Webology (ISSN: 1735-188X)
Volume 19, Number 6, 2022

Inhibitory Activity Of Essential Oils Of Curcuma Longa And Zingiber Officinale Against Burkholderia Glumae

Alexander Pérez- Cordero[1], Edin Arroyo Canchila[1], Donicer E Montes- Vergara[2]

[1]Grupo Bioprospección Agropecuaria, Laboratorio de investigaciones microbiológicas, Facultad de Ciencias Agropecuarias, Universidad de Sucre, Sincelejo, Sucre Colombia.

[2]Departamento de Zootecnia, Facultad de Ciencias Agropecuarias, Universidad de Sucre, Sincelejo, Sucre-Colombia.

* Correspondence: Author: Alexander Pérez- Cordero

ABSTRACT

Essential oils are organic compounds that during the last century have gained relevance in the pharmaceutical sector due to their antimicrobial properties, becoming viable alternative solutions for certain phytopathology. Rice plants are attacked by Burkholderia glumae, which causes bacterial blast. Therefore, the aim of this study was to evaluate in vitro the inhibitory activity of the essential oils of C. longa and Z. officinale on B. glumae. The plants were collected in a rural area in the municipality of Tolú Viejo-Sucre. Fresh rhizomes were washed with water and selected, then sliced and the essential oil was extracted from 1 kg of material from each species by the microwave-assisted hydrodistillation technique. The bacterial inoculum was mass seeded on KIN B agar plates. To evaluate inhibitory activity, the agar diffusion method was used using filter paper discs and DMSO as solvent. The dilutions of the oils were 1:1, 1:5 and 1:10 and 15 µl of each dilution was placed aseptically on the paper. The determination of the chemical components of the essential oils was carried out using the instrumental technique of Gas Chromatography with Mass Selective Detector (GC/MS). Five extractions of the essences were made from 200 g of sample, yielding an average of 0.50 g of oil per extraction from the rhizomes of C. longa, with a yield of 0.25 %, and 0.85 g of oil from the rhizomes of Z. officinale, with a yield of 0.43%. At 1:10 and 1:5 dilutions for the two oils there was no inhibitory activity and only at 1:1 dilution for the two oils there were inhibition halos showing slight inhibitory activity. There is inhibitory activity when the concentrations in the two types of oils exceed values of 500000 ppm, which suggests evaluating other variables that may be affecting the performance and efficiency of these oils to inhibit the growth of this phytopathogens.

Keywords: Essential oils, secondary metabolites, antibacterial activity, rice cultivation.

INTRODUCTIÓN

In recent years there has been a growing interest in the use of biologically active organic compounds extracted from plant species that have the ability to kill pathogenic micro-organisms by themselves, mainly due to the resistance that micro-organisms have developed to antibiotics (1). Essential oils, also called volatile compounds, are aromatic, oily liquids obtained from plant material (flowers, buds, seeds, leaves, bark, herbs, wood, fruits and roots). Essential oils are composed of lipophilic substances, mainly monoterpenes and sesquiterpenes, and their oxygenated derivatives (alcohols, aldehydes, ketones, phenols, acids, esters and ethers) in varying amounts (2). They have traditionally been used by the cosmetics industry in the production of perfumes, although during the last century they have gained importance in the pharmaceutical sector due to their antimicrobial properties (3).

Essential oils are widely distributed in a large variety of plants in more than 60 families (4), which have been reported to produce essential oils with some antimicrobial activity. An example of this group of plants are those belonging to the family Zingiberaceae, of the order Zingiberales of the monocotyledons, which has more than a thousand species in 50 genera. Many plants belonging to this family contain aromatic oils, the most representative being Curcuma longa and Zingiber officinale, described for their economic and ethnobotanical importance. Turmeric plants (Curcuma longa) have been attributed antioxidant (5), hepatoprotective (6), anticarcinogenic (7) and chemoprotective (8) properties. The rhizomes of the plant contain 2.44% essential oil (9), consisting of a mixture of terpenes and turmerone as the main components, which gives it potential antimicrobial activity (10, 11). Ginger (Zingiber officinale) has been studied to contain up to 3% of an aromatic essential oil whose main constituents are sesquiterpenoids, with zingiberene as the major component. Its characteristic odour and flavour is caused by a mixture of zingerone, shogaols and gingerols which have been reported to have certain antibacterial properties (12).

