]. The leaf is the organ that most responds to environmental radiation, being therefore chosen by many authors as object of study of spectral quality and quality effect in plants [34, 33, 24, 22, 35]. Different plant species are adapted to different lighting conditions, with an amount of light to be optimum to perform photosynthesis. Plants usually have morphoanatomic features related to these different light environments. Such characteristics provide the best use of the incident light and protection of photosynthetic apparatus [21]. During its life, plants can be exposed to different lighting conditions, so it is possible to notice that most plants have developed adaptation mechanisms, mainly anatomical, of the individual to the new climatic conditions [22]. This flexibility occurs even at a cellular level, as in the case of different organizations and developmental changes in mesophyll chloroplasts [21, 22].

Some advantages of photoautotrophic micropropagation at the expense of natural light compared to the conventional method of micropropagation include plant growth increase. Due to the removal of sucrose from the culture medium, there are improvements in the physiological characteristics of the plant, once the cultivation environmental conditions are more natural, reducing plant stress during acclimatization, increasing the percentage of survival of seedlings [48, 49, 50], elimination of lighting costs and reduced costs for repairs and maintenance, and also possibility of use of simplified facilities reducing construction costs [51].

In conventional micropropagation, the heterotrophic or fotomixotrófica nature of plant growth is directly or indirectly responsible for most of the factors related to the cost of production of micropropagated plants [50]. Explants are cultured in flasks without gas exchange and with high relative humidity (about 98%), high ethylene concentration, low CO2 concentration (which decreases from 3.000 to 1 mol 9.000μmol the dark period to less of 100μmol mol-1 during the photoperiod), and low photon flux density of photosynthetically active. That means that low lightning (40 - 50μmol m-2 s-1) and with sucrose as a major source of metabolic energy [52], once the explants show low photosynthetic rate [50]. The nutrient medium used is composed of essential and

plants is MS medium (Murashige and Skoog) [56], which has as essential nutrients: inorganic salts, carbohydrates, vitamins and growth regulators.

Many explants or in-vitro plants have the ability to grow photoautotrophicly, i.e. without sucrose in the culture medium and under environmental conditions, which promote photosynthesis [50]. The photosynthetic process, contrary to what occurs in the respiratory chain, need an external source of energy, without which it does not occur, showing that there is a strong relationship between light and plants, photosynthetic organisms. Since, during photosynthesis, light is not only a regulation factor, it acts as an important component in the biochemical reaction [21], being able to interfere in several physiological factors, such as chlorophyll production, stem stretching regulation, in enzymes production and other substances such as anthocyanins, a type of flavonoid [21]. In this sense, it is understood that the light is a determining factor for plants to be able to, from inorganic molecules, synthesize carbohydrates.

Kalanchoe pinnata Description

The Brazilian flora is extremely rich, because in it there are thousands of species of medicinal plants, and among these plants there is *Kalanchoe pinnata*, which despite being widely used by the population, still has few studies proving its medicinal properties. It is a perennial plant, therefore easily found in several climates and regions of the world, and easy handling due to their morphological characteristics (Table 2).

The different species of the genus Kalanchoe (Crassulaceae family) are known in popular medicine in many countries as it can be observed in Table 1. As used in treatment of inflammatory processes and in several diseases. This fact favors the search for new bioactive molecules. Among the main active principles of *Kalanchoe pinnata*, there are the polyphenols [59], which are flavonoids, antioxidants, mucilage and others (Table 3). Among the present flavonoids, there is quercetina43, which has shown significant action in the leishmaniasis treatment [41].

Quercetin and kaempferol are flavonoids widely spread throughout the plant kingdom and have significant anti-inflammatory action, that can be attributed to inhibition of the enzymes phospholipase A2 (PLA2), 8 lipo-oxygenates to, cyclooxygenase and inhibition of nitric oxide production, through modulation of enzyme iNOS [35, 37, 59].

