International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.10, pp 445-459, 2016

In Vitro and *In Vivo* Growth, Chemical and Antimicrobial Studies for *Plectranthus amboinicus* plant.

Mohammad M.EL-Zefzafy[1], Gouda T. M. Dawoud[2*], and T. H. El-Morsy[3]

[1]Medicinal Plants, [2]Phytochemistry and [3]Microbiology Departments, Applied Research Center for Medicinal Plant, National Organization for Drug Control and Research (NODCAR), Egypt

Abstract : The effect of some factors on micro propagation of *P. amboinicus* plant *in vitro*. The results clearly showed that, White's medium recorded the best results in most of growth parameters was recorded in nodes explant comparing with MS and B5 media. BA 1.0 mg/ l in addition to White's medium was significant increasing of shoot number/ explant, while White's medium free hormone (control) was recorded the highest values in survival and rooting percentage, shoot number/ explant and shoot length cm / explant comparing with IBA (from 0.5 to 4.0 mg/l.). Also data showed that, Peat moss and sand interaction (growing media) were recorded the best result in acclimatization of rooted plants comparing with peat moss or sand individually. in a greenhouse of controlled temperature, as well as studying the effect of different seasons in and out normal greenhouse conditions, The results clearly showed the effect of temperature on the survival percentage of plants during acclimatization with significant difference ($p<0.05$), as the highest significant percentages of survival were obtained with the degrees 31 and 36 ºC compared to the other treatments. Interestingly, the results indicated that the summer and autumn showed higher percentages of survival and growth rate compared to the other seasons. However, the best result was observed in the summer. The quantity of essential oil of plant was ≥ 0.6 % (V/W) of yellowish volatile oil, from analysis of the oil using GC and GC/MS; it was obvious that it contained 23 compounds where Limonene represented the highest percentage (40%) while Junipene was the lowest one (0.54 %). The antioxidant activity, total phenolic compounds and flavonoid contents were investigated in plants grown in and out the normal greenhouse. The obtained results outside the greenhouse were 38 %, 77 µg and 65 µg, while the results inside the greenhouse were 29 %, 35 µg % and 31µg %, respectively. The antimicrobial activity against yeasts and bacteria was assessed in broth micro-dilution assays to determine antibiotic sensitivity testing and the minimum inhibitory concentration (MIC) necessary to inhibit microbial growth, our results showed that essential oil of *P. amboinicus* could be used as a tool for the developing novel and more efficacious antimicrobial agents.

Keywords: Tissue culture, Phytochemical, Essential oil, Antimicrobial and *Plectranthus amboinicus*.

Introduction

The family Lamiaceae contains several genera, such as sage (*Salvia*), basil (*Ocimum*) and mint (*Mentha*), with a rich diversity of ethno botanical uses. Another important genus is *Plectranthus*, a large genus containing about 300 species found in Tropical Africa, Asia and Australia (Paton et. al.,[1]). The genus *Plectranthus* belongs to the mint and sage consists of annual or perennial herbs or shrubs with herbaceous stems

and leaves, sometimes semi-succulent or succulent, with usually terminal, spike-like inflorescences of two-lipped flowers. There are ± 350 species spread throughout the tropical and warm regions of the old World, in Africa, Madagascar, Asia, India, Australia and a few Pacific islands. There are 53 species in southern Africa, occurring mainly in the southeastern and eastern parts and absent from the Northern Cape It is a good plant for containers and hanging baskets and can be grown indoors.

P. ambiguus leaves are used in traditional medicine to treat colds. (Potgieter, et. al.,[2]). An antioxidant activity-guided fractionation of *Plectranthus amboinicus* resulted in the isolation of ladanein (5, 6-dihydroxy-7, 4 - dimethoxyflavone) **(**Liu and Ruedi,[3]). In South Africa, tea made from the leaves of *P. laxiflorus* is taken for coughs and colds (Hutchings et. al., [4]; Rabe and Staden,[5]) and an infusion of the crushed leaves of *P. ambiguus* is mixed with a little hot water and sipped for coughs (Hulme,[6]). Classically plant growth regulators are considered as one of the most important factors affecting cell growth, differentiation and metabolism formation in plant cell and tissue cutlers (Liang et. al.,[7]). As with both auxin and cytokinins – mediated plant responses are accompanied by enhanced nucleic acid and protein metabolism. However, so far there is no unequivocal evidence that any of their growth, promoting plant hormones act directly on the protein synthesizing system of the plant (Datta,[8]).

The growing medium container into which *in vitro* rooted plantlets are transplanted is important for good survival, inhibitors or dramatic shift in pH in a medium can adversely affect root growth and thus transplanting success (Jones,[9]). Most laboratories and nurseries transplanting into a uniform medium that adequately supports the plants, has a suitable pH, is well buffered, reproducible and sufficiently porous to allow adequate drainage and serration (Miller,[10]). Peat was selected because it is inexpensive and easy to use, and is known to be an efficient barrier material for treating waste water, (Rasmussen et. al.,[11]). Sand dunes are the best source due to their physical properties, being medium or the coarse sand particles which provide optimum adjustments in media texture, certainly sand is the least expensive of all inorganic amendments which makes it a valuable amendment for both potting and propagation media (Wilson and Stoffella,[12]). Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, and ischemia, reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS. (Cook and Samman,[13]). Monoterpenoids, sesquiterpenoids, diterpenoids and phenolic have been reported in species of *Plectranthus*. The abietane diterpenoids are the most diverse of the diterpenoids isolated from species of *Plectranthus* (Liu and Ruedi,[3]) *P. amboinicus* consist of many important constituents and widely used for medicinal purposes. It was proven that the essential oil possess biological activity against several bacteria and yeast. (Rashmi et. al.,[14]) The species of genus *Plectranthus* are always cited as antimicrobial agents that can be used to treat several infections. (El-hawary et.al.,[15]; Jiyauddin et. al.,[16]), Helmy and Bakr[17] stated that *P. amboinicus* has been used traditionally for treatments of burns, insect bites and malaria fever. Moreover its antimicrobial activity was evaluated. Phenolic compounds represent the main constituents of *P. amboinicus*. Chandrappa et. al., [18] Showed that ethanolic and hot water extract of the leaves of *P. amboinicus* have antibacterial property against both Gram positive and Gram negative organisms. Also, Gurgel, et. al., [19]; Praveena and Pradeep [20] proved that the hydro alcoholic extract from the leaves *of P.amboinicus* had antibacterial activity against methicillin resistant *Staphylococcus aureus*.

The objective of this study were shoot and root formation and from node stem explant *in vitro*, acclimatization determination of active constituent and antimicrobial activity of essential oil from *P. amboinicus*.

Materials and Methods

I- Materials:

1-Aerial parts of *P. amboinicus* were collected from National Organization for Drug Control and Research (NODCAR) Medicinal Plants Dept., in March 2016.

