Research Article

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Free Radical Scavenging Activity of Essential Oil of *Eugenia caryophyllata* from Amboina Island and Derivatives of Eugenol

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Abstract: Essential oil from *Eugenia caryophyllata* was normally used to heal many different diseases. Various chemical compositions of essential oil distilled and steamed of Moluccas *Eugenia caryophyllata* has been investigated by many different researchers. Even though an intensive research has been carried out of the local chemotypes, a very detail study has not been fully investigated to find out the complete chemical compounds from the plant essential oil and its content associated with their biological activities. In present paper, we assess the free radical scavenging of *E. caryophyllata* collected from Moluccas islands, Indonesia. Essential oil was extracted from leaves, buds, and stems of plant by steam distillation and analyzed using GC-FID and GC-MS. The result showed that free radical activity of essential oil, main constituent and its derivatives were analyzed using *in vitro* method. Essential oil activity from stem obtained as $(0.82 \pm 0.15 \mu\text{g/mL})$ was higher than that from bud and leaf possessing both 1,1-diphenyl-2-picrylhydrazyl (DPPH) and (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging assays by synergism of eugenol, eugenyl acetate, β -caryophyllene and humulene. The activity of isoeugenol (**2**) ($3.59 \pm 0.54 \mu\text{M}$) and ($5.0 \pm 0.53 \mu\text{M}$) scavenging DPPH and ABTS, respectively, as derivatives eugenol was higher than (**3**), (**4**) and (**5**). Although (**6**) was active originally, it was inactive after conversion of the ester. While the change of the double bond of location to conjugation structure caused more activity scavenging radicals than the starting molecule.

Keywords: Amboina island; free radical scavenging; isomethyl eugenol; methyl eugenol hydroxy.

1 Introduction

Natural product compounds, including essential oils (EOs) distilled from *Eugenia caryophyllata* have various chemical contents depending on local distribution of the plants. There are three parts of clove tree in famous Moluccas islands of Indonesia such as buds, leaves, and stems can produce clove oil by using steam distillation. Indonesia, Tanzania, Sri Lanka, Madagascar are world largest clove-producing countries on earth. While, India had a limited scale of clove product [1]. The primary contents of clove oil are eugenol, β -caryophyllene, α -humulene, eugenyl acetate, and humulene epoxide [2-3]. Even though there were many compounds found in clove oil closely related to geographical location, and the plant tissue, eugenol remains the primary constituent of clove oil [4-5]. In 2018 [6], Da Silva et al., found that by modifying the molecular structure of an active substance it can be used as an anti tumor and anti infective drug.

Eugenol, a major compound inside clove oil, is generally isolated by using a distillation process with a reduced pressure. Moreover, the eugenol hydroxyl and allyl groups can be changed to other functional groups. On the other hand, eugenol as one of phenolic compounds can easily be converted into methyl eugenol by the use of a methylation reaction of eugenol with DMS [7-8]. While another method to produce methyl eugenol is by the process of eugenol with dimethyl carbonate (DMC) with the help of Phase transfer catalyst (PTC) [9-14]. The reaction of isoeugenol with acetic anhydride forms isoeugenol acetate [15-16]. Isomerization of alkenyl-alkoxybenzenes, especially, linked to a migration process of a double bond in the alkenyl group of alkenyl-alkoxybenzenes from a not conjugated position with the benzene ring to a conjugated position with the benzene ring by the use of

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Scheme 1: Derivatives of Eugenol.

a base catalyst. Eugenol and methyleugenol, which have an allyl group can be converted into an isomer by using KOH-alcoholic, or without the use of an alcoholic solvent in order to yield isoeugenol and isomethyl eugenol, respectively [17-19]. While the synthesis of secondary alcohols that changes the double bond to be a hydroxyl group shown in scheme 1 can be conducted using few types of techniques of oxymercuration-demercuration of olefin by adding mercury acetate and NaBH_4 based on *in situ* according to ref. [20-21]. The other method to produce a secondary alcohol was by changing the allyl group of methyl eugenol through additional formic acid, and then followed by hydrolysis treatment [22].