Table 1- Taxonomy of *Kalanchoe pinnata (Lamarck) Persoon:*

Kingdom		Plantae (Plantas)
sub Kingdom		Tracheobionta (vascular plant)
Super division		Magnolioliophyta (Flowering plant)
Class		Rosidae
Order		Saxifragales
Family		Crassulaceae Stonecrop family [39]
Genre		Kalanchoe
Species		*Kalanchoe pinnata* (*Lam.*) *Per* [14]
Synonyms		Verea pinnata, Crassuvia floripendia, C. calyculata, Cotyledon calycina, Bryophyllum calycinum, Crassula pinnata, Sedum madagascariense, B. germinans, C. rhizophilla. [13]
Source		Uncertain. It is believed to be the Mauritius Islands, Africa, India and Indian Ocean islands
Distribution		It is distributed through-hout India and grown in wild gardens in the hills of northern and western India, Deccan and Bengal [18]. In Brazil, they can be found from Sao Paulo to Bahia, mainly in the coastal zone.
Regional Names	Brazil	Saião, Folha-da-fortuna, Coirama, Roda-da-fortuna, Folha-da-costa, Folha-grossa, Erva-da-costa
	India	Zakhm-hayat
	Arabia	kushnulhayat
	Walking stick	Koppata
	Sanskrit	Asthi-bhaksha
	telugu	Simajamudu
	Tamil	Ranakalli
	kannada	Ganduklinga
	malayalam	Elamurunga
	Persian and Urdu	Chubehayat [17, 19]

Table 2 : Morphological characteristics of *Kalanchoe pinnata*:

Dimension	Sublenhosa plants, perennial fleshy, 1.5 meters high; succulent stems, hermaphrodite, tubular, penduladas, pale green or yellow reddish.
Seeds	Oblong small soft - ellipsoid, striatum bad, soft. The leaves often produce, at the ends of the lateral nerves, buttons furnished with roots, stems and leaves, which drop off at the same time become new plants [13].
Sheets	Juicy, Dimensions: length 7-20cm 4-9cm wide on average, 10 to 25 leaves, or occasionally when lower generally have 8-12 and 6-8 in size, the upper usually 3-5 or sometimes 7 - leaflets, long and pointed, the united petioles by a ridge around the stem. Leaflets ovate or elliptical, serrated [17].
Fruits	With 10-14 mm long, produce numerous seeds per fruit in closed chalice and corolla. [13]
Trunk	succulent stems, hollow, rarely branched. [13]

One of the main features of *Kalanchoe pinnata* is its ease in micropropagation in aquatic environments and on land, having high adaptability to different climates and easy handling conditions (Table 3). Due to these factors, it was decided to replace the synthetic medium MS [56] for water and earth through composted with natural bio-fertilizer and humus, and bio-organic materials used in biofertilizers, provided from waste solids reuse fruit.

According to Fukuoka [59], the natural fertilization is defined by natural conditions, soil regeneration with the soil itself, returning to the soil what it hasgiven, such as fruits, vegetables and grains. The soil is enriched progressively mind, and the content of natural mind-nutrients present is balanced with the help of natural fertilizer from the own culture developed in this soil. In Table 3 below the optimal growth conditions for the *Kalanchoe pinnata.*

Table 3. Characteristics of a better climate adaptation and management:

Life Cycle	Perennial
Climate	Enjoy warm weather
luminosity	Enjoy light full sun and half-shade
pruning	Not necessary, but cut dry parts can be carried out, and to contain the spread, remove new growth if you want
Cultivation	It must be used substrate that has good drainage, which does not accumulate water which will cause the death of the plant. Mixing Tip: two pieces of coarse sand construction, a part of common garden and land part of the plant. It supports soil with low humidity, and also develops in water
Fertilization	At the site preparation, apply about two NPK tablespoons of 04-14-08 formula per square meter
Propagation	Vegetative from seedlings, and shoot buds formed along the sheet margins, producing young plants [40]
Medicinal plant	Her daughter medicinal properties have been used in the treatment of various diseases.
Chemical constituents	Mucilage, tannins, organic acids, minerals, quercetin glycosides [41, 43]. bufadienolídeos [44, 45].
Toxic plant	Special care should be taken with small children, pets and master-mind with grazing animals
Use	Quite showy ornamental effect, is very well in rock gardens forming together with other succulents

The objective of the research was to investigate the development of the crop in water and soil with humus (natural biofertilizers) of *Kalanchoe pinnata* leaves, (Lam Pers), kept under natural light (photoperiod).