2- All media hormones were purchased from Sigma chemical Co., St. Lewis, USA.

3- Chemicals

All chemicals, solvents and reagents used were of analytical and pure grade. DPPH was purchased from Fluke chemical company (Switzerland). Rutin and Gallic acid standards were purchased from Sigma chemical Co., St. Lewis, USA.

4-A Series of bacterial and fungal strains available in stock culture of Microbiology Department, National Organization for Drug Control and Research (NODCAR), were used for antibiotic sensitivity testing.

II- Methods:

Part 1 – *In vitro* and *In vivo* growth of *P. amboinicus*:

This work was carried out in Applied Research Center of Medicinal Plants (Tissue Culture and Phytochemistry labs.), National Organization for Drug Control and Research (NODCAR), Giza, Egypt, during the period of 2014 – 2016 To study the effect of some factors on micro propagation in *P. amboinicus* plant *in vitro*. Nodes explant used as start material obtained from applied research center of medicinal plant farm, explants kept in anti-oxidant solution (150 mg/l ascorbic acid +100 mg/l citric acid) for three hours and washed several time by tap water, then were rinsed with a small amount of liquid soap for 15 minutes for assuring the removal of most external contamination, and rinsed again under running tap water for 30 minutes to remove all the remaining detergent ,after that the explants were dipped again in anti-oxidant solution (150 mg/l ascorbic acid +100 mg/l citric acid) for two hours ,surface sterilization began under aseptic condition in laminar air flow cabinet . Nodes explants (2cm) were rinsed for 20 minutes in 12.5% Clorox (0.625%) then rinsed in sterile distilled water (three times) to remove all traces of the disinfectant .The sterile explants were planted in sterile jars containing 20 ml of tested medium and supplemented with 30g/l sucrose and solidified by 2g/l phytagel. The pH value was adjusted to 5.7 –5.8 by adding suitable amount of 1 N HCl and 1 N KOH by using the pH meter prior to autoclaving at 1.3 kg/cm^2 for 20 minutes. The cultures were incubated in a growth chamber under 25$\pm$2˚C in light intensity (2000 Lux) of cool white fluorescent lamps for 16 hr. light/day in all tested treatments. The treatments were as follow.

I-T**he work designing**:

1- Shoot and root production from node stem explant of *P. amboinicus in vitro* culture.

A. Effect of media type (MS, White's and B5) on survival percentage and growth of node explants. Node explants were cultured on different media type individually to study their effect on survival percentage and growth (starting stage). **(**Murasige and Skoog,[21], White's,[22] and Gamborge et. al.,[23]). Data were recorded after 8 weeks incubation period.

B. Effect of cytokinins (Kinetin and Benzyl adenine) on growth of shoots. Shoots (1 cm) were obtained from node explants grown on White's medium in starting stage and re-cultured on different levels of Kin or BA in the rate (0.5 ,1.0 and 2.0 mg /l.) individually in addition to White's medium to study their effect on growth of shoot (multiplication stage). Data were recorded after 8 weeks incubation period.

C. Effect of auxin, Indole – 3 – butyric acid (IBA) on rooting of shoots. shoots and nodes (2cm) were obtained from explants grown on White's medium supplemented with BA 1.0 mg/l. in multiplication stage and re-cultured on different levels of (IBA- 0.0, 0.1, 1.0, 2.0 and 4.0 mg/l.) in addition to White's medium to study their effect on rooting of explants (rooting stage). Data were recorded after 8 weeks incubation period. Data were recorded after 8 weeks incubation period. Growth measurement: Survival %, Shoot number/ explant, Shoot length cm / explant, Leaf number/ explant, Plant strength, Rooting %, Root length cm / explant and Root number/ explant. Plant strength was estimated (as score) and presented as follow according the method described by (Pattino,[24]),

(a) Negative growth result = 1 (b) below average growth = 2 (c) average growth = 3 (d) above average growth (e) excellent growth =5.

II- Acclimatization and plant growth of *P. amboinicus*:

A. Effect of growing media, peat moss and sand on plants acclimatization. Harmony plants were obtained from rooting stage were transplanted to green house in plastic pot 14 cm full of peat moss + sand as follow (1/0, 0/1, 1/1, 1/4, 1/8, 2/1, 4/1 and 8/1- V/V) to study their effect on acclimatization of plants Data were recorded after 8 weeks incubation period.

B. Effect of various degrees of temperature on acclimatization and growth of *P. amboinicus* in greenhouse under control. Plantlets obtained from rooting stage in average tall (4.5 – 5 cm) and fresh weight (3.22) g / plant and root number (5.6) were washed from phytagel under running tap water and soaked in solution of fungicide (0.2 % Benlet), then transferred to the greenhouse under control in plastic containers (5 cm) full of peat moss and sand (1/1–V/V) for acclimatization to study the effect of various degrees of temperature (20 ºC , 25 ºC ,30 ºC and 35 ºC) on acclimatization of plants. Data were recorded after 8 weeks of incubation. Plants were covered with plastic bags and maintained in the greenhouse at different temperature degrees. Twenty five replicates were made for each treatment Data (survival percentage, plant height (cm), fresh weight g / plant, root number, root fresh weight g/plant and plant growth rate) were recorded after six weeks from culturing at greenhouse under control.

C. Effect of various seasons on plant growth and development of *P. amboinicus* cultured in and out normal greenhouse. This experiment was conducted to study the effect of various seasons on vegetative growth and development of *P. amboinicus* cultured in greenhouse. Twenty five replicates of acclimatized plants with average tall 15.77cm, fresh weight 21.88 g/plant and root number 9.89/plant, were transferred to plastic containers (50cm) full of peat moss/sand at equal volumes for more growth and development in uncontrolled greenhouse. While other twenty five individuals were grown out the greenhouse conditions to study the effect of various seasons (spring, summer, autumn and winter). Data were recorded after the end of each season. Twenty five replicates of each group were estimated for survival %, plant height (cm), fresh weight (g) and plant growth rate. These data were recorded after three months at the end of each season.

Statistical analysis: Data of all experiments were statistically analyzed by one factorial randomized complete design using (SAS,[25]) package. The least significant difference among levels of each treatment were compared using L.S.D. test at 5% level according to **(**Steel and Torrie, [26]).

Part II - Phytochemical methods for *P. amboinicus*:

1- Determination the % essential oil from *P. amboinicus*:

(100g) from dried aerial of the plants were separately ground, and submitted to hydro distillation for 3 h using a Clevenger-type apparatus according to the method recommended by the Egyptian Pharmacopoeia[27]. The obtained essential oils were dried over anhydrous sodium sulphate and after filtration determination the % then stored at 4 °C until analyzed.

2-Gas chromatography and Gas Chromatography-Mass Spectrometry.

2.1- Gas Chromatography.

