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The Natural Alternatives: The Impact of Plant Extracts on Snowbush (*Breynia disticha* Forst.) Cuttings' Morpho-Physiological and Biochemical Characteristics

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Abstract: The utilization of cuttings remains a prevalent method for vegetative propagation in various plant species. Synthetic rooting hormones have conventionally been employed to enhance the rooting process; however, their high cost and potential environmental risks have necessitated the exploration of natural alternative compounds. In this study, the efficacy of natural plant extracts (pure honey, aloe extract, willow extract, moringa extract, and cinnamon powder) and synthetic auxin derivatives (indole-3-butyric acid and α-naphthaleneacetic acid) at a concentration of 2000 ppm was compared for their influence on rooting and vegetative growth of snowbush (*Breynia disticha*) cuttings, a significant ornamental and medicinal shrub. Results demonstrated that while the synthetic hormones produced the highest survival percentages and root number, the roots were consistently short, thick, friable, and largely void of secondary roots. Conversely, the alternative natural plant extracts, particularly aloe extract, exhibited promising outcomes, showcasing good rooting percentage and root number, significantly increased root length, and fresh and dry root weight. Furthermore, aloe extract demonstrated the potential to enhance shoot length and alter the chemical constituents of the cuttings. The highest values of total phenols and nitrogen percentage were recorded with cuttings treated with indole-3-butyric acid, followed by cuttings treated with aloe extract. While using aloe extract, we recorded the highest values of total carbohydrates, phosphorus, and potassium percentages. Notably, the highest endogenous levels of indole acetic acid and gibberellic acid, along with the lowest abscisic acid concentration in cutting tissues, were associated with the highest rooting percentage observed with indole-3-butyric acid, followed by the use of aloe extract. We hypothesized that natural plant extracts, specifically aloe extract, have potential as a viable alternative for synthetic auxins in promoting successful rooting and vegetative growth in snowbush cuttings. Further research is warranted to explore the underlying mechanisms and optimize the application of these natural compounds in vegetative propagation techniques.

Keywords: synthetic hormones; natural plant extracts; pure honey; aloe extract; willow extract; moringa extract; cinnamon powder

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1. Introduction

Plant propagation is a pivotal part of both the horticultural and agricultural industries. With several propagation techniques (sexually and asexually), vegetative propagation by 'cuttings' is still the preferred method for many species due to the fastest, most accessible, and most inexpensive asexual technique. It produces more symmetric plants entirely identical to maternal plants [1–6]. Various factors influence the rooting of the cuttings. The path of adventitious root (AR) formation is a complicated process, often requiring special environmental and physiological conditions of the maternal plant, the time of cutting preparation, the tissue of the cuttings, and hormonal stimuli, whose understanding is pivotal in successful commercial propagation of the plants [7,8]. One of these critical factors affecting rooting formation is hormonal compounds (plant growth regulators). Synthetic plant hormones are often known as plant growth regulators (PGRs), classified into five major groups: gibberellins, cytokinins, auxins, ethylene, and abscisic acid. Auxins such as indole butyric acid are particularly important for rooting the cutting [9]. Recently there has been an effort to replace synthetic plant hormones with natural compounds because of the high cost of synthetic plant hormones, their chemical nature causing environmental risks, and the danger of toxicity in plants, animals, and humans due to the application of overdoses [10]. Thus, it is essential to use alternative natural plant extracts rich in natural antioxidants and plant hormones to ameliorate and stimulate the rooting of cuttings. Different alternative hormones may be used, such as coconut water, moringa extract, willow tea, and honey [11]. Among many natural alternatives, honey has been used, as it is a natural source of numerous vitamins like vitamin C and B1, found in the root initiation stage in the cuttings of different plants [12]. Many plant species' roots have been promoted in the presence of vitamins [13]. Also, natural botanical extracts like *Aloe vera* are rich in phytohormones and can promote and improve numerous plant species' growth [14]. *Aloe vera* gel extract contains essential vitamins, amino acids, enzymes, natural sugars, nutrients, lignin, and plant hormones such as gibberellin and auxins. It contains organic acids such as salicylic acid, a plant root growth promoter [15–17].

Further, agricultural scientists are interested in moringa because it is a rich source of macro- and micro-nutrients, hormones, and antioxidants. *Moringa oleifera* extracts are deemed to be very advantageous for better seed germination, vigorous growth, and deeper root development [18,19]. These may be because the leaf extract of moringa contains amino acids, inorganic compounds, hormones (i.e., gibberellins, indol-3-acetic acid, and cytokinins), some natural antioxidants (i.e., pigments, phenols, flavonoids, proline, and ascorbic acid), proteins, and soluble sugars. It also contains vitamins such as E, C, B1, B2, B3, and A and nutrients such as K, P, Fe, Ca, Mg, and Na [20,21]. Recently, willow extracts have been shown to manifest bio-stimulating effects in the growth of plants, such as flowering, stress mediation, and easier root cutting. This may be due to its indole butyric acid content and the high levels of phytohormone salicylic acid [22,23]. Also, the studies authenticated the presence of many bioactive secondary compounds such as polyphenols (flavonoids, lignans, phenolic acids, tannins, and proanthocyanidins), isoprenoids, and, most importantly, numerous salicylate compounds (salicylic acid, salicin, and saligenin) [24–26]. Other natural plant extracts were also used, such as cinnamon powder, which is regarded as a biological control factor with high potential, particularly as an anti-microbial and as a rooting agent, which is useful for encouraging the formation of roots in a variety of plants [27].