Extracts of developed sheets were evaluated by spectrophotometry, as the concentration of flavonoids, which is, the minimum necessary to justify the

medical activities of the extracts of *Kalanchoe pinnata* leaves described in Table 4.

Table 4: Medicinal Uses Worldwide:

Brazil		Furunculous [20], Intestinal problems, arthritis, ulcers, athlete's foot, abscesses, bubos, bronchitis, burns, calluses, conjunctivitis, corns, dermatosis, coughs, earaches, eczema, fever, urinary insufficiency, rheumatism, itch, glaucoma, infections, headache, kidney stones, scurvy, tumor, wart, sedative, whooping cough, wounds, inset stings, lymphatic disorders, mouth sores, respiratory infections, mouth sores, erysipelas [10]. Immunosuppressant, leishmaniasis [41, 43], diabetes [41, 42, 43] hemostatic, antiseptic, healing topical, inflammation (stomach pain) [15, 16]
USA		Chicken pox, stomachache, fevers [10]
Mexico		Inflammations, wounds, eye infections, headaches, menstrual disorders [10]
Ecuador		Bruises, broken bones [10]
Guatemala		Diarrhea, pain, skin problems, aches [10]
Nicaragua		aches, burns, colds, pain, fever, headache, respiratory infections, coughs, childbirth [10]
Bangladesh		Coughs, fever, constipations, mucus, epilepsy [11]
Peru		Bacterial infections, boils, broken bones, ulcers, urethritis, sore, skin problems, nausea, migraine, intestinal problems, eye infections, epilepsy, gas, headache, heartburn, inflammation, cancer (lymphoma), bronchitis, conjunctivitis, coughs, earaches [10]
South America		Asthma, tumors, headaches, colds, earaches, chest colds, sores, strains [10]
Nigeria		Coughs, earaches, pimples, inflammation, eczema10, cut umbilical cord in new born baby [7]
West Indies		Ulcers, menstrual disorders, urinary disorder, hypertension [10]
Other places		Arthritis, asthma, burns, bruises, constipation, malnutrition, headaches, migraines, nephritis, respiratory infections, earaches, diabetes, paralysis, rheumatism, swelling [10], and to induce vomiting of blood, expel worms, cut umbilical cord in new born baby [7]
Vietnam		Anti-inflammatory and antibacterial [8]
India	Orissa	Diarrhea [5, 2]
	Karnataka	Leaf juice externally, applied to scabies and leucoderma and leaf decoction applied over, cuts to stop bleending [10, 12]
	Maharashtra	The leaves in used against cough dysentery [4]
	in Himalaya	Leaves are applied on wound, bruises, insect bite, swelling [9]
	Arunachal Pradesh	Leaf extract is taken in empty stomach is used in the treatment urinary bladder stones and fewer in children's [3]

II. METHODS AND MATERIAL

A. Humus preparation

The papaya, banana, orange, mandarin and mango residue were gathered and milled with the aid of an organic waste processor in order to form a biomass of fruits. They were then added to water ratio gradient in order to obtain the best uniformity for the process of fermentation the kefir grains, for cleaving complex sugars and free reducing sugars, providing among these microelements to facilitating uptake by plants. During the fermentation, the fruit biomass was placed in glass containers covered with fabric. Subsequent to fermentation, biomass was added to the earth for the humus formation, through composting method. Fermented biomass was evaluated to their chemical composition of trace elements as well as dosage of total ash, moisture, protein, carbohydrates, glucose, fructose, fiber and lipids.