Essential oils were analyzed using an Agilent Technologies 6890 gas chromatograph, equipped with a flame ionization detection (FID) detector and HP5 column (30m x 0.25mm x 0.25µm film thickness).Injector and detector temperatures were set at 225 °C and 275 °C respectively. oven temperature gradually raised from 60 to 250 °C at a initial rate of 10 °C/min.Nitrogen (purity 99.9%) was the carrier gas, at a flow rate of 1 mL/min. Diluted sample (1/100 in n-hexane, v/v) of 1.0 µL was injected in the split mode (ratio 1:10). Quantitative data were obtained electronically from FID area percent data without the use of correction factors. Peak integration and quantification were performed automatically with HP chemistation software. A checking of the integration of each peak is carried out and corrected manually if necessary.

2.2. Gas chromatography/mass spectrometry (GC-MS).

Analysis of essential oils was performed by using GC/MS-5989B with the following conditions: Column: HP5-MS, 30 m, 0.53 mm ID, 1.5 µm film. Carrier gas: Helium (purity 99.99999%) at flow rate 1ml/min. Ionization mode: EL (70 eV). Temperature program: 60^0C (static for 2 min.), then gradually increasing (160 ^{0}C at a rate of 2 ^{0}C/ min.) up to 250 °C (static for 7.5 min.). Detector temperature 250 ^{0}C and Injector temperature 225°C. MS was adjusted with an emission current of 10 µA and electron multiplier voltage at 1500 V. Trap temperature was 250 °C and mass scanning was from 50 to 550 amu. The components were identified based on (Searched library: Wiley 275. LIB and NIST 02 version 2.62). The retention indices were determined by comparison of their mass spectra on both columns with those stored in Wiley 275. LIB and NIST 02 version 2.62 or with mass spectra from literature (Adams, [28]).

3. Determination of Total antioxidant activity determination by using Free radical scavenging assay.

3-1. Extraction procedure for 2 grams of *P. **amboinicus*** powder under investigation were ground and mixed with 50 mL of 95% ethanol. The mixture was stored in the dark at 4 °C for 4 h and was shaken to ensure complete extraction. Then, it was filtered (Whatman 113), and the extract was stored in the dark at 4 °C for a maximum of 3 days until analysis.

3-2.Determination the free radical scavenging activity of **P. amboinicus** extracts was determined by UV–Vis. The extract solutions were prepared through dilution with ethanol 95%. A fixed volume of plant extract solution (4.75 mL) at different dilutions was mixed with 250 µL of DPPH 1 mM in ethanol 95% and stored in the dark at room temperature for 30 min. At the end of the reaction period, the same solution was used to measure UV–Vis spectra by the discoloration of a methanolic solution of DPPH (2, 2 diphenyl -2- picryl hydrazyl) according to Astudillo et al. [29]; Daniele et al. [30] and Viturro et al. [31].

4. Determination of total phenolic contents.

The concentration of total phenolic compounds in the different extracts were determined spectrophotometrically using the Folin-Ciocalteu reagent which is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of total phenolic contents using Gallic as reference material according to (Frankle and Meyer [32], Donald et al.,[33] and Julkunen, [34]).

5. Determination of total flavonoids.

Determination of the total flavonoid contents in the different extracts were done colorimetrically by using aluminum chloride solution. Standard curve was done using different concentrations of rutin in methanol (six serial 2 fold dilution to give 100-3.2 µg /ml). 100µl of each extract (previously prepared) were added to a 96 micro well plate and then 100µl of 2% aluminum chloride solution in methanol. After 10min, their absorbance was measured using HP spectrophotometer at 415nm using methanol as blank and the concentration of total flavonoids was calculated according to (Karawy and Aboutable,[35] and Chiang *et al.*,[36]).

Part III- Anti microbial Screening studies for volatile oil of *P. amboinicus*.

1-Antimicrobial Activity by using Disk Diffusion Assay:

Eleven strains of bacteria (Gram-positive and Gram-negative), yeast and fungi were used in the antimicrobial assays. *Escherichia coli* – (ATCC 10538); *E. coli* - (ATCC 14169); *Pseudomonas aeruginosa* – (ATCC 27853); *P. aeruginosa* - (ACCT 9027); *Micrococcus luteus*- (ATCC 9341); *Staphylococcus aureus*- (ATCC 25923 and 6538); *Bacillus subtulis* (ATCC 6633), and two fungi, *Aspergillus niger*. (ATCC 16404), and *Fusarium oxysporum* (ATCC 48112), and *Candida albicans* (ATCC 10231) were used. The previously prepared essential oil were diluted 1/3 v/v in dimethyl sulphoxide (E-Merck), 30 µl of each (containing 10 µl of pure oil) were used in the test. Dimethyl sulphoxide (50 µl) was used as a negative control. The agar diffusion method (Bauer et. al., [37]) was applied using trypticase soy agar (Difco) medium inoculated with the bacterial or fungal suspension of the test organisms. Discs 5 mm in diameter were impregnated with the oils or the control. Then the discs were placed onto the surface of the culture medium. Discs of ceftriaxone and clotrimazole were used as standard antibacterial and antifungal agent, respectively. The plates were incubated at 35-37 ºC for 24-48 hours in case of bacteria, 25 ºC for 48 hours in case of filamentous fungi, while yeasts were incubated at 30 ºC for 24-48 hours.

After incubation, the diameters of inhibition zones were recorded in millimeters and the results were compiled in tables (9).

2- Antimicrobial Activity by using MIC Testing;

The minimum inhibitory concentrations (MIC) of the tested oil sample against the same microorganisms in Disk Diffusion Assay were also determined by micro dilution method (Hammer *et. al.,* [38]).

Results and Discussions

Part 1 – *In vitro* and *In vivo* growth of *P. amboinicus*

A-Effect of media type (MS, White's and B5) on survival percentage and growth of node explants.

The effect of different type of media MS, White's and B5 during the starting stage are presented in Table (1). Survival, rooting percentage and shoot strength were recorded the highest values when node explants cultured on White's medium were (97, 98 % and 4.51 respectively) while with MS medium survival, rooting percentage and shoot strength were recorded (25, 0.0 % and 1.91, respectively) and but B5 medium Survival, rooting percentage and shoot strength were recorded (55, 42 % and 3.99, respectively). In this concern showed that, White's medium proved enhancing the growth compared to MS or B5 media, it may be for the high level of MgSo4 in White's medium composition. $MgSo_4$ is the central element in chlorophyll molecules and is important as an enzyme activator **(Kumar, [39])**.