Bacterial diseases in plants have become very important due to the economic losses caused by the decrease in agricultural production, often caused by poor implementation of control measures; post-harvest damage caused by bacterial pathogens in plant products is also considerable (13). Rice, for example, is the main cereal used as a source of food, more than 50% of the world's population benefits from this product, and in Colombia it occupies the first place in terms of economic value among short-cycle crops (14). Rice plants are attacked by many diseases caused by multiple phytopathogens resulting in low yields and crop losses worldwide. Bacterial panicle blast of rice caused by Burkholderia glumae, reported in different countries around the world, causes yield reductions of up to 75% in severely affected regions, because the phytopathogen directly affects grain filling (15). The application of pesticides for the control of this disease has been a technique used, but it has not been effective in its results and also carries an imminent danger for the environment (16).

In accordance with the above, and taking into account the need to find new antibacterial agents to manage this type of disease in a natural and more efficient way, the potential of essential oils from representative species of the zingiberaceae family was used to evaluate the antibacterial activity of these oils on B. glumae. Therefore, the aim of this study was to evaluate in vitro the inhibitory activity of the essential oils of C. longa and Z. officinale on B. glumae, which causes bacterial panicle blast in rice in the department of Sucre-Colombia.

MATERIALS AND METHODS

Collection of plant material and taxonomic identification

The plants were collected in the municipality of Tolú Viejo, in the rural area of Piche at 86 m above sea level in the department of Sucre and were subsequently transported and classified in the herbarium of the University of Sucre for their respective identification and assignment of the Voucher code.

Extraction of essential oils

Fresh rhizomes of each species were washed with water and selected to guarantee the good condition of the samples and then they were transferred to the microbiological research laboratory of the University of Sucre under refrigeration at a temperature of 4 ºC; The rhizomes were then cut into slices and the essential oil was extracted from 1 kg of material from each species. This procedure was carried out in a modified Clevenger microwave-assisted hydrodistillation equipment for 1 hour per 200 grams of material, immersed in 100 ml of water in the case of turmeric rhizomes and 500 ml of water for ginger; the essential oil obtained from each sample was separated from the aqueous layer, dried with anhydrous sodium sulphate, filtered and stored at 8°C, with this product inhibitory activity was evaluated on the phytopathogens B. glumae and an aliquot was sent to the National University of Colombia to establish its chemical composition.

Determination of the antibacterial activity of essential oils

Preparation del inoculum. The bacterial strain of B. glumae (ATCC 4026-1) isolated from rice cultures, on loan from CIAT (Tropical Agriculture Research Centre) was grown in a 24 h culture at 30˚ C, in King B broth, and adjusted to a concentration of 105 CFU/ml with sterile saline. The bacterial inoculum was mass seeded on KIN B agar plates (peptone proteose (20 g/L), K2 HPO4 (1.5 g/L), MgSO4 7H2O (1.5 g/L), glycerol (15.0 ml/L), agar (15 g/L), using a sterile cotton swab, to achieve uniform microbial growth. The plates inoculated with the bacteria were covered with filter paper discs, approximately 5 mm in diameter, on which the essential oils prepared as follows were applied.

To evaluate the sensitivity of this microorganism to essential oils, the agar diffusion method was used, according to the recommendations of Prabuseenivasan et al (17). The essential oils were dissolved in a 10% dimethyl sulfoxide (DMSO) solution, added with Tween 80 (0.5%

v/v), sterilized by filtration (0.45µm Whatman membrane filter). The dilutions of the oils were 1:1, 1:5 and 1:10 and 15 µl of each of the concentrations of the essential oils were aseptically placed on the paper. DMSO was used on one of the filter paper discs as a negative control to rule out the antimicrobial activity of the filter paper. In addition, an oxalicylic acid (antibiotic) disc was used as a reference or positive control. The plates were left for 30 min at room temperature to allow diffusion of the essential oil and then incubated at 30° C for 24 h. After the incubation period, growth inhibition halos (dhi) were measured in millimetres using a caliper. Oil activity was classified as marked (dhi ≥16mm), moderate (12mm ≤ dhi < 16mm), mild (8 mm ≤ dhi < 12mm) or no activity (dhi < 8 mm), according to the ranges of the scale used by Sanchez et al. (18). Analyses were carried out in triplicate.