Breynia disticha (snowbush) is an important medicinal and ornamental shrub in the family Phyllanthaceae. Snowbush may be used as a specimen for its beautiful leaves. Also, it forms a colorful hedge as a border shrub in the garden, and branches are resilient enough to disperse along the wall [28,29]. Ethnomedically, snowbush is used in curing headaches, toothaches, and tooth infections [30]. *Breynia disticha* leaf extract contained reasonable quantities of alkaloids, glycosides, flavonoids, starch, and tannins with moderate saponins and proteins. So, naturally, the extract of snowbush is an analgesic, antimicrobial, antioxidant, and anti-inflammatory agent and could have therapeutic potential in manually treating various chronic diseases such as malaria [31]. To expand the cultivation of *Breynia disticha* in Egypt, it is generally propagated vegetatively by cutting, since it is hard to obtain seeds in the Egyptian climate.

Furthermore, the pretreatments required for good germination and the lengthy germination period for seeds made it difficult to propagate this plant [32]. Also, vegetative propagation by stem cutting is tricky. So, the objective of the present research is to evaluate the effects of alternative growth regulators using some natural products like honeybee, aloe gel, moringa extract, willow extract, and cinnamon powder on the rooting of difficult-to-root cuttings of *Breynia disticha* by analyzing the morphology, physiology, and biochemistry changes.

2. Materials and Methods

2.1. Plant Material and Cutting Preparation

Several newly produced healthy and disease-free shoots of *Breynia disticha* (approximate age of two months) were collected within two seasons on the 1st of April from adult donor plants growing in the nursery of ornamental plants at Mansoura University. In the early morning, cuttings about 20–25 cm in length were clipped while the plant cells were fully turgid and immediately placed in flasks full of water until use. Terminal softwood cuttings (7 cm long having 2–3 nodes) were selected for propagation by rooting cuttings.

2.2. Planting and Caring for Cuttings

The cuttings' bases were disinfected by dipping them for five seconds into an alcoholic solution (ethanol 70%) to prevent the appearance of fungal diseases before being treated with the different treatments, i.e., control (distilled water), two auxin derivatives (α-naphthaleneacetic acid (NAA) and indole-3-butyric acid (IBA)) at the concentration of 2000 ppm for each auxin alone, natural honey (75 and 100%), aloe vera gel (75 and 100%), moringa leaf extract (75 and 100%), willow leaf extract (75 and 100%), and cinnamon powder. The uniform length of snowbush cuttings was dipped in the treatment solution for 20 min, while for the treatment of cinnamon powder, the cuttings were rolled in it to create a thin layer at the base.

Treated cuttings were planted in plastic pots (5 cm) filled with a mixture medium of sand: peat moss: and vermiculite (1:1:1, $v/v/v$). All the planted pots were arranged in glass containers (100 $\times$ 60 $\times$ 25 cm) in the experimental room. Irrigation of cuttings during the experiment was carried out by filling the bottom of the glass containers with a thin layer of water (0.5 cm in height) and misting as needed. The glass containers were covered with a clear polyethylene sheet to keep the relative humidity between 85 and 95% and incubated under cool white fluorescent lights of 2500 Lux, with a 16 h/day photoperiod at 25 $\pm$ 2 °C in the experimental room for 40 days.

2.3. Experimental Design and Data Recording

The experiment layout was a completely randomized block design with four replications, each containing four pots for each treatment (4 $\times$ 4 = 16 pots for each treatment).

For all treatments, after 40 days of planting, data recorded were rooting percentage, root number, root length, stem length, and fresh and dry weight of roots (g). Also, nitrogen (g/kg) was determined by the modified Micro Kjeldahl method as described by Pregl [33], phosphorus (g/kg) according to Jackson [34], and potassium (g/kg) according to Black [35]. Meanwhile, 1.5–2.0 cm of each sample of the cutting bases was taken and dried to determine the total carbohydrate (%) and phenol content (mg/g DW). Total carbohydrates (%) were estimated colorimetrically using the anthrone sulphuric acid method described by Fales [36]. The total soluble phenol content was calculated as mg/g DW using the Folin reagent and colorimetric method by using a spectrophotometer at a wavelength of 730 nm described by A.O.A.C [37] and modified by Daniel and George [38] and a standard curve of pyrogallol.

In addition, endogenous hormones of indole-3-acetic acid (IAA), gibberellic acid (GA_3), and abscisic acid (ABA) were determined according to Chen and Yang [39] in tissues of fresh cutting bases (the basal 5 cm portions of the cuttings), which were isolated during the second season and stored at −20 °C until the analysis. Each sample was ground to a powder by mortar and pestle. A total of 0.6 g of each sample was extracted with 18 mL Butylated hydroxytoluene (BHT-MeOH) solution (0.5 g/L) after one hour at 4 °C, then the extract was filtered, and 9 mL of 5 Mm k-phosphate buffer (pH 6.5) was added to each sample. The extract was centrifuged at 5000 r/min and 4 °C for 15 min and the supernatant was collected. Then fresh cold methanol was poured into the remnant and extracted three times with the aforementioned methods. The total methanolic extract was dried in a rotary evaporator and dissolved in 10 mL methanol. Endogenous hormones (IAA, GA3, and ABA) were determined using HPLC (Perkin-Elmer Binary LC Pump, Waltham, MA, USA) by the injection of the extract into a reverse-phase HPLC, with a methanol gradient in 0.6% acetic acid.

2.4. Statistical Analysis

A simple experiment randomized complete block design was used. Data were subjected to analysis of variance (ANOVA) using a one-way analysis ANOVA using the Costat v. 6.303 [40] program. The multiple comparison test was performed using Duncan's multiple range test (a = 0.05).