Table (1): Effect of media type (MS, White's and B5) on survival % and growth of node explants

Media type	Survival %	shoot number/ explant	shoot length cm / explant	leaf number/ explant	Rooting %	Root number/ explant	shoot strength
MS	25	1.11	1.09	0.98	0.0	0.0	1.98
White's	97	3.51	5.33	6.11	98	3.17	4.51
B5	55	2.19	2.61	3.71	43	1.91	3.99
LSD at 5%	**-**	**0.152**	**0.121**	**0.321**	**-**	**1.014**	**0.101**

B-Effect of cytokinins (Kinetin and Benzyladenine) on growth of shoots.

The results of the effect of cytokinins (Kin and BA) on growth response are showed in Table (2) White's medium supplemented with BA 1.0 mg/l was achieved the highest significant response in shoot number/explant (13.32) , while White's medium free hormone (control) recorded the highest significant values in survival (100), shoot length cm / explant (5.56) , leaf number/ explant (6.66) and rooting % (100), the lower response in shoot number / explant , shoot length cm/ explant and leaf number / explant were achieved with 2.07 mg/l for Kin and BA individually. **Turker *et al.,* [40]**, mentioned that in *Verbascum thapsus*, all explants tested formed shoots with either BA or Kin, over all the number/ explant was lower for all explants on media with Kin when compared to BA.

Table (2): Effect of cytokinins [Kinetin (Kin) and Benzyl adenine (BA)] on growth response.

Treatment mg/l	Survival %	Number of shoot lets/explant	Length of shoot lets cm/explant	Leaf number /explant	Rooting %
Control	**100**	3.59	5.56	6.66	100
Kin					
0.5	98	6.59	3.89	4.74	41.33
1.0	95	9.52	2.97	3.96	0.0
2.0	91	3.21	1.11	1.09	0.0
BA					
0.5	96	9.93	3.15	3.11	15.37
1.0	92	13.32	1.97	1.02	0.0
2.0	88	2.07	1.21	4.74	0.0
LSD at 5%	**-**	**1.254**	**0.221**	**0.72**	**0.131**

C- Effect of auxin, Indole – 3 – butyric acid (IBA) on rooting of shoots.

Data of the effect IBA on the rooting percentage are showed in Table (3), medium control (White's medium free hormone) was recorded the maximum response of survival & rooting percentage (100 %), root number/explant (7.93) and root length cm / explant (7.22). Increasing levels of IBA from 0.5 to 4.0 mg \l in addition to White's medium caused decreasing in parameters were recorded. In this concern, Caruso [41] observed

that when stem segments of *Verbascum thapsus* were grown on a sample nutrient medium without growth regulators , they gave a morphogenetic response, this kind of bud formation in *Verbascum thapsus* was interpreted as resulting from the interaction of endogenous growth regulators which emanate from vascular tissues in the inter nodal segments. Razdan, [42] mentioned that, White's medium is one of the earliest plant tissue culture media originally formulated for root culture. All shoots began rooting on medium without plant growth regulator, those subsequently transferred to rooting media containing IBA produced on increased root number / explant after the rooting treatment , and no difference in survival (95%) was observed when elongated shoots were placed on medium without IBA and subsequently transferred to rotting media for *Echinacea purpurea* (Koroch *et al.* [43]). Based on these results these results, IBA treated is unnecessary for root induction.

Table (3): Effect of auxin, Indole – 3– butyric acid (IBA) on rooting of shoots.

Treatments IBA (mg/l)	Survival %	Rooting %	Root number/ explant	shoot length cm / explant
control	100	100	7.93	7.22
0.5	97	96.24	7.01	6.11
1.0	94	92.33	6.54	5.63
2.0	91	88.01	6.08	5.49
4.0	88	66.66	6.01	5.37
LSD at 5%	**-**	**-**	**0.232**	**0.121**

D- Effect of growing media, peat moss and sand on plants acclimatization.

The effects of growing media (peat moss and sand) on plants acclimatization are presented in Table (4). Survival percentage and root number / explant were recorded the maximum response and root number/ explant (100 %, 8.44, 8.66 and 8.88 respectively) with peat moss /sand (1/1, 1/4, 1/8 and 2/1 -V/V) while the lowest value of survival percentage (55) was recorded under peat moss treatment individually, and sand treatment individually was achieved 95%. In shoot length cm / explant, peat moss / sand (1/1, ¼ and 1/8 V/V) were recorded the maximum significant value as follow (13.33, 15.55 and 14.88, respectively), while in root length cm / explant peat moss / sand (1/1 and ¼ -V/V) were recorded the maximum significant value (9.11, 9.55 and 9.74 , respectively) , the minimum value of shoot and root length cm/explant were recorded under peat moss individually. From previously data showed that, sand medium in addition to peat moss medium is an important for ***P. amboinicus*** acclimatization. This data were agreement with **Rasmussen et. al.,**[11] were showing that Peat moss was mixed with sand to decrease the chance of formation of anaerobic conditions created by microbial decomposition of the organic material, and to optimize hydraulic conditions

Table (4) Effect of growing media, peat moss and sand on plants acclimatization.

Medium composition V/V		Survival %	Shoot length cm / explant	Root length cm / explant	Root number / explant
Peat moss	sand				
1	0	55	4.22	5.01	2.01
0	1	95	7.33	7.72	7.72
1	1	100	13.33	9.55	8.44
1	4	100	15.55	9.77	8.66
1	8	100	14.88	9.11	8.88
2	1	90	12.44	5.85	4.65
4	1	86	10.43	4.66	3.79
8	1	72	5.77	3.44	2.55
L.S.D. at 5%		**-**	**1.102**	**0.345**	**0.324**

E-Effect of various degrees of temperature on acclimatization and growth of *P. amboinicus* in greenhouse under control.

Shoots produced from multiplication stage by tissue culture without roots were transferred from planting media to the greenhouse under control in plastic containers (5 cm) full of peat moss and sand (1/4-V/V) at different temperature degrees for six weeks for root formation and data of the plant growth were shown in Table (5) Data shows that, temperature degree levels (21, 26, 31 and 36 ºC) improved the acclimatization and plants growth in greenhouse under control. In case of temperature degrees 31 and 36 ºC significantly recorded the maximum values of survival percentage which was 100% with both of them. The same degrees observed the best plant height (20.77, and 27.99cm), fresh weight plant (14.33 and 17.22 g/plant), root number (9.66 and 12.22) root fresh weight (3.38 and 4.85 g/plant) and plant growth rate (130.99 and 160.22 %). The lower degree temperature showed lower effects on all treatment as shown in Table (5).

Amat [44] which reviewed that *Stevia rebaudiana* growth requires mild temperature between 15 and 38 degree and relative humidity of about 80 %, supposed that at the higher temperature, respiration exceeded Fig synthesis. The formation of adventitious roots on shoots is temperature dependant, for example no_roots formed on young shoot tips of Asparagus at 0, 10 or 15 ºC, but 22 % of cuttings rooted at 20 ºC, and 45 % at 25 ºC (Gorter, 1965), Most *plectranthus* are adapted to live in the summer rainfall forest, and have to deal with low light, trampling, winter drought, and root competition. They are fast-growing, which enables them to move quickly into areas of good light and /or nutrients **(**Bessi [45] and Bowden, [46]).