Free radicals existence produced in plants are very important to initiate chain reactions that heal damage cells, and cause a death cell alive. In general, many deathly diseases such as cardiovascular and neurodegenerative diseases were because of damage cells. The free radicals in plants can fight against the such effects due to antioxidant substances [23]. Eugenol as one of antioxidant compounds is powerful to fight oxidative stress for a better health. Therefore, the broad spectra of biological activities of eugenol in this research work was to find out its derivatives by chemical processes of isomerization, substitution and addition reactions, respectively. We present this novel work that connects between the specific compounds found in *E. caryophyllata* from Amboina island, and its EOs.

2 Experimental

2.1 Equipment

The equipment used in this research consisted of three groups of apparatuses. The first group was from fractional distillation under reduced pressure, electric

heaters, evaporators and tools of Buchii laboratory glassware. The second ones were spectroscopy instruments of Spectrophotometer PD-303S, Apel, Japan, Infra Red spectrophotometer (FTIR-8400S, Shimadzu), and ^1H -NMR Spectrophotometer (JEOL-MY 400). Finally, Gas Chromatography 2010 Shimadzu (GC), and Mass Spectrophotometer QP 2010 Plus (MS), Shimadzu (GC-MS) were employed to identify the compounds.

2.2 Materials

There were bud, leaf, stem as parts of *E. caryophyllata* taken from local garden in Amboina Island as raw materials.

2.3 The Essential Oil Isolation and Synthesis Derivatives of Eugenol

The volatile oils of clove were obtained by conventional distillation [24]. The dried materials (1.00 kg) of bud, leaf (1.00 kg) and stem (0.60 kg) were treated by steam distillation for 6 hours. The composition of essential oil was analysed using GC. The isolation of eugenol (1) from stem clove oil was done by using a sodium hydroxide (NaOH) solution to obtain sodium eugenolate, followed by acidification with 20% hydrochloric acid (HCl), and then distilled under pressure reduction at $1200^\circ\text{C}/10$ mmHg yielding eugenol (1) as large as 74.09% [19,24]. The spectral data corresponded with the earlier reports [17,18]. The isomerization of eugenol (1) with KOH in a solvent ethylene glycol monomethyl ether was refluxed at 140°C for 4 hours, followed by distillation under the pressure of 10 mmHg at 120 - 122°C yielding isoeugenol (2) with the content of 69.72% [18]. The methylation of eugenol (1) was carried out using DMS at the 103°C for 90 minutes, followed by distilling under pressure at $140^\circ\text{C}/20$ mmHg which yielded methyl eugenol with the amount of 62.08% [8,22].

The spectral data was fitted with that given in previous reports [8,16,17,22]. The isomerization of methyleugenol (3) with KOH, and without any solvent at the 140°C for 4 hours yields isomethyleugenol (4) (66.68%). The compound was then identified using GC yielding *cis*-isomethyleugenol (9.80%) and *trans*-isomethyleugenol (90.20%) [16-17]. The oxymercuration-demercuration of methyl eugenol (3) was carried out with $\text{Hg}(\text{OAc})_2$ - NaBH_4 , followed by distillation process with reduced pressure reduction at 146°C/10 mmHg to obtain methyleugenyl alcohol (5) (78.03%) [20-21]. The spectral data was comparable with that in ref. [8,22]. The isoeugenyl acetate was synthesized from isoeugenol (2) and acetate anhydrous using sodium acetate as a catalyst. The mixture was refluxed at 120°C for 90 min, followed by recrystallization in methanol to obtain isoeugenyl acetate (6) with yield as high as 63.08% at b.p 65°C [15].

2.4 DPPH and ABTS Free Radical Scavenging Assays

2.4.1 DPPH free radical scavenging assay

The DPPH activity was investigated with the method described in Ref. [25]. The test sample (10 mg) was dissolved in 1 mL methanol. The reaction mixture consisted of 1 mL of DPPH 6×10^{-5} M and 33 μL sample solution in methanol. After 20 min incubation at 37°C, the absorbance values of the sample caused by the reaction in the prepared mixture were measured at a wavelength of 515 nm using a spectrophotometer PD-303S, Apel, Japan. The blank samples comprised of 33 mL of methanol in a DPPH solution was also measured at the same wavelength. The experiment was performed in triplicate. While the percentage of inhibition was calculated using equation:

$$\text{Percentage of inhibition} = -\text{Abs}_{\text{test}} / \text{Abs}_{\text{control}} \times 100 \quad (1)$$

In Eq. (1), the $\text{Abs}_{\text{control}}$ was absorbance of blank, while the Abs_{test} were absorbance of all the samples. A lower absorbance in the sample was indicated as a higher DPPH scavenging activity. The concentration dependence of IC_{50} value was determined from EOs and its derivatives prepared with the DPPH radical to 50% of the control. Ascorbic acid was used as a standard.