3. Results and Discussions

3.1. Effect of the Rooting Treatments on Growth Parameters of Snowbush Cuttings

3.1.1. Rooting Behavior (Rooting Percentage, Root Number, and Root Length)

In the present study, a comparison was made among two synthetic auxins individually (2000 ppm of NAA or IBA) and five different alternative natural plant extracts on cuttings of snowbush. Four parameters were evaluated to study the effect of other treatments on rooting behavior, i.e., rooting percentage, root number, and root length, as shown in Table 1. It is essential to mention that the control treatment in which the cuttings were dipped in distilled water failed to induce roots, most leaves fell off, and cuttings turned brown and then withered after about 15 days. Data presented in Table 1 clearly show that using the two synthetic plant hormones NAA or IBA at a concentration of 2000 ppm achieved the highest rooting percentage of 87.5 and 81.2%, respectively, for the first season and 93.8 and 87.5%, respectively, for the second season, followed by a rooting percentage of 75% with cuttings treated by either 75 or 100% aloe extract or willow extract at 100% for the first season, and a percentage of 81.2% in the second season with aloe extract at 100%, with insignificant difference between values. In comparison, cuttings treated with cinnamon produced the lowest values of rooting percentage in both seasons (25.0 and 18.8%), respectively, compared with the other treatments.

Examining the effect of the different treatments on root number per cutting, the results in Table 1 and Figure 1 show a trend more or less similar to the previous parameters. The two synthetic plant hormones, NAA and IBA, recorded the highest significant root number per cutting in both seasons, at 21.69 and 8.83 roots and 22.08 and 8.66 roots, respectively. The following positive effect on root number was recorded with aloe extract at a concentration of 100%. There was a significant difference among all alternative natural plant extracts since it was 5.98 and 6.14 roots in the two seasons, respectively. Otherwise, the weakest effect in this regard was recorded with cuttings treated with honey at a concentration of 100% and cinnamon in the two seasons.

For root length, as shown in Table 1 and Figure 1, it is important to mention that although cuttings treated by the synthetic plant hormones NAA and IBA produced the highest mean root number, the roots were constantly very short, thick, and friable without or with a small number of secondary roots during both seasons. The data clearly show that alternative natural plant extracts had the upper hand in that respect, since all alternative natural plant extracts had a significantly greater increment effect on root length than the two synthetic plant hormones NAA and IBA. The highest considerable root lengths in the two seasons of 6.04 and 6.35 cm were obtained with aloe extract at a concentration of

100%. The present investigation showed that the rooting behavior of snowbush cuttings varied widely when cuttings were treated with synthetic plant hormones and alternative natural plant extracts. Generally, control treatment failed to promote any rooting behavior growth. These results align with those of [41] on *Zanthoxylum aramatum*, who reported that the control set of branch cuttings had not produced any roots. Also, tip cuttings treated with IBA increased the rooting percentage compared with the untreated cuttings for *Conocarpus erectus* and *Rosmarinus officinalis*, respectively [42–44].

Conversely, synthetic plant hormones significantly had the upper hand regarding rooting percentage and root number. Stimulation of rooting from cuttings treated by growth hormones may be due to motivating carbohydrate synthesis in leaves, hydrolysis of starch, and enhancing the speed of movement and translocation of sugar to the base of cuttings [45]. The ability of auxin to stimulate protein synthesis and the production of DNA and RNA, both of which are necessary for cell division, may account for its ability to promote the induction and development of adventitious roots [46].

The influence of auxins in promoting adventitious root formation is well-documented in several studies on *Buphorbia pulcherrima* [47], *Vitex negundo* [48], *Rosa indica* [49], *Zanthoxylum aramatum*, and *Myrtus communis* [50]. Most of them reported that the best rooting promoter is IBA, possibly due to its fast auxin activity and an enzymatic system of relatively slow destruction [51]. In addition, the stimulatory effect of IBA on the adventitious root formation may be attributed to its impact on the swelling of tissues, cell elongation, cell division, development of root primordial, and formation of adventitious roots [52,53]. Furthermore, exogenous IBA application increased vascular cambial activity, resulting in histological processes leading to root initiation and primordial development [54]. Moreover, IBA treatments may be able to regulate cuttings' endogenous auxin levels [55].

On the other hand, alternative natural plant extracts, especially aloe and willow extracts, showed promising results in that area as they recorded good rooting percentages and root numbers associated with the significantly highest root length. This can be corroborated by the conclusion of [56], who noted that the gel of *Aloe vera* leaves can be recommended as an alternative root-inducing substance that helps to induce the rooting of *Citrus aurantifolia* stem cuttings. The different concentrations of willow bark extract tested, 1.06 µL/L, totally acquired the best results for stem cutting propagation in lavender and chrysanthemum, as it reduced the time taken for adventitious root formation in stem cuttings [57].

Table 1. Effect of different auxins and natural plant extracts on rooting percentage, root number, root length, and shoot length of *Breynia disticha* cutting during two seasons.