Table (5): Effect of temperature degree levels on acclimatization and growth of in vitro obtained *P. amboinicus* after 5 weeks in greenhouse under control.

Temperature ºC	Survival %	Plant height cm	Root number / explant	Root length cm / explant	Fresh weight g/plant	Plant growth rate
21 ºC	**75**	**6.88**	**5.79**	**2.98**	5.77	25.98
26 ºC	**93**	**12.44**	**7.33**	**4.61**	9.88	75.51
31 ºC	**100**	**20.77**	**9.66**	**7.71**	14.33	130.99
36 ºC	**100**	**27.99**	**12.22**	**9.55**	17.22	160.22
LSD at 5%	**-**	**3.252**	**1.923**	**1.321**	**1.914**	**15.101**

F- Effect of various seasons on growth and development of *P. amboinicus* cultured in and out normal greenhouse conditions.

This experiment was conducted to study the effect of various seasons (spring, summer, autumn and winter, respectively) on the survival percentage, vegetative growth and development for those plants cultured in and out normal greenhouse conditions. As shown in table (6) the recorded data revealed that the maximum values in plant height cm, (29.44, 59.52, 43.21 and 18.88, respectively), fresh weight g/ herb of plant (63.89, 122.97, 111.68 and 27.77, respectively), and plant growth rate (44.74 %, 153.96 %, 131.09 and 22.33 %, respectively). These results have a significant differences ($P<0.05$) among the various seasons (spring, summer, autumn and winter respectively). While out greenhouse conditions. The maximum effect of various seasons (spring, summer, autumn and winter) on growth, development and survival percentage was recorded as showed in table (6). The recorded data has a significant differences ($P<0.05$) in various seasons (spring, summer, autumn and winter respectively). The good plant growth requires mild relatively high temperature and enough light which is always available between summers to autumn**.** (Van Jaarsveld, [47] 2006)

Table (6): Effect of various seasons (spring, summer, autumn and winter) on survival percentage and vegetative growth of *P. amboinicus* cultured in and out normal greenhouse conditions.

Seasons	Survival %	Av.plant height cm	Av. fresh weight g/ herb of plant	Plant growth rate
Plant growth inside normal greenhouse (under controlled				
Spring	94	29.44	63.89	44.74
Summer	100	59.52	122.97	153.96
Autumn	100	43.21	111.68	131.09
Winter	88	18.88	27.77	22.33
Plant growth in outside normal greenhouse				
Spring	87	24.22	53.89	34.74
Summer	100	44.55	162.97	153.96
Autumn	100	33.66	141.88	131.09
Winter	82	15.33	21.77	22.33
LSD at 5%	**-**	**3.254**	**4.221**	**2.725**

Part II - Phytochemical methods for *P. amboinicus*:

1-Investigation of essential oil from *P. amboinicus* including:

P. amboinicus is rich in essential oil (i.e. > 0.6% volatile oil on a dry weight basis), the GC chromatogram as shown in Fig (1) and identifiedby GC/MS as shown in Tab (7) revealed the presence of the following compounds α-thugen, α–pinene, camphene, β-pinene, β-myrcene, limonene, thymol, α-cubebene,thymol acetate, α-copaene, junipene, β-cubebene, trans-caryophllene, α-humulene, dodecen-1-ol, β-selinene,α-zingiberene,α-Selinene, α-amorphene , cis-calamenene, delta-cadinene, caryophyllene oxide and pentadecanoic acid. It is obvious that limonene represented the highest percentage (42%) for followed by β-myrcene (11.3%) while was the lowest one was Junipene (00.54%). **Fabíola et al** [48] showed that the essential oils from *Plectranthus amboinicus, Plectranthus ornatus*, and *Plectranthus barbatus* were investigated for their chemical composition. The major components found were carvacrol (54.4% *P.amboinicus*) and eugenol

(22.9%—*P.ornatus* and 25.1%—*P. barbatus*).**Yaouba et al** [49] showed that the main components found in *P. glandulosus* leaves oil were terpinolene (30.8%), fenchone (13.2%), terpene 4-ol (11%) and piperitenone oxide (8%) **Mahesh et al** [50] proved that the chemical composition of the essential oil obtained from the leaves of *Plectranthus incanus* Link, with the yield of 0.6% (w/v), was analyzed by GC and GC/MS. A total of 16 constituents, representing 95.2% of the oil, were identified. The major components of the oil were fenchone (6.0%), piperitone oxide (32.4%), piperitenone (3.0%) and piperitenone oxide (41.5%). **Mwangl etal** [51] studies the essential oil isolated by hydro distillation from leaves of *Plectranthus tenuiflorus* was analyzer by GC and GC–MS. A total of 17 compounds accounting for 72.3% of the oil were identified. Carvacrol (14.3%) α-terpinene (10.2%) and *p*-cymene (10.9%) were the major constituents. The oil had low quantities of oxygenates terpenes. **Abdel-Mogib et al** [52] showed that the genus Plectranthus is rich in essential oil (i.e. > 0.5% volatile oil on a dry weight basis).The main constituents of essential oils of Plectranthus are mono- and sesquiterpenes.