B. Determination of the chemical composition of biomass fruit

The chemical composition of biomass (consisting of papaya, orange, mango, banana and tangerine waste)

was determined according to the methodology of the AOAC [57]: moisture (925.45); protein (960.52); lipids (920.39) and ash (923.03). The conversion factor used for determining the protein content was 6.25. The energy value was calculated using the general factors of Atwater and considering the energy from dietary fiber (DF) [58]. The analyzes were performed in triplicate and the results expressed in g per 100 g of sample in the integral base. The DF content was determined according to AOAC method 991.43 [57]. The analysis was done in quadruplicate and the results expressed as g / 100g sample basis. Dosages of glucose and fructose were made in triplicate by Waters chromatograph column for detection of sugars (Shodex SC 1011); refractive index detector; Mobile phase: EDTA-Ca 0,187g / l. Chromatography conditions: column temperature: 72 ° C; detector temperature: 45C, mobile phase flow: 0.6 ml / min. Detector sensitivity 32, Volume injection: 10µL, running time: 30 minutes.

C. Photoautotrophic Micropropagation

The leaves of *Kalanchoe pinnata*, initially developed in soil, were transferred to individual containers in which were placed submerged in water. The jars were kept at room temperature, covered by a synthetic porous fabric (TNT), provided direct daylight (photoperiod). Initially, the dimensions and leaflets of each sheet were measured. After a period of seven to ten days of permanence in the same water, the size of sprouts from the leaflets and their roots were measured as shown in Fig. 1, and the water was changed. Each bud, with its leaves and roots were transferred to the humus (prepared from biomass fruit fermented with kefir and composted It was transferred 0.5 ml of *K. pinnata* extract solution into a 25 mL volumetric flask and was added 0.100 mL of 10% aluminium chloride with 4.3 ml of grain alcohol. We adopted the same experimental procedure dilutions, described below, for the preparation of the absorbance of the response curve as a function of the concentration (mg / ml) ranging from 60 to 260 (mg / mL) of total flavonoids equivalent of catechin using the equation of the line from the calibration curve of the standard secondary reference catechin being evaluated linearity.

Linearity

To check the linearity, it was elaborated analytical curve with the stock solution of standard secondary reference with soil). Then, the micropropagation development monitoring was made, measuring the dimensions of the roots and formation, and the growth of buds in relation to the cultivation time.

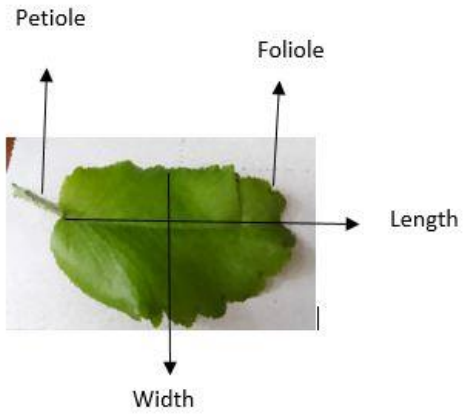

Figure 1. Illustrative Photo *Kalanchoe pinnata*. Source: Author's photo

D. Preparation of the Kalanchoe pinnata leaves extract

There were extractions of leaves developed to evaluate the concentrations of flavonoids. The alcoholic extract of 100 g of cereal leaf was macerated in ethanol, and transferred quantitatively into 100 mL volumetric flask, and the final volume with grain alcohol.

E. Determination of total flavonoid, catechin equivalents

catechin (purity = 98%) in the concentration of 500,0µg / ml in cereal alcohol. From the stock solution dilutions were prepared at concentrations of 0.084; 0.05; 0.028; 0.017; 0.0076 g / L, which was reacted with 0.100 mL of 10% aluminum chloride solution (v / v), completing the volume with ethanolic grain alcohol. After 40 minutes of rest, we proceeded to read in a spectrophotometer at 510 nm using as white cereal alcohol solvent plus aluminum chloride solution. Elaborated the analytical curve, checking the linearity of the method, using visual observation and appropriate statistical analysis, obtaining - the equation of the line and the linear correlation coefficient. Equation 1 is the equation of the calibration curve of the standard secondary reference

catechin, where y is absorbance and x is the concentration of standard secondary reference catechin:

y = 10.121 x – 0, 0475 (**1**)
r2 = 0, 9936

From the obtaining of a line to the calibration curve and the correlation coefficient (r2) .9936 was verified the linearity of the method.