Treatments	Rooting %	Root Number/ Cutting	Root Length (cm)	Shoot Length (cm)	Rooting %	Root Number/ Cutting	Root Length (cm)	Shoot Length (cm)
	1st Season				2nd Season			
Control	0.0 g	0.0 h	0.0 h	0.0 i	0.0 g	0.0 g	0.0 j	0.0 h
NAA at 2000 ppm	87.5 ± 7.2 a	21.69 ± 1.10 a	0.82 ± 0.03 g	11.17 ± 0.14 e	93.8 ± 6.25 a	22.08 ± 0.79 a	0.85 ± 0.04 i	10.93 ± 0.11 ef
IBA at 2000 ppm	81.2 ± 6.2 ab	8.83 ± 0.39 b	2.13 ± 0.02 f	11.34 ± 0.13 e	87.5 ± 7.21 a	8.66 ± 0.13 b	2.03 ± 0.09 h	11.27 ± 0.14 de
Honey 75%	37.5 ± 7.2 ef	2.63 ± 0.23 f	3.21 ± 0.10 e	10.39 ± 0.16 g	31.2 ± 6.25 ef	2.75 ± 0.14 e	3.26 ± 0.08 g	10.62 ± 0.20 f
Honey 100%	43.8 ± 6.2 e	1.25 ± 0.14 g	4.18 ± 0.03 c	10.82 ± 0.17 f	43.8 ± 6.25 de	1.50 ± 0.20 f	4.25 ± 0.14 de	10.69 ± 0.24 f
Aloe extract 75%	75.0 ± 0.0 abc	4.33 ± 0.13 de	5.04 ± 0.12 b	12.85 ± 0.12 c	68.8 ± 6.25 bc	4.41 ± 0.15 d	5.46 ± 0.07 b	12.80 ± 0.16 b
Aloe extract 100%	75.0 ± 0.0 abc	5.98 ± 0.48 c	6.04 ± 0.02 a	13.24 ± 0.02 b	81.2 ± 6.25 ab	6.14 ± 0.21 c	6.35 ± 0.05 a	13.25 ± 0.12 b
Willow extract 75%	68.8 ± 6.2 bc	3.42 ± 0.20 def	4.26 ± 0.10 c	12.69 ± 0.06 c	62.5 ± 7.21 c	3.54 ± 0.28 de	4.11 ± 0.15 e	11.93 ± 0.04 c
Willow extract 100%	75.0 ± 0.0 abc	4.58 ± 0.14 d	4.90 ± 0.03 b	11.99 ± 0.06 d	68.8 ± 6.25 bc	4.29 ± 0.17 d	4.62 ± 0.09 c	11.64 ± 0.10 cd
Moringa extract 75%	50.0 ± 0.0 de	3.13 ± 0.12 ef	3.10 ± 0.08 e	13.35 ± 0.09 ab	50.0 ± 0.0 cd	3.33 ± 0.23 e	3.24 ± 0.08 g	13.82 ± 0.26 a
Moringa extract 100%	62.5 ± 7.2 cd	4.17 ± 0.09 ed	3.72 ± 0.09 d	13.59 ± 0.06 a	56.2 ± 6.25 cd	4.21 ± 0.12 d	3.77 ± 0.04 f	13.84 ± 0.14 a
Cinnamon	25.0 ± 0.0 f	1.38 ± 0.23 g	4.08 ± 0.14 c	9.79 ± 0.08 h	18.8 ± 6.25 f	1.25 ± 0.14 f	4.48 ± 0.08 cd	9.25 ± 0.26 g

Means followed by the same letter within columns are not significantly different at the $p < 0.05$ level of significance using Duncan's multiple range test. Values reported are the means ± standard error (n = 6).

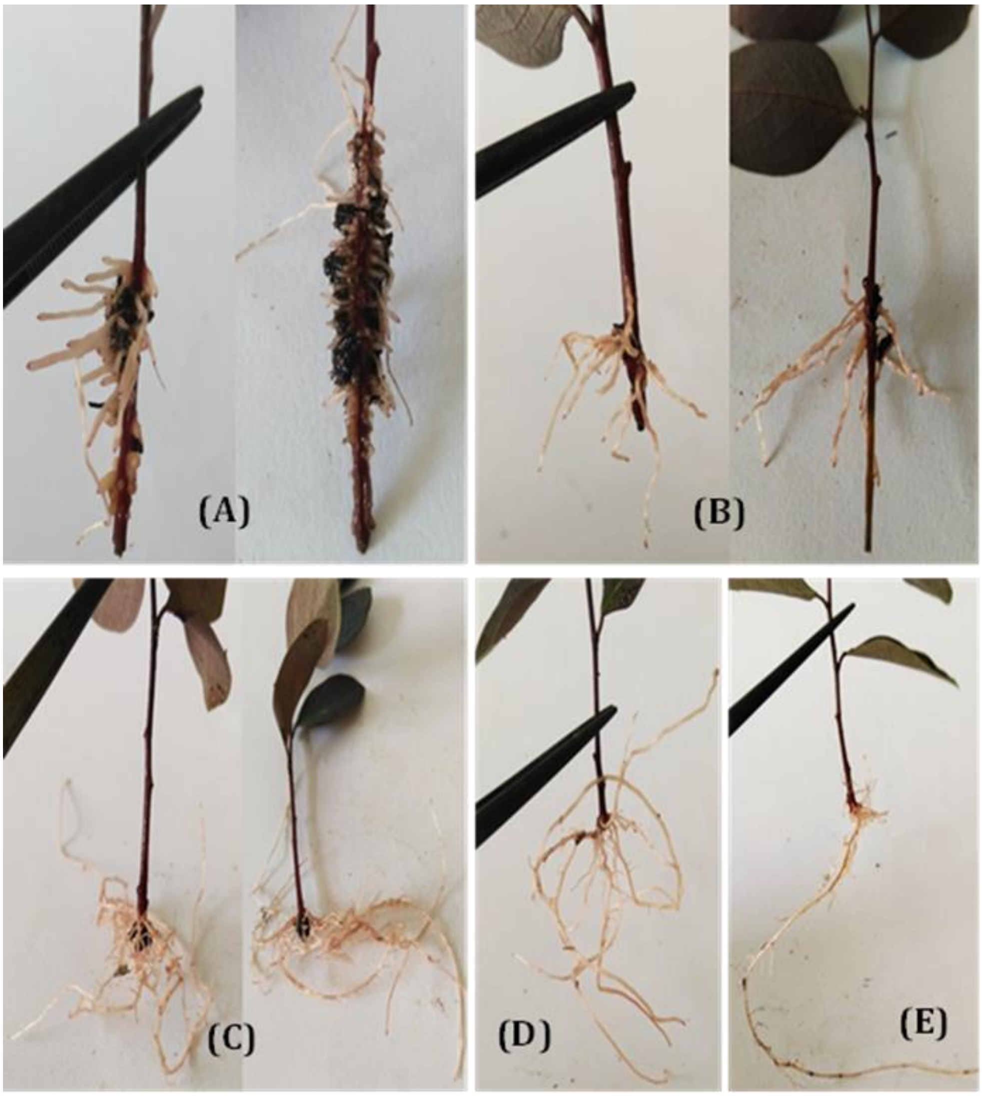

Figure 1. Effect of different auxins and natural plant extracts on rooting behavior of *Breynia disticha* cuttings. (**A**) Cutting + NAA at 2000 ppm. (**B**) Cutting + IBA at 2000 ppm. (**C**) Cutting + aloe extract at 100%. (**D**) Cutting + willow extract at 100%. (**E**) Cutting + honey at 100%.