Analysis by gas chromatography coupled to mass spectrometer (GC/MS). The determination of the chemical components of the essential oils was carried out by the instrumental technique of Gas Chromatography with Mass Selective Detector (GC/MS), using an Agilent 6890N gas chromatography equipment coupled to an Agilent 5973N mass selective detector and with the injector in splitless mode. The Kovats indices were determined on a DB_1MS non-polar capillary column using Helium as carrier gas. The furnace programming was divided into four steps: initially the temperature was set to 50°C and held there for 0.33 min, then the temperature was increased at a rate of 5°C/min to 150°C and held there for 4.34 min, then the temperature was increased at a rate of 10°C/min to 250°C and held for 3 min and finally the temperature was increased at a rate of 15°C/min to 300°C and held for 4 min; the whole run lasted 45 min.

The tentative identification of the registered compounds was established according to their mass spectra, using the database with the highest matching probability for the NIST02.L and NIST5a.L databases greater than 90% or alternatively using the NIST98.L database. All important information such as retention time and area percentage was generated by MSD ChemStation software.

RESULTS AND DISCUSSION

Scientific classification of plant material. Rhizomes and whole plants of the species under study were taken to the facilities of the University of Sucre where the respective extraction of the oils and taxonomic identification were carried out in the herbarium of the University of Sucre where they were classified as Zingiber officinale and Curcuma longa, identified with voucher codes 000900 and 000901 respectively (Figure 1).

Figure 1. Taxonomic identification: Zingiber officinale (A) and Curcuma longa (B).

Extraction of essential oils

Five extractions of the essential oil were made by hydrodistillation from 200 g of each of the two species, yielding on average 0.50 g of a pale yellow liquid for the rhizomes of C. longa, with a yield of 0.25 % per extraction (Table 1); and 0.85 g of a greenish yellow liquid for the rhizomes of Z. officinale, with a yield of 0.43 % (Table 2).

Table 1. Results of hydrodistillation extractions for rhizomes of Curcuma longa.

Extractions	Vegetal mass (g)	Time (h)	Oíl Mass (g)	Yield (%)
1	200	1	0,50	0,25
2	200	1	0,48	0,24
3	200	1	0,52	0,26
4	200	1	0,49	0,245
5	200	1	0,51	0,255
ẋ	200	1	0,50	0,25

Table 2. Results of hydrodistillation extractions for rhizomes of Zingiber officinale.

Extractions	Vegetal mass (g)	Time (h)	Oíl Mass (g)	Yield (%)
1	200	1	0,86	0,43
2	200	1	0,88	0,44
3	200	1	0,86	0,43
4	200	1	0,85	0,425

5	200	1	0,85	0,425
ẋ	200	1	0,86	0,43

ẋ: Mean; Rdto: Yield.

These yield results compared to other studies such as the one carried out by Meza, et al. 2009 (9) show a relatively low production percentage with respect to the yield in the oil extraction process, which suggests the optimization of the extraction technique or other ways of preparing the plant material, being advisable to dry the sample beforehand to increase the biomass contained in the hydrodistillation balloon and thus increase the production of oils per kg of sample.

Determination of the antibacterial activity of the essential oils

A study of the antibacterial activity of the essential oils obtained against B. glumae was carried out, using dilutions of 1:1, 1:5 and 1:10 for each of the oils. A slight antibacterial activity was observed in all cases, being greater as the concentration increased, but when the treatments were compared with the positive control, the latter showed a greater inhibitory effect against B. glumae than the essential oils (figures 2, 3 and 4).

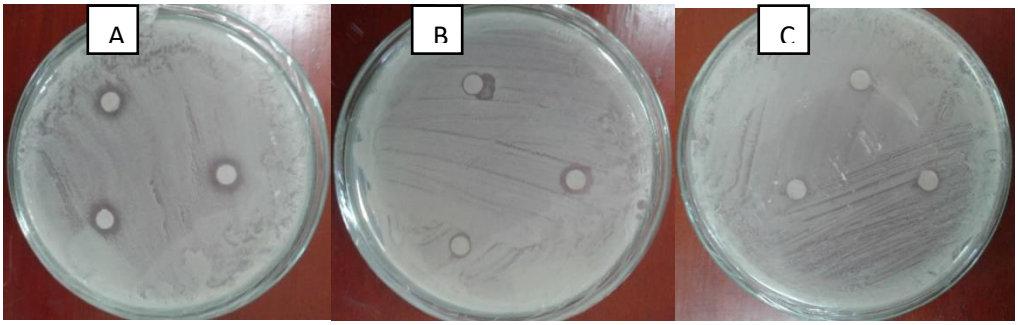

Figure 2. Antibacterial activity of Curcuma longa essential oil against Burkholderia glumae: 1:1 dilution (A), 1:5 dilution (B) and 1:10 dilution (C).