2.4.2 ABTS free radical scavenging assay

The ABTS radical scavenging assay was carried out according to the method described by Fitriana et al.

[26]. By oxidating ABTS with potassium persulfate, the ABTS radical was obtained. In order to form ABTS cation solution, 5 mL ABTS (7 mm) was mixed with 100 μL of potassium persulphate, and then incubated for 12 hours. Furthermore, the working solution was prepared with a certain amount of the former solution following by adding the phosphate buffer saline solution until the absorbance as large as (0.70 ± 0.001) at 734 nm. The freshly prepared working solution was then kept in the dark for 30 minutes. A volume of 1 mL of different concentrations of plant extract was mixed thoroughly with 1 mL of working solution, and kept in the dark for 10 minutes. The absorbance of the test sample was measured at the same wave length of 734 nm. of the control sample. The percentages of inhibition were calculated using Eq. (1) in which the $\text{Abs}_{\text{control}}$ was absorbance of blank, while the Abs_{test} were absorbance of all the samples. A lower absorbance in the sample was indicated as a higher ABTS scavenging activity. The concentration dependence of IC_{50} value was determined from EOs and its derivatives prepared with the ABTS radical to 50% of the control. Ascorbic acid was used as a standard.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and Discussion

3.1 Chemistry

The starting material eugenol (1) was isolated from clove stem oil. The isomerization of (1) with KOH in ethylene glycol monomethyl ether produced (2). Methylation of eugenol (1) with dimethyl sulphate (DMS) produces methyleugenol (3) and isomerization of (3) with KOH without solvent produced isomethyleugenol (4). Oxymercuration-demercuration of methyleugenol with $\text{Hg}(\text{OAc})_2$ - NaBH_4 , produced methyleugenyl alcohol (5) and esterification of (2) with anhydrous acetate produces isoeugenyl acetate (6) as shown in Scheme 1.

3.2 Free Radical Scavenging Activities

To evaluate the antioxidant properties of essential oil, eugenol and derivatives, two methods of DPPH and ABTS were employed. The positive control used in this research was ascorbic acid. The inhibition concentration, IC_{50} was measured for the antioxidant activity and the compounds, respectively. The results of scavenging activities are

Figure 1: Scavenging effect of different essential oils and ascorbic acid (A) DPPH, (B) ABTS assays at concentration 319.45 µg/mL and 99.00 µg/mL. Mean values are significantly average $SD \pm n=3$.

Table 1: IC_{50} value free radical scavenging of essential oil.

	DPPH (µg/mL)	ABTS (µg/mL)
Essential oil from stem (EOS)	0.82 ± 0.15	0.81 ± 0.16
Essential oil from bud (EOB)	1.18 ± 0.56	0.54 ± 0.77
Essential oil from leaf (EOL)	1.16 ± 0.74	0.66 ± 0.67
Ascorbic acid (positive control)	1.38 ± 0.65	0.74 ± 0.54

listed in Table 1. The sample amount needed in order to decrease the absorption of DPPH and ABTS by 50% was then calculated graphically by plotting % of inhibition against the concentration in µg/mL.

The free radical scavenging activity of the essential oil on the DPPH scavenging was observed at 319.45 µg/mL to 9.98 µg/mL concentration. All of the essential oils, regardless of distillation steam method, showed DPPH scavenging activity that increased in a concentration dependent manner. As shown in Figure 1A, all the essential oils, including the reference ascorbic acid (AA), exhibit intense DPPH radical scavenging activity at low concentration 9.98 µg/mL. The percentage of inhibition of DPPH from stem essential oil was high ($99.00 \pm 1.05\%$), followed by bud ($98.80 \pm 0.96\%$), and leaf ($98.10 \pm 0.76\%$). The value of IC_{50} value showing high activity of DPPH was (0.82 ± 0.15), (1.18 ± 0.56) and (1.16 ± 0.65), for stem, bud, leaf, respectively.