Extracts from aloe and willow are known to contain many biological compounds. *Aloe vera* extract contains sugars, enzymes, vitamins, amino acids, nutrients, plant sterols, gibberellins, and salicylic acid, which are associated with improvements in growth (especially root growth), oil production, and plant mineral composition [58]. While the precise mechanisms of action of this plant extract are unknown, many of its ingredients have been found to affect root growth or nutrient intake features that may impact cuttings' root development. Sugars have been demonstrated to stimulate roots in cuttings when added to the rooting medium by supplying the plant with a carbon source [59]. At the same time, amino acids have been linked to increased nutrient uptake via chelation [60,61]. Also, plant hormones, such as the rooting hormone salicylic acid, have been discovered in Aloe vera extract [58]. Aloe vera extract contains hormone-like compounds that may contribute to these effects [62].

In addition to biostimulants, *Aloe vera* extract contains plant nutrients (potassium, calcium, sodium, magnesium, copper, chromium, manganese, zinc, and selenium) [16], likely contributing to the observed advantageous effects. Nutrients have been proven to be taken up by the cuttings' stems and promote faster growth (after adventitious root emergence) when nutrients are applied directly after excision [63]. This is in line with the observation that *Aloe vera* extract treatment gave a greater effect size for root branching (secondary roots) than the number of adventitious root formations, which may be attributed to biostimulants, plant nutrients, or some combination thereof. Similarly, willow extract contains many bioactive secondary compounds such as polyphenols (lignans, phenolic acids, flavonoids, proanthocyanidins, and tannins), terpenoids, and, most notably, many salicylate compounds (salicylic acid, salicin, and saligenin) [28]. So, the effect of willow extract in root stimulation has been attributed to the phytochemical catechol [23,64], but this impact is more commonly correlated with the phytohormone salicylic acid, which is a primary compound in willow extract [26,65]. Salicylic acid is a phenolic signaling molecule and is related to the regulation of improving root growth and development [66,67]. Salicylic acid possibly affects rooting directly through interactions with rooting-related hormones. Exogenous SA combined with the prorating hormone IAA has been shown to promote the hormone's actions and enhance adventitious root development more than IAA alone [66]. Also, salicylic acid has been shown to regulate jasmonic acid (JA), the wound response hormone, which is negatively and positively connected with adventitious root formation [68,69]. Although JA and IAA exist in the base of stem cuttings [69,70], it is unknown if exogenous SA can be taken up and interact with these molecular processes. Therefore, either indirectly by stress mediation or directly by carbon supply or hormones, the aloe and willow extracts confer root-stimulating effects when applied to cuttings.

3.1.2. Shoot Length

Results presented in Table 1 show that most treatments of natural plant extracts at all rates significantly affected shoot length compared to the two synthetic plant hormones used and the natural extracts, except for cinnamon and honey, which recorded the lowest values of shoot length in the two seasons. The highest shoot length in both seasons resulted from the 100% moringa extract treatments, at 13.59 cm and 13.84 cm, respectively. The following positive effect on shoot length was recorded with the same natural extract at 75%, followed by aloe extract at 100% in both seasons. These results can be attributed to the fact that moringa extract contains a lot of growth hormones, mainly cytokinins like Zeatin, which was shown to have a high concentration of between 5 and 200 mcg/g in the leaves and encourage growth parameters, including plant height, stem diameter, and leaf number [71,72]. Also, cytokinins significantly impact the enhancement of cell division, elongation, and modification in apical dominance in plants [73]. Additionally, improved rooting performance may be responsible for improving shoot characteristics by enabling rooted cuttings to absorb more nutrients and water, which will contribute to enhanced vegetative growth. [74]. Similar results were obtained by [75,76], who found that treating tip cuttings with IBA or bio-stimulants enhanced measures of the roots and shoots compared to untreated cuttings.

3.1.3. Root Fresh and Dry Weight

The data in Table 2 reveal that cuttings treated with aloe extract at 100% gave the highest value of root fresh and dry weight in the two seasons (0.492 and 0.039 g and 0.487 and 0.040 g, respectively). Also, the same plant extract at 75% gave a subsequent positive effect, followed by willow extract at 100%, while treating cuttings with 2000 ppm NAA produced precisely the significantly lowest values of root fresh and dry weight in both seasons compared with the other treatments. These results may be attributed to aloe and willow extracts, which appear to have plant hormone properties, e.g., salicylic acid [28,58], which is related to the regulation of root growth and development [76,77]. Also, it has been shown that exogenous SA improves the actions of the endogenous rooting hormone IAA, which results in the motivation of DNA, RNA, and protein synthesis [55,66].

Table 2. Effect of different auxins and natural plant extracts on root fresh weight and root dry weight of *Breynia disticha* cutting during two seasons.

Treatments	Root Fresh Weight (mg)	Root Dry Weight (mg)	Root Fresh Weight (mg)	Root Dry Weight (mg)
	1st Season		2nd Season	
Control	0.0 i	0.0 h	0.0 j	0.0 h
NAA at 2000 ppm	65 ± 6 h	6 g	95 ± 3 i	9 g
IBA at 2000 ppm	360 ± 7 c	31 bc	352 ± 1 d	30 d
Honey 75%	162 ± 3 f	19 e	180 ± 7 g	21 e
Honey 100%	109 ± 5 g	102 f	133 ± 102 h	14 f
Aloe extract 75%	456 ± 8 b	37 a	463 ± 14 b	37 b
Aloe extract 100%	492 ± 6 a	39 a	487 ± 7 a	40 a
Willow extract 75%	380 ± 10 c	30 c	352 ± 12 d	31 cd
Willow extract 100%	446 ± 15 b	34 b	422 ± 9 c	34 bc
Moringa extract 75%	286 ± 4 e	19 e	256 ± 6 f	17 f
Moringa extract 100%	311 ± 9 d	27 d	289 ± 4 e	23 e
Cinnamon	112 ± 7 g	14 f	133 ± 6 h	15 f

Means followed by the same letter within columns are not significantly different at the $p < 0.05$ level of significance using Duncan's multiple range test. Values reported are the means ± standard error (n = 6).