III. RESULT AND DISCUSSION

A. Quantification of equivalent total flavonoids catechin in *Kalanchoe pinnata* extract.

In Table 5 it can be seen the results of quantification of total flavonoids, catechin equivalents in *Kalanchoe pinnata* extract, with an average of 42.39 mg / mL.

Table 5 – spectrophotometric dosage of the equivalent total flavonoids catechin present in *Kalanchoe pinnata* extract to 510nm. **EKP**: *Kalanchoe pinnata* extract .: **CF**: concentration of total flavonoid, catechin equivalents.

Concentration of EKp (µg/mL)	Absorbance	CF µg/mL)
60,0	0,2230	17,34
100,0	0,3320	28,00
140,0	0,4219	36,00
180,0	0,5543	50,00
220,0	0,6030	54,00
260,0	0,7456	69,00
		Average: 42,39

B. *Kalanchoe pinnata* Micropropagation

Water-soaked sheets produced roots from all the leaflets, and buds (buds) with leaves in most leaflets, from 7 to 10 days. In a similar period, the sheet cultivated on earth developed, on average, two shoots 10 days and 40 days from the main stem height reached an average of 8 cm from each bud, 8 new sheets for each sprout dimensions Similar to that of the mother sheet. In water, the leaves are multiple and diminutive dimensions, three times smaller, and remains in this size and had to be transferred to new culture in water or on land.