As shown in Figure 1B, the free radical scavenging activity of the essential oil on the ABTS scavenging were observed at concentration of 99.00 µg/mL to 0.77 µg/

mL. The percentage of inhibition of ABTS from bud essential oil was high ($93.53 \pm 0.68\%$), followed by leaf ($92.33 \pm 0.34\%$) and stem ($91.94 \pm 0.68\%$). The value of IC_{50} showing high activity of ABTS was (0.54 ± 0.77), (0.66 ± 0.67) and (0.81 ± 0.16), for bud, leaf, stem, respectively.

The higher antioxidant activity of oil of *E. caryophyllata* EOS could be related to the synergistic property associated with the high content of eugenol (97.75%) with other constituents viz., β -caryophyllene (0.90%), eugenyl acetate (0.76%) and caryophyllene oxide (0.59%) reported previously (Sohilait et al, 2018). The presence of eugenyl acetate (20.93%) synergic with eugenol (75.30%), β -caryophyllene (3.0%), humulene (0.37%) and caryophyllene oxide (0.40%) in EOB makes its ABTS activity higher than EOL's. On the other hand, the absence of eugenyl acetate in EOB could cause higher antioxidant activity of scavenging ABTS than EOL's. Humulene in EOS had no indicator of the presence of antioxidant activity, which may be due to the absence of participating electron delocalization group or protons. The presence of β -caryophyllene, caryophyllene oxide and humulene from the EOs could show no effect of antioxidant activity [27-28]. The essential oils of eugenol were compared to a known synthetic antioxidant ascorbic acid (Table 1). The oil of *E. caryophyllata* showed better antioxidant property than pure eugenol. The important facts found are the nonspecific responses and synergistic effects of the compounds from essential oil. For this reason, a bioassay-direct fractionation of an active extract was not always contribute to the isolation of active compounds. An

Figure 2: Inhibition of eugenol and its derivatives at DPPH and ABTS. Values were performed in triplicate and represented as mean $\pm$ SD. Abbreviation: AA, ascorbic acid.

Table 2: Radical scavenging activity of eugenol and its derivatives.

Compounds	DPPH (μ M)	ABTS (μ M)
Eugenol (1)	6.34 $\pm$ 0.79	6.76 $\pm$ 3.13
Isoeugenol (2)	3.59 $\pm$ 0.54	5.0 $\pm$ 0.53
Methyl eugenol (3)	7.80 $\pm$ 1.34	6.79 $\pm$ 1.05
Isomethyl eugenol (4)	8.53 $\pm$ 1.28	9.88 $\pm$ 0.86
Methyl eugenyl hydroxy (5)	>100	>100
Isoeugenyl acetate (6)	-	-
Ascorbic acid (positive control)	4.71 $\pm$ 1.05	4.65 $\pm$ 1.64

apparent loss of activity on separation of synergistically acting components of low part potency cannot be easily distinguished from the loss of activity resulting from the chemical changes induced by particular isolation technique. In this work, we summarized that stem, bud and leaf of *E.caryophyllata* from Amboina Island can be used as a source for use as a food additive, preservative, and as an antiseptic.

The free radical scavenging activity of the eugenol was then measured using a DPPH assay reported in the literature [6]. When the scavenging activity of each compound was quantified by the colorization of DPPH at 515 nm after 20 min-incubation at 37°C, compound isoeugenol exhibited radical scavenging activity, even though its activity was higher than that of derivatives compounds (Table 2). Secondary alcohols generally have little scavenging effects. The result showed that isoeugenyl acetate 6 has no activity, indicating that the radical

scavenging effect was caused by the hydroxyl attack of the benzene ring of compound (1).

The antioxidant activity of eugenol derivatives was investigated with DPPH and ABTS. One of the best methods for screening the antioxidant activity of a substance was radical scavenging activity. The capture capability of free radicals induced by eugenol derivatives (3-5) against DPPH and ABTS were analyzed in parameter IC_{50} , which represents the concentration required to capture 50% of the radicals in the medium. Phenolic compounds, such as eugenol, have the ability of transferring electron or hydrogen atoms by neutralizing free radicals which can block the oxidative process [29-30]. The results depicted in Table 2 showed that all the derivatives (3-6) presented higher IC_{50} than eugenol. Such cause was due to the structural modification resulted in substances with lower DPPH scavenging effects. Compound (2) was produced by isomerization of compound (1).