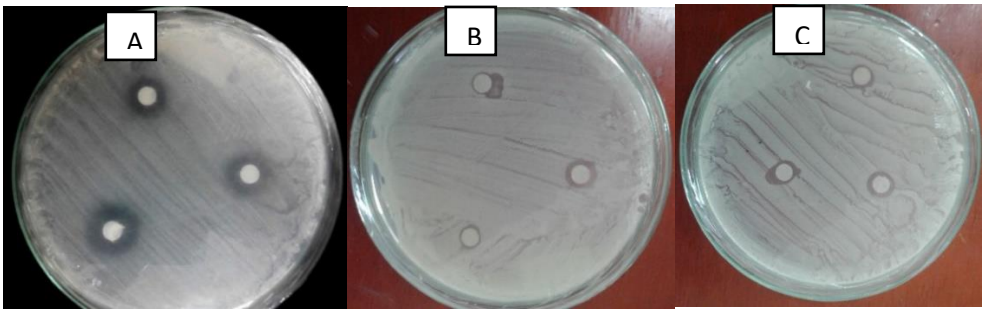

Figure 3. Antibacterial activity of Zingiber officinale essential oil against Burkholderia glumae: 1:1 dilution (A), 1:5 dilution (B) and 1:10 dilution (C).

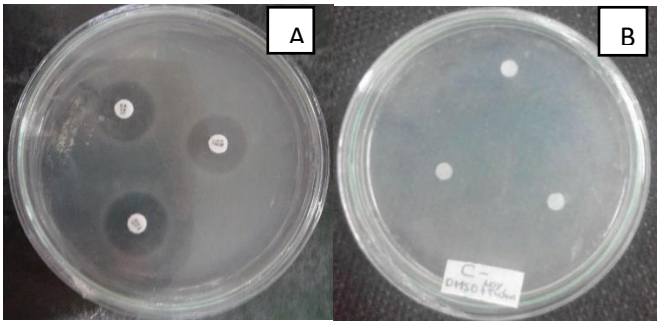

Figure 4. Positive control-oxalicylic acid (A) and negative control-(DMSO) 10%, + Tween 80 (0.5% v/v) (B).

Figures 2 and 3 show qualitative results on the antibacterial activity of essential oils obtained from rhizomes of C. longa and Z. officinale species against B. glumae. In all dilutions used, a slight inhibitory activity against B. glumae was observed, with larger inhibition halos in the more concentrated dilutions for both turmeric and ginger rhizomes, but in the 1:10 and 1:5 dilutions for the two oils, according to the ranges of the scale used by Sánchez et al. 2009 (18) there was no marked inhibition because none of the treatments showed inhibition halos greater than 8 mm, and only the 1:1 dilution for the two oils showed inhibition halos that determined a light type of activity, i.e. the lowest degree of inhibition (8 mm on average for C. longa oil and 10 mm for Z. officinale oil). According to the above, it can be concluded that at the concentrations used, these essential oils did not have a considerable effect of antimicrobial activity, and inhibitory activity only began to be noted when the concentrations in the two types of oils exceeded values of 500000 ppm, which is considered a high concentration and therefore not viable in terms of biological control studies on this phytopathogens using the essential oils of these two plant species. For this reason, it is suggested that other variables be evaluated in future studies to determine comparisons that will allow sufficient data to be gathered to confirm or completely rule out the inhibitory activity of these oils against B. glumae, such as the optimization of the extraction technique or the use of other techniques, cutting and drying methods, extraction times, variation in the biogeographical region from which the plants were taken, among others.

Chemical analysis by gas chromatography coupled to mass spectrometry (GC-MS). The differences in the abundance of each component of the essence, compared to those reported by other authors, emphasize the great variability in the chemical composition of the essence of both turmeric and ginger and the importance of determining the chemical composition that corresponds to a certain biological effect. The results of the chemical analysis of the two types of oils for this case show that there is a great variability of compounds, so much so that the majority compounds do not present very high values in comparison with other studies (Table 3 and 4).