C. Statistics of *K. pinnata*.micropropagation

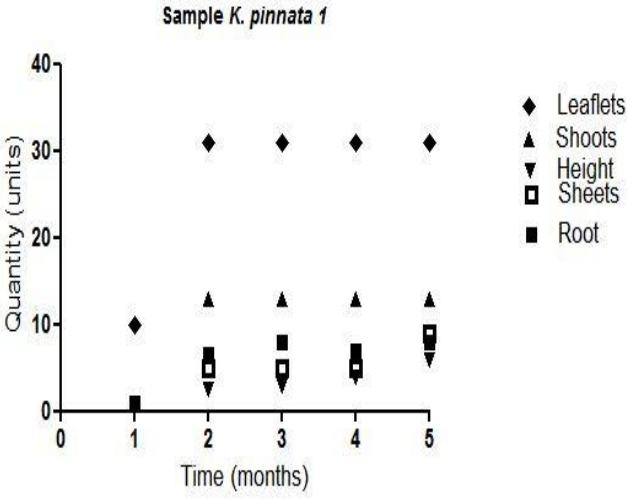

Figure 2. K. *pinnata* with 11.5cm long and 6.5cm wide; ANOVA: p < 0.05 ***

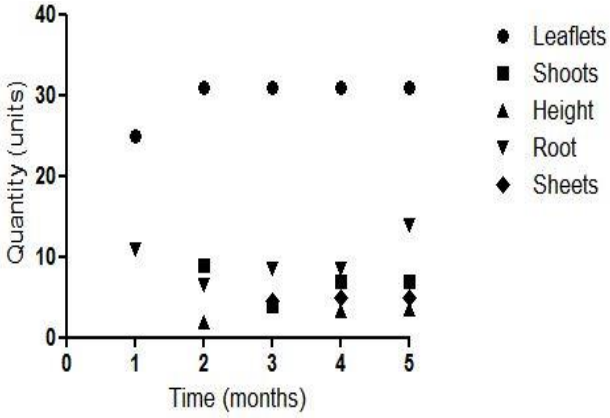

Figure 3. *K. pinnata with 15.7 cm long and 10 cm wide; ANOVA: p <0.05 ****

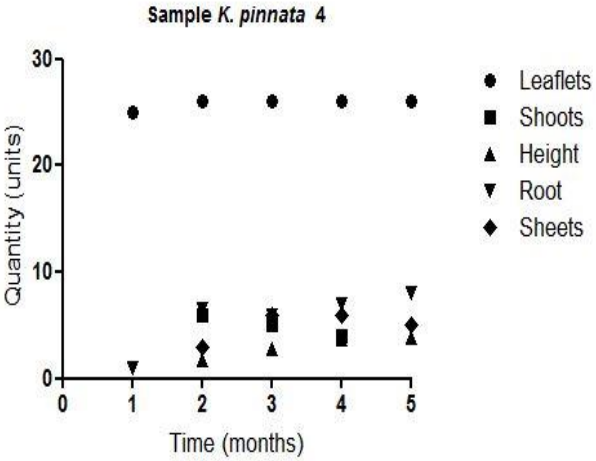

Figure 5. *K. pinnata with 14cm long and 9 cm wide; ANOVA: p <0.05 ****

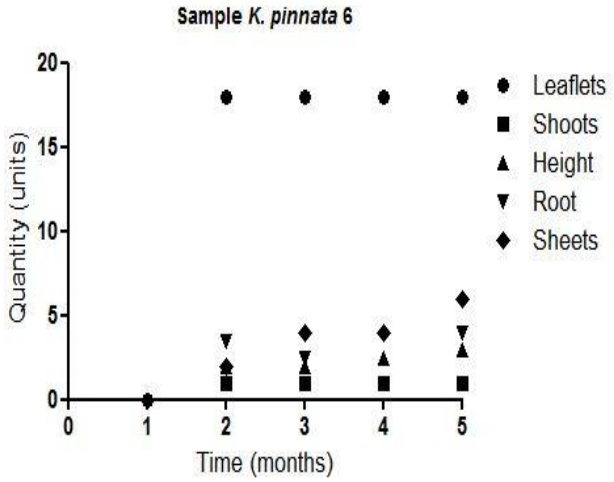

Figure 7. *K. pinnata with 18cm in length and 12.5 in width;*
*ANOVA: p <0.05 ****