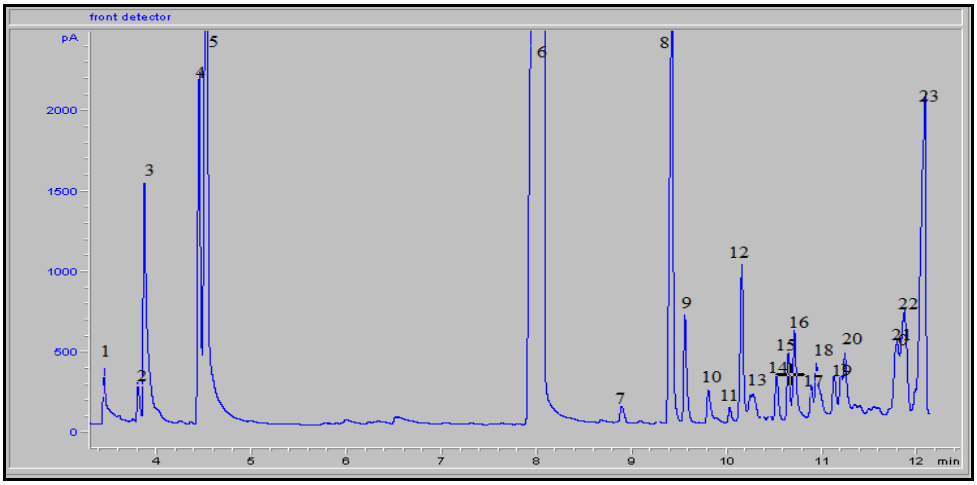

Fig (1) GC chromatogram for oil of *Plectranthus amboinicus.*

Table (7) GC/ MS analysis of essential oil *Plectranthus amboinicus*

No	KI(Kovats Retention Indices)	Name	%
1	931	Thujne	01.20
2	933	α pinene	00.70
3	953	Camphene	04.70
4	980	β pinene	04.10
5	990	β Myrcene	11.30
6	1029	Limonene	42.00
7	1290	Thymol	00.70
8	1351	α Cubebene	9.80
9	1355	Thymol acetate	01.50
10	1376	α Copaene	00.74
11	1386	Junipene	00.54
12	1390	β Cubebene	02.14
13	1410	Trans-caryophllene	00.95
14	1449	α Humulene	00.85
15	1461	Dodecen-1-ol	00.89
16	1485	β Selinene	01.75
17	1492	α Zingiberene	00.84
18	1495	α Selinene	01.60
19	1498	α Amorphene	01.40
20	1521	Cis-Calamenene	01.80
21	1530	Delta-Cadinene	03.00
22	1577	Caryophyllene oxide	01.50
23	1866	Pentadecanoic acid	06.00
Total	-	-	100.00

Kovats Retention Indices according to the mass spectrum (MS) and by comparison of KI with the literature.

II - % Antioxidant activity, Total phenolic compounds and Total flavonoids in different extracts of the studied plants as percentage of extracts of *P. amboinicus*.

As shown in table (8), the antioxidant activity, total phenolic compounds and flavoniod contents were investigated through out greenhouse and in greenhouse cultivated ***P. amboinicus*** groups. The obtained results were appeared 38 %, 77 µg and 29%, 35 µg %, while the results of in greenhouse were 25 %, 55 µg % and 28µg %, respectively.

It has been mentioned that, the antioxidant activity of plants might be due to their phenolic compounds (Cook and Samman,[13]). Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action. Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity (Frankel and Meyer [32]). Currently available synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinone and Gallic acid esters, have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity (Barlow, [53]).

Table (8) % Antioxidant activity (by using of DPPH method), Total phenolic compounds (Measured by Folin Ciocalteu and Total flavonoids (Measured by $AlCl_3$) in different extracts of the studied plants as percentage of extracts of *P. amboinicus*.

Name of extract	% Antioxidant activity	Total Phenolic contents µ g %	Total Flavonoids contents µ g %
***P. amboinicus* growth** out greenhouse	38 %	77	65
***P. amboinicus* growth** In greenhouse	29 %	35	31
Mother plant growth out greenhouse	30 %	70	55
Mother plant growth In greenhouse	25 %	30	28

Part III- Anti microbial Screening studies for volatile oil of *P. amboinicus*.

1- Antimicrobial Activity by using Disk Diffusion and MIC Assays:

As shown in (Tables 9 and 10) that all the test organisms except *Pseudomonas aeruginosa (ACCT 27853) and Pseudomonas aeruginosa (ACCT 9027)* are most susceptible towards oil comparing with other organisms tested. The abundant of phytochemical constituents in *P. amboinicus* oil as mentioned in (Tables 7) might contribute for the abilities of the extracts to inhibit the microbial growth successfully even with the small concentration tested (containing 10 µl of pure oil). But, the abilities of the *P. amboinicus* water extract to kill the microorganisms somehow difficult for *, Pseudomonas aeruginosa(ACCT 27853) ,Pseudomonas aeruginosa (ACCT 9027), Escherichia coli (ATCC 10538) and Escherichia coli (ATCC 14169)* (9.0 to 6.0 µg/ml) as described by MIC values in (table 10). Nascimento *et al.* [54] reported that *B. subtilis* has the protective endospores so that it can tolerate in the extreme conditions easily. Based on (Table 10), *P. amboinicus* root extract were considered susceptible to the all microorganisms tested especially fungus, yeast and some strains of bacteria. In this study, the abundant of phytochemical exist in the plant as in *P. amboinicus* whole plant didn't assure the susceptibility of the bacteria towards the plant extracts. The spread antibiotic-resistant microorganisms plus the complexity of the phytochemical itself might be the reasons for the *P. amboinicus* oil tolerant to the some bacterial tested. The susceptibility of the test microorganism is related to inhibition zone size in millimeters via agar well diffusion assay. Microorganisms are termed susceptible to the plant extract when the zone inhibition is equal to or more than 3 mm (≥3) in diameter, or resistant with a zone of inhibition less than 3 mm (<3) therapies Nascimento et. al., [54]. Among the microorganisms tested are more resistant to the *P. amboinicus* oil, *Pseudomonas aeruginosa* (ACCT 27853*) ,Pseudomonas aeruginosa (ACCT 9027)*, *Escherichia coli (ATCC 10538)* and *Escherichia coli (ATCC 14169)* are less susceptible to the action of antimicrobials activities more than fungal organisms and yeast since the less susceptible microorganisms possess an outer layer of membrane which could protect cell wall and restrict hydrophobic compounds from diffuse through the lipopolysaccharide covering (Vaara, [55]). And this agreement with Nara et.al., [56]. However, the formation of a clear zone of inhibition from agar-well diffusion assay could not indicate the effectiveness of

the antibacterial activity (Friedman *et al.,*[57]), the micro-dilution method has provided a potentially useful technique for MIC of large numbers of test samples. Its advantages over diffusion techniques include increased sensitivity for small quantities of extract and ability to between bacteriostatic and bactericidal, (Ncube *et al.* [58]).

Table (9). Antibacterial activity of the essential oils of *P. amboinicus*

Test organisms	Diameter of zone of inhibition (mm) distinguish		
	EO	**Ceftriaxon.**	**Clotrimazole**
Pseudomonas aeruginosa(ACCT 27853)	R	22	-
Pseudomonas aeruginosa(ACCT 9027)	R	22	-
Escherichia coli(ATCC 10538)	5	23	-
Escherichia coli(ATCC 14169)	7	30	-
Micrococcus luteus(ATCC 9341)	15	25	-
Bacillus subtulis(ATCC 6633)	4	26	-
Staphylococcus aureus(ATCC 6538)	21	29	-
Staphylococcus aureus(ATCC 25923)	17	29	-
Candida albicans(ATCC 10231)	19	-	21
Aspergillus niger(ATCC 16404)	13	-	19
Fusarium oxysporum(ATCC 48112)	10	-	17

*****The results are the mean of 3 readings. Key, R= Resistant (absence of inhibition of microbial growth at 100ug/ml) H= halo of inhibition (mm) EO= Essential oil; Cef. = 0.2UI/ml; 10mg; - = not determined

According to El-hawary *et al.* [15] and Claribel Luciano-Montalvo *et, al.,*[59], the MIC of extract against the tested microbes was found that oil of the leaves showed high antifungal activity against all the tested fungi and yeast compared to clotrimazole as standard .The findings indicated that the oil of *P. amboinicus* contain phenolic compounds that have antimicrobial activity comparable to other medicinal plants**.**