3.2. Effect of the Rooting Treatments on Chemical Analysis of Snowbush Cuttings

3.2.1. Total Carbohydrates and Total Soluble Phenols

The data in Table 3 show that the highest significant value of total carbohydrate percentage during both seasons (29.79 and 31.61%) was recorded with cuttings treated with aloe extract at 100% compared with all other treatments, while the highest contents of total soluble phenols were recorded when cuttings were treated with IBA at a concentration of 2000 ppm in both seasons (4.08 and 4.16 mg/g DW), respectively, followed by treatment of aloe extract at 100% without significant differences. On the other hand, the cinnamon treatment recorded the lowest total carbohydrate percentage and total soluble phenols (19.88 and 18.29% and 2.46 and 2.15 mg/g DW) during the two seasons, respectively.

These results may be ascribed to the fact that aloe extract is rich in essential amino acids, natural sugars, lignin, enzymes, macronutrients, micronutrients, vitamins, and plant hormones such as auxins and gibberellin, and contains organic acids such as salicylic acid [15,16], which enhances growth characteristcs and chemical constituents. Generally, cuttings treated with aloe extract at 100% had higher levels of total phenols and carbohydrates, which led to appropriate rooting percentages and root and shoot measurements. These results are in accordance with some researchers who declared that higher levels of total phenols and carbohydrates could motivate adventitious root formation and improve root development [76–78].

The positive relationship between root ability and the high contents of carbohydrates may be because carbohydrates have been considered the principal source of energy and carbon [79] as well as cell structural materials for the initiation of root primordial [80]. The concentration of carbohydrates in cutting tissues may be influenced by auxins, which can enhance starch hydrolysis and carbohydrate mobilization from leaves and upper stems towards the rooting zone [81]. Previously, carbohydrate breakdown was found in lemon cuttings during the early phases of root development, as well as an increase in catalase and peroxidase activity accompanied by carbohydrate breakdown [82]. Furthermore, sufficient carbohydrates and nitrogenous bases work with others in synthesizing the building blocks for nucleic acids (DNA and RNA). These biochemical compounds are the primary source in synthesizing protein, carbohydrate, and fat metabolism, which is necessary for normal cell division [83]. On the other hand, many researchers reported that the phenolic compounds act as auxin cofactors in promoting adventitious root formation. In addition, phenolic chemicals have a role in the production of AR by preventing peroxidase [84–86]. This enzyme can act as an IAA oxidase by destroying the endogenous auxin IAA, a rooting inducer. Accordingly, the increment in polyphenolic compounds has improved rooting ability as well as shoot and root characteristics by its direct impact on limiting auxin oxidation, increasing the amount of auxin accessible for inducing roots [87].

Table 3. Effect of different auxins and natural plant extracts on total carbohydrates and total soluble phenols of *Breynia disticha* cutting during two seasons.

Treatments	Total Carbohydrates (%)	Total Soluble Phenols (mg/g DW)	Total Carbohydrates (%)	Total Soluble Phenols (mg/g DW)
	1st Season		2nd Season	
NAA at 2000 ppm	20.63 ± 0.16 hi	3.64 ± 0.02 cd	20.43 ± 0.29 g	3.71 ± 0.16 c
IBA at 2000 ppm	25.43 ± 0.47 c	4.08 ± 0.04 a	25.25 ± 0.074 c	4.16 ± 0.05 a
Honey 75%	21.30 ± 0.30 gh	2.54 ± 0.06 g	21.23 ± 0.30 fg	2.52 ± 0.04 e
Honey 100%	22.06 ± 0.11 fg	2.78 ± 0.09 f	22.11 ± 0.04 ef	2.58 ± 0.03 e
Aloe extract 75%	27.86 ± 0.46 b	3.95 ± 0.03 ab	27.67 ± 0.29 b	3.94 ± 0.05 b
Aloe extract 100%	29.79 ± 0.09 a	4.04 ± 0.06 a	31.61 ± 0.52 a	4.14 ± 0.03 a
Willow extract 75%	23.60 ± 0.31 d	3.73 ± 0.10 bcd	23.58 ± 0.29 d	3.85 ± 0.03 bc
Willow extract 100%	25.85 ± 0.42 c	3.84 ± 0.07 abc	26.22 ± 0.39 c	3.96 ± 0.02 b
Moringa extract 75%	22.35 ± 0.07 ef	3.38 ± 0.07 e	21.24 ± 0.09 fg	3.49 ± 0.02 d
Moringa extract 100%	23.15 ± 0.44 de	3.55 ± 0.10 de	22.38 ± 0.60 e	3.69 ± 0.02 c
Cinnamon	19.88 ± 0.12 i	2.46 ± 0.14 g	18.29 ± 0.31 h	2.15 ± 0.02 f

Means followed by the same letter within columns are not significantly different at the $p < 0.05$ level of significance using Duncan's multiple range test. Values reported are the means ± standard error ($n = 6$).

3.2.2. Nitrogen, Phosphorus, and Potassium

It appears from the data in Table 4 that the highest value of nitrogen percentage (1.440 and 1.438 N %) in both seasons was recorded with cuttings treated by 2000 ppm IBA followed by the cutting treated with aloe extract at 100% in the two seasons (1.355 and 1.403 N %), respectively, while using aloe extract at 100% in the two seasons recorded the highest value of phosphorus and potassium percentage (0.335 and 0.348 P % and 1.475% and 1.525%), followed by treatment of IBA at a concentration of 2000 ppm. Conversely, the cinnamon treatment recorded the lowest nitrogen, phosphorus, and potassium percentage content compared with other treatments during the two seasons. The beneficial effect of aloe extract may be ascribed to its very high content of many biological compounds, such as amino acids and nutrients, which are associated with improvements in plant mineral composition [58]. Amino acids have also been related to enhanced nutritional absorption [61].