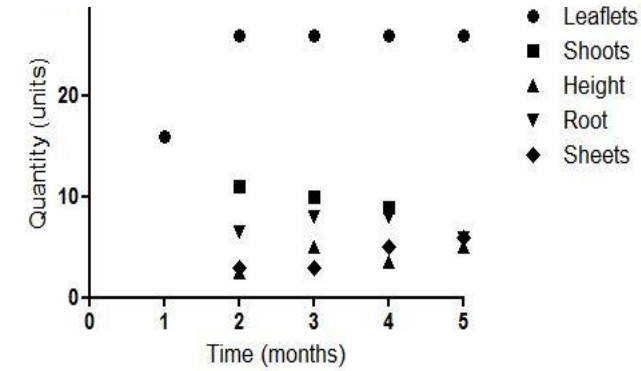

Figure 4. *K. pinnata in length and 13cm 9.7 cm wide;*
*ANOVA: p <0.05 ****

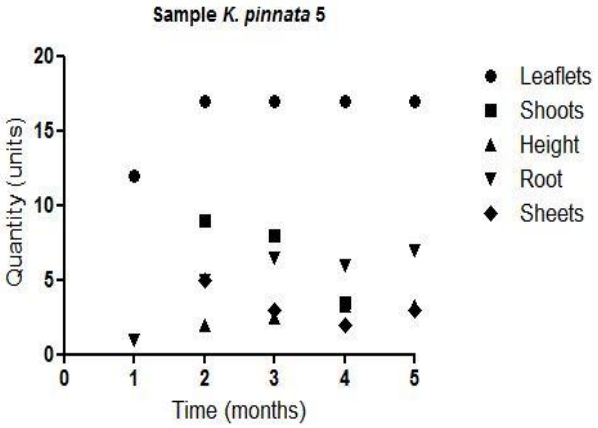

Figure 6. *K. pinnata with 11 cm long and 5.5 cm wide; ANOVA: p <0.05 ****

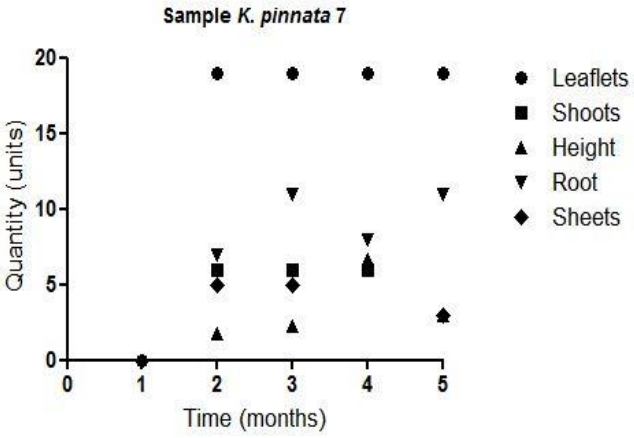

Figure 8. *K. pinnata* with 10,5cm long and 5,4wide; ANOVA: P < 0, 05 ***

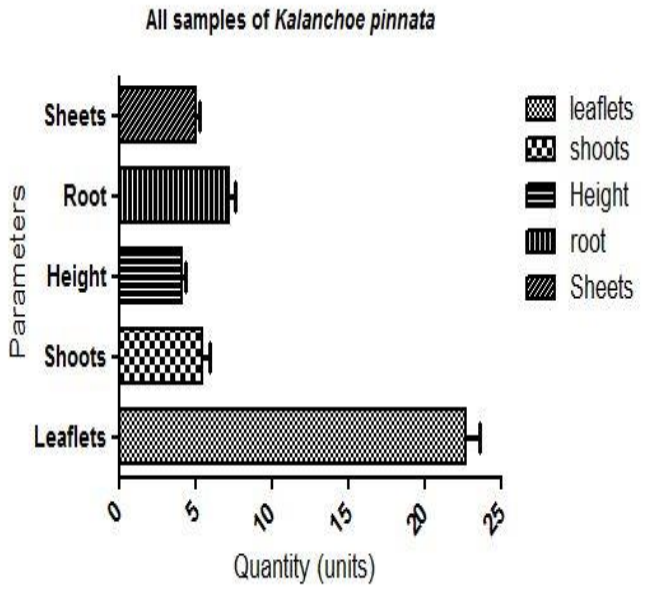

Figure 9. *K. pinnata (all sheets with varying sizes) ANOVA: P <0.05 ****

A. Determination of the chemical composition of biomass fruit

Table 6 - Proximate analysis of biomass of fruits

Biomass fruit (g/100 g)	**(I)**	**(II)**	**(III)**	**(IV)**	**Average**
Carbohydrates	4	4,9	4,8	--	4,56
Lipids	0,02	0,03	0,02	--	0,023
Proteins	0,4	0,5	0,8	--	0,56
Ash	2	1,98	2	--	1,99
Humidity **(%)**	89	89	89	--	89
Fibers	5	4,8	4,9	4,9	4,9
Glucose **(g/L)**	7,756	7,54	7,15	--	7,483
Fructose **(g/L)**	9,107	8,80	8,31	--	8.756

IV. CONCLUSION

The micropropagation of *Kalanchoe pinnata* proved to be auspicious, since it presented a high capacity for reproduction by fotoautotrophic micropropagation in water with the use of natural light, and soil humus with derived bio-organic fertilizer. Observe a satisfactory value the concentration of micronutrients present in the biomass of fruits needed for plant nutrition, this fact makes possible the replacement of synthetic media, which implies lower costs allowing obtaining of one rich source of active ingredients for herbal, water extracts (poultices) or cereal alcohol (tinctures).

The photoautotrophic plant micropropagation, in addition to increasing the growth of in vitro explants, also minimizes the risk of microbial contamination, reduces production costs, improves the physiological characteristics of the plant and facilitates their acclimatization to ex vitro conditions due to have been grown in conditions natural.

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