Table 3. Major secondary metabolites of Curcuma longa

IDENTIFICATION	RT	%ÁREA
Pineno	7,140	5,13
β-Pineno	7,528	1,57
3-Thujeno	8,040	25,57
Terpileno	8,465	5,25
Eucaliptol	8,779	7,98
trans-β-Ocimeno	9,050	0,75
γ-Terpineno	9,364	3,25
Terpinoleno	10,374	19,09
1,5,8-p-Mentatrieno	10,710	0,08
cis-p-Ment-2-en-1-ol	10,901	0,12
4-Metil Ciclohexeno	11,925	0,21
4-Carvomentenol	12,327	1,12
Sabinol	12,890	0,35
trans-Piperitol	13,095	0,11
1-(2-metoxi-fenil)-etanol	13,805	0,79
p-ment-1-en-3-ona	14,032	0,13
Linalool butirato	14,434	0,32
2-Undecanona	15,341	0,29
Etanona, 1-(2-tienil)	15,905	0,92
Metil cinnamato	17,302	0,38
Geranil acetato	17,587	0,20
3-Metil-5-pirazolidinona	17,777	0,34
Cariofileno	18,897	0,33
cis-β-Farneseno	19,796	1,76
α-Farneseno	21,011	0,98
β-Sesquifelandreno	21,442	0,43
Nerolidol	22,445	0,32
α-Bisabolol	23,279	0,15
cis-β-Santalol	23,710	0,15
α-Bisabolol	24,149	0,27
Tumerona	25,985	7,12
Curlona	26,739	3,07

RT: retention time

Table 4. Major secondary metabolites of Zingiber officinale

IDENTIFICATION	RT	% AREA
1R-α-Pineno	6,189	1,91
Canfeno	6,548	4,38
β-Pineno	7,491	1,50
α-Felandreno	7,784	0,33
γ-Terpineno	8,567	7,65
Linalool	10,248	1,23
Borneol	11,991	1,88
(+)-α-Terpineol (p-ment-1-en-8-ol)	12,642	2,39
Citral	15,261	15,40
2,6-Dimetil-2,6-octadien-8-il acetato	17,777	1,93
Curcumeno	20,433	4,21
Biciclo[3.1.1]hept-2-eno, 2,6-dimetil-6-(4-metil-3-pentenil)	20,959	5,99
1,3-Ciclohexadieno, 5-(1,5-dimetil-4-hexenil)-2-metil-, [S-(R*,S*)]-	21,091	4,94
α-Farneseno	21,384	5,95
β-Sesquifelandreno	21,823	4,53
Eremofileno (7CI)	22,598	1,41
Dehidrolinalool	24,376	1,36
β-Eudesmol	25,473	1,13
α-Cedreno	**26,622**	**1,91**

RT: retention time

Table 3 shows the chemical composition of C. longa oil, with the major components being 3-Thujene (25.57 %) and Terpinolene (19.09 %). Table 4 shows the chemical composition of Z. officinale oil, the major component of which is citral (15.40%).

In the chemical composition of turmeric essential oil, the dominant presence of monoterpenes and sesquiterpenes was to be expected, with a notable predominance of the former (3 thujene, for example); in the case of ginger, the presence of terpenoids was dominant, where their concentrations were highly variable, with citral being the major component. It is known that these components, e.g. citral and thumerone, have been reported to have antimicrobial functions (10), however, it is deduced that being in very low concentrations they were not determinant for optimal inhibitory activity, so very high concentrations of both oils had to be used to obtain considerable antimicrobial effects.

These variations may be associated with environmental conditions, species, phenological state of the plant, part of the plant used to extract the essence, harvesting time, extraction method, oil extraction time, among others (19). In addition, antibacterial activity is often

correlated with the chemical composition of essential oils, and numerous studies have suggested that the greatest contribution to the antimicrobial effect is made by their oxygenated components (20), but these components were found in very low concentrations in both species in the present study, which suggests further evaluation of the aforementioned variations in subsequent studies.

ACKNOWLEDGEMENTS

The authors would like to thank the Microbiological Research Laboratory, part of the Agricultural Bioprospecting Research Group, Faculty of Agricultural Sciences, University of Sucre, Colombia.