Table 4. Effect of different auxins and natural plant extracts on nitrogen, phosphorus, and potassium percentage of *Breynia disticha* cutting during two seasons.

Treatments	N (g/kg)	P(g/kg)	K (g/kg)	N (g/kg)	P (g/kg)	K (g/kg)
	1st Season			2nd Season		
NAA at 2000 ppm	11.65 ± 0.17 d	2.13 ± 0.14 d	9.32 ± 0.25 de	10.95 ± 0.32 b	2.03 ± 0.04 e	10.85 ± 0.15 de
IBA at 2000 ppm	14.40 ± 0.20 a	2.88 ± 0.06 b	14.25 ± 0.85 a	14.38 ± 0.22 a	2.98 ± 0.07 b	14.00 ± 0.40 ab
Honey 75%	9.73 ± 0.54 e	2.55 ± 0.06 c	8.25 ± 0.62 ef	8.60 ± 0.35 c	2.48 ± 0.08 cd	10.25 ± 0.62 ef
Honey 100%	1.48 ± 0.30 e	2.75 ± 0.05 b	11.50 ± 0.28 bc	10.23 ± 0.19 b	2.75 ± 0.05 bc	13.00 ± 0.40 bc
Aloe extract 75%	12.50 ± 0.21 c	3.18 ± 0.04 a	13.75 ± 0.47 a	13.95 ± 0.43 a	3.18 ± 0.04 ab	15.25 ± 0.25 a
Aloe extract 100%	13.55 ± 0.37 b	3.35 ± 0.02 a	14.75 ± 0.47 a	14.37 ± 0.35 a	3.48 ± 0.06 a	15.25 ± 0.25 a
Willow extract 75%	1.48 ± 0.34 e	2.85 ± 0.02 b	9.25 ± 0.62 de	10.15 ± 0.27 b	3.00 ± 0.04 b	11.25 ± 0.47 de
Willow extract 100%	11.50 ± 0.25 d	2.95 ± 0.02 b	11.75 ± 0.10 b	10.65 ± 0.23 b	2.95 ± 0.06 b	13.25 ± 0.47 bc
Moringa extract 75%	6.53 0.24±g	2.00 ± 0.04 de	10.25 ± 0.32 cd	6.93 ± 0.17 d	2.10 ± 0.04 de	12.00 ± 0.40 cd
Moringa extract 100%	8.65 ± 0.17 f	2.75 ± 0.02 b	12.38 ± 0.23 b	8.85 ± 0.17 c	2.75 ± 0.06 bc	13.25 ± 0.47 bc
Cinnamon	5.73 ± 0.20 g	1.90 ± 0.07 e	7.28 ± 0.26 f	4.95 ± 0.21 e	1.52 ± 0.44 f	9.38 ± 0.83 f

Means followed by the same letter within columns are not significantly different at the $p < 0.05$ level of significance using Duncan's multiple range test. Values reported are the means ± standard error ($n = 6$).

3.2.3. Plant Endogenous Hormones (IAA, GA3, and ABA)

The data in Table 5 and Figure 2 present the HPLC analysis of the samples extracted from the basal root zone of snowbush cuttings in the second season. It was evident from the data that there was a relationship between rooting behavior and endogenous IAA, GA_3, and ABA. Rooting percentage and root number per cutting were increased with increasing endogenous IAA and GA_3 content and decreasing endogenous ABA.

Table 5. Effect of different auxins and natural plant extracts on plant endogenous hormones (IAA, GA3, and ABA μg/g f.w.) of *Breynia disticha* cutting.

Treatments	**IAA (μg/g f.w.)**	**GA_3(μg/g f.w.)**	**ABA(μg/g f.w.)**
		2nd Season	
IBA at 2000 ppm	249.7 ± 6.6 a	376.4 ± 14.3 a	0.87 ± 0.07 d
Honey 100%	62.9 ± 0.9 e	92.4 ± 1.7 e	2.62 ± 0.10 a
Aloe extract 100%	203.0 ± 7.0 b	283.5 ± 8.6 b	1.04 ± 0.01 d
Willow extract 100%	126.9 ± 5.4 c	234.9 ± 7.7 c	1.36 ± 0.09 c
Moringa extract 100%	99.4 ± 1.1 d	185.0 ± 2.6 d	1.71 ± 0.10 b
Cinnamon	53.1 ± 0.9 e	89.5 ± 1.2 e	2.77 ± 0.02 a

Means followed by the same letter within columns are not significantly different at the $p < 0.05$ level of significance using Duncan's multiple range test. Values reported are the means ± standard error ($n = 6$).

So, the cuttings treated with IBA at 2000 ppm gave the best rooting percentage and root number associated with the highest level of endogenous IAA and GA_3 content (249.7 and 376.4 μg/g f.w, respectively) as well as the lowest level of endogenous ABA content (0.867 μg/g f.w.), followed by the cuttings which were treated with aloe extract at 100%. On the other hand, cuttings treated with cinnamon, which gave the lowest rooting percentage and root number, recorded the lowest level of endogenous IAA content and GA_3 (53.1 and 89.5 μg/g f.w., respectively) and the highest level of endogenous ABA content (2.77 μg/g f.w.), followed by plant cuttings treated with 100% honey. The same trend was obtained in the second season. These results are in harmony with the findings of [88–90], indicating a positive correlation between endogenous IAA content and the rooting ability of cuttings.