Table 10. Minimum inhibitory concentration (MIC) *P. amboinicus*

Test Organism	MIC (µg/ml)
	EO
Pseudomonas aeruginosa(ACCT 27853)	7.1
Pseudomonas aeruginosa (ACCT 9027)	9.0
Escherichia coli (ATCC 10538)	8.2
Escherichia coli (ATCC 14169)	6.0
Micrococcus luteus (ATCC 9341)	1.5
Bacillus subtulis (ATCC 6633)	2.1
Staphylococcus aureus (ATCC 6538)	0.5
Staphylococcus aureus (ATCC 25923)	0.37
Candida albicans (ATCC 10231)	1.4
Aspergillus niger (ATCC 16404)	1.2
Fusarium oxysporum (ATCC 48112)	1.45

References

1. Paton, A.J., Springate, D., Suddee, S., Otieno, D., Grayer, R.J., Harley, M.M., Willis, F., Simmonds, M.S.J., Powell, M.P. and V. Savolainen (2004). Phylogeny and evolution of basils and allies (Ocimeae, Labiatae) based on three plastid DNA regions. Molecular Phylogenetics and Evolution 31, 277–299
2. Potgieter, C. J.; Edwards, T.J.; Miller, R. M. and Staden, J. V. (1999).Pollination of seven *Plectranthus spp*. (Lamiaceae) in southern Natal, South Africa. Plant Systematics and Evolution. 218: 99- 112
3. Liu G, Ruedi P 1996. Phyllocladanes (13-beta-kauranes) from *Plectranthus ambiguus. Phytochemistry 41*: 1563-1568
4. Hutchings, A., Scott, A.H., Lewis, G., A. Cunningham (1996). Zulu Medicinal Plants. An inventory. University of Natal Press, Pietermaritzburg
5. Rabe, T. and J. Staden (1998). Screening of Plectranthus species for antibacterial activity. South African Journal of Botany 64, 62–65
6. Hulme, M.M. (1954).Wild Flowers of Natal. Shulter&Shooter, Pietermaritzburg
7. Liang, S., Zhong, J. and T. Yoshida (1991). Review of plant cell culture technology for producing useful products (part 1). Ind. Microbial. 21(3), 27-31
8. Datta, S.C. (1994).Plant Physiology. Published by H.S. Popali for Wiley Eastern Limited, Ansari road. Printed in India
9. Jones, J.B. (1982). How can we get micro cutting out of the lab .Comb. Proc. Intl Plant Pro. Soc., 32: 322-327
10. Miller, D. (1983). Weaning and growing on of micro propagation plants, Comb Intel Plant Prop Soc 33: 253-25
11. Rasmussen, G., Fremmersvik, G. and R.A. Olsen (2002). Treatment of creosote-contaminated groundwater in a peat/sand permeable barrier—a column studies. Journal of Hazardous Materials B93. 285–306
12. Wilson, S.B. and P.J. Stoffella (2006). Using compost for contain production of ornamental wetland and flat wood species native to Florida. Native Plants Journal (7). (3): 293-300
13. Cook, N.C. and Samman, S. (1996). Flavonoids Chemistry, metabolism, cardio-protective effects, and dietary sources, Nutrition Biochemistry, 7: 66-76
14. Rashmi SK, Sourish Karmakar and Shanta Banerjee. (2011). Uropathogen Resistant Essential Oils of *Coleus aromaticus* and *Ocimum sanctum*. International Journal of Pharmaceutical Science and Research, 2(8): 2168-2172
15. El-hawary Seham S. and El-sofany Rabie H., (2013). Polyphenolics Contents and Biological Activity of *Plectranthus amboinicus* (Lour) Spreng Growing in Egypt (*Lamiaceae*). Journal of Pharmacology, 2012; 2(3): 45-56
16. Jiyauddin K., Samer A. D., Darashhni T., Rasha S., Jawad A., M. Kaleemullah S. Budiasih1, Rasny M. R., M. Qamar1, Hamid K., Sakina R., Junainah A. H. Fadli A. and Eddy Y. (2015).Comparison of antibiotic activity of Ocimum (*Tenuiflorum L*. and *Plectranthus amboinicus* (LOUR SPRENG) Against the clinical pathogens *Staphylococcus aureus , Pseudomonas aeruginosa* and *E. coli.* (13beta-kauranes) from *Plectranthus ambiguous*. Phytochemistry 41, 1563–1568
17. Helmy Mai M. and Riham O. Bakr (2014). *In vitro* Comparison of the Antimicrobial Activity of Five Herbal Extracts, and Selected Mouthwashes Marketed in Egypt against Cariogenic Streptococcus Mutans. Egyptian Journal of Medical Microbiology, January 2014 Vol. 23, No. 1
18. Chandrappa, M. S. Harsha, R. Dinesha R.and T. S. S. Gow- da, (2010). "Antibacterial Activity of *Coleus aromaticus* Leaves," *International Journal of Pharmacy and Pharmaceutical Sciences*, Vol. 2, 2010, pp. 63-66
19. Gurgel, A. P. A. D., daSilva, J. G., Grangeiro, A. R. S., Xavier, H. S. Oliviera, R. A. G. Pereira M. S. V. and de Souza, I. A., (2009). Antibacterial Effects of *Plectranthus amboinicus* [Lour.] Spreng (Lamiaceae) in Methicillin Resistant *Staphylococcus aureus* (MRSA), *Latin American Journal of Pharmacy*, Vol. 28, No. 3, 2009, pp. 460-464
20. Praveena Bhatt and Pradeep S. Negi, (2012). Antioxidant and Antibacterial Activities in the Leaf Extracts of Indian Borage (*Plectranthus amboinicus*) *Food and Nutrition Sciences*, 3, 146-152
21. Murasige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol. Plant,15: 473-497
22. White, P.R. (1963): The cultivation of animal and plant cells (2nd ed.) .2 Ronald Press, New York, pp. 1-239