REFERENCES

1. Daferera DJ, Ziogas BN, Polissiou MG. (2003). The effectiveness of plant essential oils in the growth of Botrytis cinerea, Fusarium sp. and Clavibacter michiganensis subsp. michiganensis. Crop Protection. 22: 39–44.
2. Fornari T, Vicente G, Vazquez E, Garcia-Risco MR, Reglero G. (2012). Isolation of essential oil from different plants and herbs by supercritical fluid extraction. J Chromatogr A, 1250, 34-48.
3. Burt SA. (2004). Essential oils: their antibacterial properties and potential applications in foods – a review. International Journal of Food Microbiology. 94: 223-253.
4. Martínez A. (2003). Aceites esenciales, facultad de química farmacéutica, {en línea} {12 de diciembre de 2009) disponible en (http://farmacia.udea.edu.co/~ff/esencias2001b.pdf)
5. Cousins M, Adelberg J, Chen F, Rieck J. (2007). Antioxidant capacity of fresh and dried rhizomes from four clones of turmeric (Curcuma longa L.) grown in vitro. [Versión electrónica]. Industrial Crops & Products. 25: 129-135
6. Medina A, García R, Ramos, L. Efecto antihepatotóxico de la Cúrcuma longa. Extraído el 23 de febrero del 2012 de www.ilustrados.com/publicaciones/EEF.
7. López-Lazaro M. (2008). Anticancer and carcinogenic properties of curcumin: Considerations for its clinical development as a cancer chemopreventive and chemotherapeutic agent Molecular Nutrition and. Food Research. 52: S103 – S127.
8. Anand P, Thomas SG, Jaikumar A, Kunnumakkara B, Sundaram C, Kuzhuvelil B. Harikumar , Sung B , et al. (2008). Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. Biochemical Pharmacology 76: 1590–1611.
9. Mesa M, Ramírez-Tortosa M C, Aguilera CM, Ramírez-Boscá A, Gil A (2000). Efectos farmacológicos y nutricionales de los extractos de Curcuma longa L. y de los cucuminoides. Ars Pharmaceutica. 41: 307-321.

10. Asghari G, Mostajeran A, Shebli M (2009). Curcuminoid and essential oil components of turmeric at different stages of growth cultivated in Iran. Research in Pharmaceutical Sciences. 4: 55-61.
11. Kalemba D, Kunicka (2003). Antibacterial and antifungal properties of essential oils. Current Medicinal Chemistry 10: 813-29.
12. O'Hara, Mary; Kiefer, David Farrell, Kim Kemper, Kathi (1998). "A Review of Commonly Used Medicinal Herbs". Archives of Family Medicine 7 (6): 523–536. doi:10.1001/archfami.7.6.523. PMID 9821826.
13. Sigee DC. (2005). Bacterial plant pathology: Freshwater microbiology: biodiversity and dynamic interactions. Cambridge University Press. Editorial Cambridge University Press. Vol. 1. ISBN: 978-0-521-61967-7. pp. 1, 2, 4, 9-11.
14. FAO. (2010). Estadísticas mundiales sobre cultivos. Disponible en: http://www. Faostat. Org.
15. Laii XH, Mcclung AM, Marchettii MA. (1999.) The effects of panicle blight of rice on grain weight and viability. Agricultural. Research Service,Tektran,Texas. USDA: Abstract.
16. Shankar NB, Shashikala J, Krishna murthy YL. (2009). Study on the diversity of endophytic communities from rice (Oryza sativa L.) and their antagonistic activities in vitro. Microbiological Research; 164: 290-296.
17. Prabuseenivasan S, Jayakumar M, Ignacimuthu S. (2006). In vitro antibacterial activity. of some plant essential oils. BMC Complementary and Alternative Medicine. Vol. 6. pp.39.
18. Sánchez Y, Pino O, Correa TM, Naranjo E, Iglesia A. (2009). Estudio químico y microbiológico del aceite esencial de Piper auritum Kunth (Caisimón de Anís). Rev Protección Veg; 24(1):39-46. Consultado: 10-10-2012).
19. Imelouane B, Amhamdi H, Wathelet JP, Ankit M, Khedid K, El Bachiri A. (2009). Chemical composition and antimicrobial activity of essential oil of thyme (Thymus vulgaris L.) from eastern Morocco. J Agric Biol;11:205-208.
20. Maguna FP, Romero AM, Garro OA, Okulik NB. (2006). Actividad Antimicrobiana de un grupo de Terpenoides. Facultad de Agroindustrias, UNNE, Argentina.