The result of DPPH and ABS assays for compound (1) showed higher antioxidant properties compared to that of compounds (3-6). The highest antioxidant properties in inhibition ABTS of compound (2) is possibly due to the resonance with the aromatic ring to produce the stabilization of phenoxyl radicals. On other hand, chemical modification of the hydroxyl group resulted in substances with lower antioxidant effects. Compound (6) was fabricated by an esterification reaction on the hydroxyl group, and resulted in a strong reduction in antioxidant activity to be an inactive compound [23,31-32]. The secondary alcohol compound was produced by the oxymercuration-demercuration reaction of methyl

eugenol, and is also low in antioxidant activity. In a particular manner of eugenol, the relationship between the hydroxyl group and the antioxidant action was observed in compound (2), in which this presented IC_{50} value of ABTS inhibition is lower than eugenol.

4 Conclusions

The results of both free radical scavenging assays demonstrated that structural modifications in the eugenol molecule produced a reduction in antioxidant activity. Compounds (1) and (2) have the greatest activity as free radical scavengers because of the existence of hydroxyl group in the aromatic ring related to the essential of antioxidant activity.

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Conflict of interest: Authors declare that there is no conflict of interest among.

References

- [1] Thankumari C.K., Shivaraman K., Kandianum K et al., Bud and leaf essential oil composition of *Syzygium aromaticum* from India and Madagascar J. Spices Arom Crops, 1994, 3, 105-123.
- [2] Jirovetz L., Buchbauer G., Stoilova I et al., Chemical composition and antioxidant properties of clove leaf essential oil, J. Agric. Food chem., 2006, 54, 6303- 6307.
- [3] Gupta M., Singh D., Gularia P et al., GCMS analysis and identifications of chemical constituents of *Syzygium aromaticum*, *Brassica campestris* and cow ghee, J. Chem. Pharm. Res., 2015, 7(1), 568-572.
- [4] Srivastava A.K., Srivastava S.K., Syamsudar K.V., Bud and leaf essential oil composition of *Syzygium aromaticum* from India and Madagascar, Flavour Frag. J., 2005, 20, 51-53.
- [5] Kumar A., Shukla R., Singh P et al., Chemical composition, antifungal and antiaflatoxic activities of *Ocimum sanctum* L. essential oil and its safety assessment as plant based antimicrobial, Food and Chemical Toxicology, 2010, 48, 539-543.
- [6] Da Silva F.F.M., Monte F.J.Q., de Lemos T.L.G et al., Eugenol derivatives: synthesis, characterization, and evaluation of antibacterial and antiooxidant activities, Chemistry Central Journal, 2018, 12, 34-42.
- [7] Guenther E., *The Essential Oils* Vol. II IV B, Universitas Indonesia Publishing, Jakarta, 1990.
- [8] Sohilaith H.J., Sastrohamidjojo H., Sabirin M., et al., Synthesis of analog L- α -methyl DOPA from eugenol, Indo. J. Chem., 2005, 5(3), 198-202.
- [9] Lamoureux G., Agüero Ch., A comparison of several modern alkylating agents, Arkivoc, 2009, 1, 251-264
- [10] Tundo P., New developments in dimethyl carbonate chemistry, Pure Appl. Chem., 2001, 73, 7, 1117-24.
- [11] Ono Y., Dimethyl carbonate for environmentally benign reactions, Pure and Appl. Chem., 1996, 68(2), 367-375.
- [12] Ouk S., Thiebaud S., Borredon E., et al., *O*-Methylation of phenolic compounds with dimethyl carbonate under solid/ liquid phase transfer system, Tetrahedron Letters, 2002, 43, 2661-2663