23. Gamborge, O.L., Miller, R.A. and K. Ojima (1968). Nutrient requirement of suspension cultures of soybeans root cells. Exp. Cell Res. 50:151-158
24. Pattino, B.G. (1981). Methods In Plant Tissue Culture .Dept. of Hort. Agric, College, Maryland University., College Park, Maryland , USA., PP 8-29
25. S.A.S. (1988). Statistical analysis system SAS Users Guide: Statistical SAS Institute Inc. Editors, Cary, N.S
26. Steel, G. D. and J.H. Torrie (1980). Principles and procedures of statistics, Mc. Grow Hill Boot –Col. New York
27. Egyptian Pharmacopoeia (1984): General organization for government 3rd eds., printing office, Cairo
28. Adams, R.P (2007). Identification of Essential Oils by Gas Chromatography Quadruple Mass Spectrometry. 4th edition, Allured: Carol Stream, USA
29. Astudillo. L.; Schmedu-Hischmmann, G.; Herrea, J.P. and Cortes, M. (2000). Proimate composition and biological activity of Chilean Prosopis species.J. Sci. Food Agri., 80: 567-573
30. Daniele S., Giovanna D., Maurizio M., Mario S., and Angela F. (2012). Determination of Free Radical Scavenging Activity of Plant Extracts Through DPPH Assay: An EPR and UV–Vis Study Food Anal. Methods 5:759–766
31. Viturro, C; Moina, A. and Schmeda-Hirchmann, G (1999). Free radical scavengers from *Mutisi friesiana* (*Asteraceae*) and *Sanicula gravelens (Apiaceae*) Phytother.Res., 13:422-424
32. Frankle, E. and Meyer, A. (2000). The problem in using one dimensional methods to evaluate multifunctional food and biological antioxidants', J. Sci. Food Sci. Agric., 80, 1925-1941
33. Donald, S., Prenzier, P.D., Autolovich, M. and Robard, K., (2001).Phenolic content and antioxidant activity of olive extracts, Food Chemistry, 73: 73-84
34. Julkunen-Tiito. R. (1985). Phenolic constituents in the leaves of northern willows: Methods for the analysis of certain phenolic. J. Agric Food Chem., 33, 213-217
35. Karawya, M.S. and Aboutabl, E.A. (1982).Phytoconstituents of *Tabernaemontana cornari* Jac Q. Willd and *Dichotoma roxb*, growing in Egypt Univ. XXI (1): 41-49, 41-650
36. Chiang, L.C.; Chaiang, W.; Chang, M.Y.; Ng, L.T. and Lin, C. (2002).Antiviral activity of *Plantago major* extracts and related compounds *In vitro*. Antiviral Res., 55: 53-62
37. Bauer, A.W., Kirby, W.M., Sherris, J.C., Turck. M. (1966). Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology 1966; 45:493-496
38. Hammer KA, Carson CF, and Riley TV. (2002): *In vitro* activity of *Melaleuca alternifolia* (tea tree) oil against dermatophytes and other filamentous fungi. J Antimicrobial Chemother, 50:195-199
39. Kumar,U.(1999). Methods in Plant Tissue Culture .Published by Agro Botanica, 4 E 167, J.N. Vyas Nagra, Bikaner. New Delhi .India
40. Turker, A.U., Camber, N.D. and E.Gurel (2001). *In vitro* culture of mullein (*Verbascum thapsus* L.) In Vitro Cell .Dev .Biol. Plant .37:40-43
41. Caruso, J.N. (1971). Bud formation in excised from segments of *Verbascum thapsus* L. Amer. J. Bot. 58:429-431
42. Razdan, M .K. (1996). An Introduction to Plant Tissue Culture. Published by Raju premlani for Oxford and IBH Publishing Co. pvt. LTD, 66 Janpath. New Delhi. Calcata. India
43. Koroch, A., Juliani, H.R., Kapteyn, J. and J.E. Simon (2002). *In Vitro* regeneration of *Echinacea purpura* from leaf explant. Plant Cell, Tissue and Organ Culture .69:79-83
44. Amat, A.G. (1982). Losprincipios edulcorantes de *Stevia rebaudiana*, Bert Estado actual de su conoci miento Acta Farm Bonaer 1: 121-123
45. Bessi, M. (1924). The South African Nemestrinidae (Diptera) as represented in South African Museum. Ann. S. African Mus.19: 164-190
46. Bowden, J. (1978). Diptera. In: Werger M. J. A. (ed) Biogeography and ecology of southern Africa. Junk. The Hague. 775-796
47. Van Jaarsveld, E. (2006): The southern African *Plectranthus* and the art of turning shade into glade. Fern wood Press, Simon's Town, South Africa
48. Fabíola Fernandes Galvão Rodrigues, José Galberto Martins Costa, and Adriana Rolim Campos (2013). Study of the Interference between Plectranthus Species Essential Oils from Brazil and Aminoglycosides. Evidence-Based. Complementary and Alternative Medicine Article ID724161, 7pages
49. Yaouba Aoudou, Tatsadjieu Ngoune Léopold and Mbofung Carl Moses (2011). Mycelia growth inhibition of some *Aspergillus* and *Fusarium* species by essential oils and their potential use as

antiradical agent. Agriculture and Biology Journal of North America, ISSN Print: 2151-7517, ISSN Online: 2151-7525

50. MaheshPal, Anil Kumar, Shri Krishna Tewari (2011). Chemical composition and mosquito repellent activity of the essential oil of *Plectranthus incanus* Link Physics, Chemistry and Technology Vol. 9, No 1, pp. 57 – 64
51. Mwangl, J. W. W. Lwande andA. Hassanali (2006). Composition of essential oil of *Plectranthus tenuiflorus* (Vatke) Agnew Flavour and Fragrance Journal Volume 8, Issue 1, pages 51–52
52. Abdel-Mogib, M.; Albar, H.A. and Batterjee, S.M. (2002). Chemistry of the Genus *Plectranthus,* Molecules, *7,* 271301
53. Barlow, S.M. (1990). Toxicological aspects of antioxidants used as food additives in food antioxidants", Hudson BJF (Ed) Elsevier, London, 253-307
54. Nascimento, G.G.F., Locatelli, J., Freitas, P.C., Silva, G.L., (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic resistance bacteria. Braz. J. Microbiol. 31: 247-256
55. Vaara, M., (1992). Agents that increase the permeability of the outer membrane. Microbiol. Rev. 56: 395-411
56. Nara O. dos Santos, Bruna Mariane, João Henrique G. Lago, Patricia Sartorelli, Welton Rosa, Marisi G. Soares, Adalberto M. da Silva, Harri Lorenzi, Marcelo A. Vallim and Renata C. Pascon (2005). Assessing the Chemical Composition and Antimicrobial Activity of Essential Oils from Brazilian Plants—*Eremanthus erythropappus* (Asteraceae), *Plectrantuns barbatus*, and *P. amboinicus* (*Lamiaceae*) .Molecules, 20, 8440-8452
57. Friedman, M., Henika, P.R., and Mandrell, R.E., (2002). Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni, Escherichia coli, Listeria monocytogenes,* and *Salmonella enterica.* J. Food. Prot. 65: 1545-1560
58. Ncube, N.S., Afolayan, A.J. and Okoh, A.I. (2007). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African Journal of Biotechnology 7: 1797-1806
59. Claribel Luciano-Montalvo, Isabelle Boulogne and Jannette Gavillán-Suárez (2013): A screening for antimicrobial activities of Caribbean herbal remedies.BMC Complementary and Alternative Medicine , 13:126

*****