The increase in the content of endogenous IAA may be due to the role of IBA in the synthesis of endogenous IAA, as reported by [54], who suggested that the effects of IBA and NAA treatments on the regulation of endogenous auxin production by either the immediate modulation of IAA oxidase system or the movement of auxin protectors may be responsible for the rise in endogenous auxin concentration. Also, the superiority of aloe extract may be because it contains phytohormones like auxin and gibberellins, as well as many other efficient compounds that play a remarkable function in root formation [15,16].

Generally, phytohormones like gibberellins, auxin (IAA), and cytokinins play a significant role in root formation and plant growth [91,92]. Auxin plays an influential role in many aspects of root growth, development, and differentiation. IAA regulates the development of the primary and lateral roots [93], root apical meristem [94], root cap [95], and root vascular differentiation [96]. Also, auxin can accelerate DNA, RNA, and protein synthesis, all of which are necessary for cell division, which may explain its favorable effect on the onset of the development of adventitious roots [55].

Furthermore, gibberellic acid (GA_3) has bioactive properties that promote effects on adventitious root formation, as reported by [97–99]. GA_3 indirectly affects the promotion of AR formation by increasing endogenous auxin through stimulation of endogenous auxin synthesis and inhibition of the IAA oxidase effect [100]. Furthermore, GA regulates elongation, cell division, and protein and nucleic acid synthesis [101]. As for abscisic acid (ABA), it is known that ABA is a hormone that inhibits rooting [102]. This finding agrees with the study herein that showed a negative relationship between endogenous ABA and root development. However, the low ABA levels benefit root primordial induction [103], so the cross between auxin and abscisic acid may play an essential role in regulating root growth.

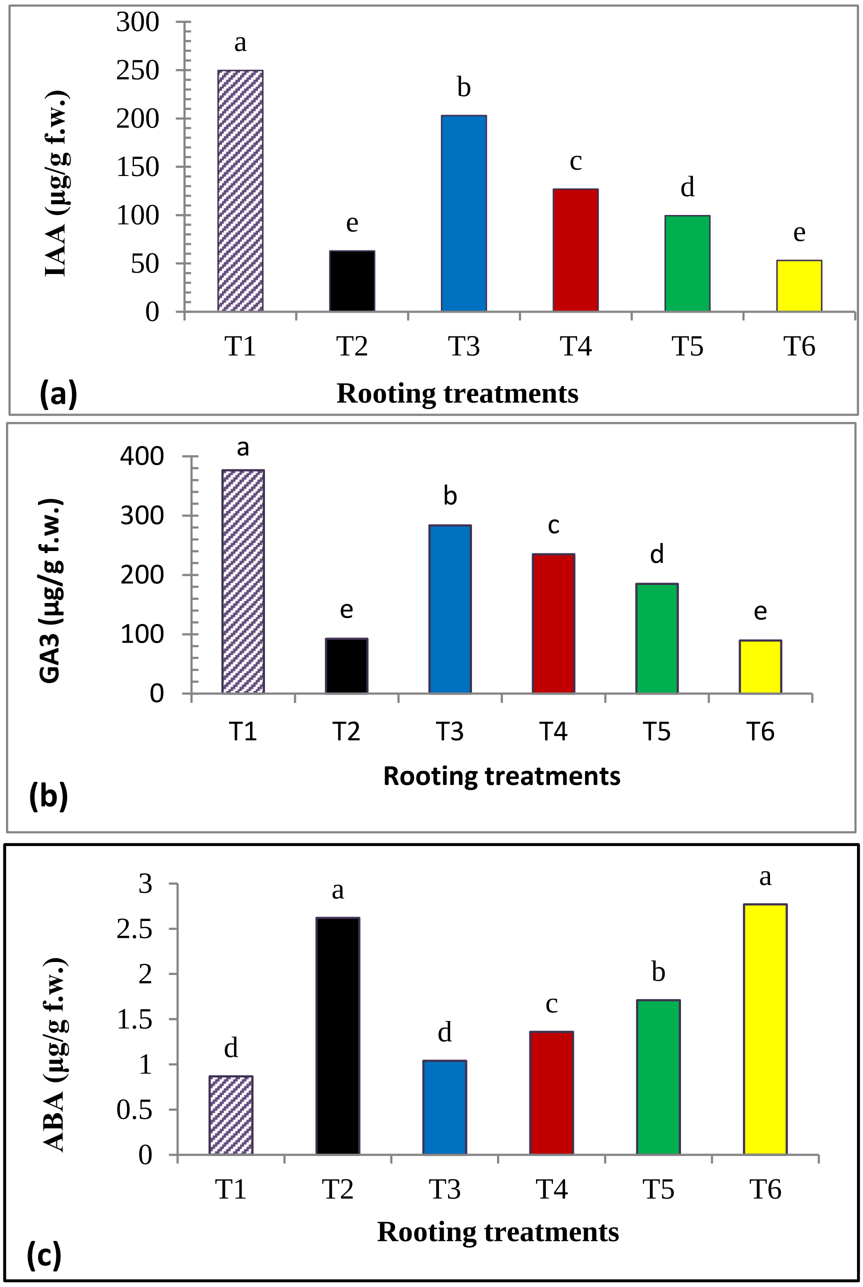

Figure 2. Indole-3-acetic acid (IAA) (**a**), gibberellins (GA_3) (**b**), and abscisic acid (ABA) (**c**) content on *Breynia disticha* cuttings in the second season. T1, IBA at 2000 ppm; T2, honey at 100%; T3, aloe extract at 100%; T4, willow extract at 100%; T5, moringa extract at 100%; and T6, cinnamon. Significant differences (a–e) over the bars indicate variations at a significance level of $p < 0.05$, determined through Duncan's multiple range test.

4. Conclusions

According to the results of this study, alternative natural plant extracts such as aloe and willow extract have a promising effect on vegetative propagation, and they can be recommended as alternative root-inducing compounds with their benefits, cost-effectiveness, and safety for the environment.

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