- [13] Wen-Chung S.W.Ch., Dell St., Repic O., 1,8-Diazabicyclo[5.4.0] undec-7-ene (DBU) and Microwave-Accelerated Green Chemistry in Methylation of Phenols, Indoles, and Benzimidazoles with Dimethyl Carbonate, Organic Letter, 2001, 3(26), 4279-4281
- [14] Tundo P. and Selva M., The Chemistry of Dimethyl Carbonate Carbonate, Acc. Chem. Res. 2002, 35, 706-716.
- [15] Lampman G.M., Sharpe S.D., A Phase Transfer Catalyzed Permanganate Oxidation. J. Chem. Educ., 1983, 60, 503-504.
- [16] Xuan Th, Luu Th., Lam T. et al., Fast and Green Microwave-Assisted Conversion of .Essential Oil Allylbenzenes into the Corresponding Aldehydes via Alkene Isomerization and Subsequent Potassium Permanganate Promoted Oxidative Alkene Group Cleavage, Molecules, 2009, 14, 3411-3424
- [17] Nagase Ts., Suzukamo G, Fukao M, Isomerization of alkenyl-alkoxybenzenes, US patent 3,852,305, Dec, 3, 1974.
- [18] Baby Ch., Microwave Isomerization of Safrole and Eugenol., Syn. Comm., 1977, 27(24), 4335-4340.
- [19] Sohilaith M.R, Sohilaith H.J, Francina E, Synthesis of 3,4-methyleedioxy isoamyl cinnamic as the sunscreen compound from culilawan oil, Ind. J. Chem. Res., 2013, 1, 1-5.
- [20] Brown H.C., Geoghegan J.P.J., The Oxymercuration-Demercuration of Representative Olefins in an Aqueous System. A Convenient Mild Procedure for Markonikov Hydration of the Carbon-Carbon Double Bond, J.Org.Chem, 1970, 6, 1844-1850.
- [21] Brown, H.C., Lynch, G.J., Oxymercuration-Demercuration of Methoxy, Hydroxy and Acetoxy-Substituted Alkenes, J. Org. Chem., 1981, 6(3), 531-538.
- [22] Sohilaith, H. J., Sastrohamidjojo, H., Sabirin, M et al., Synthesis of Secondary Alcohol Compounds from Methyleugenol, Indo. J. Chem., 2003, 3(3), 176 -178
- [23] Wang J., Xia F., Wen-Bin J et al., Efficient synthesis and antioxidant activities of N-heterocyclyl substituted Coenzyme Q analogues, Bio-Organic Chem., 2016, 68, 214-218.
- [24] Sohilaith H.J, Kainama H., GC/GC-MS Analysis, Isolation and Identification of Bark Essential Oil Components from *Cinnamomum culilawan*, Blume, Am. J. App. Chem., 2016; 4(4), 157- 160
- [25] Singh R.S.G., Negi P.S., Radha C., J. of functional Foods., Phenolic composition, antioxidant and antimicrobial activities of free and bound phenolic extracts of *Moringa oleifera* seed flour, J. of functional Foods, 2013, 5, 1883-1891.
- [26] Fitriana W., Ersam, T., Koniyoishi S et al., Antioxidant Activity of *Moringa oleifera* extracts, Indones. J. Chem., 2016, 16, 297-301.

- [27] Hammami S., Mokni R.E., Faïdi K et al., Chemical composition and antioxidant activity of essential oil from aerial parts of *Teucrium flavum* L. subsp. *flavum* growing spontaneously in Tunisia, *Nat. Prod. Res.*, 2015, 29(24), 2336-2340.
- [28] Cote H., Boucher M., Pichette A et al., Anti-Inflammatory, Antioxidant, Antibiotic, and Cytotoxic Activities of *Tanacetum vulgare* L. Essential Oil and Its Constituents, *Medicines*, 2017, 4(2), 34-43.
- [29] Hindalgo M.E., Rosa C., Antioxidant capacity of eugenol derivatives, *Quim Nova*, 2009, 32(6), 1467- 1470.
- [30] Findik E., Ceylan M., Elmastas M., Isoeugenol-based novel potent antioxidants: Chemistry Synthesis and reactivity, *Eur. J. of Med.*, 2011, 46, 4618-4624.
- [31] Baroso MR., Ramalhosa M.J., Alves R.C et al., Total antioxidant capacity of plant infusions: Assessment using electrochemical DNA-based biosensor and spectrophotometric methods, *Food control.*, 2016, 68, 153-161.
- [32] Lasha-Benamrouchea S., Madania K., Phenolic contents and antioxidant activity of orange varieties (*Citrus sinensis* L. and *Citrus aurantium* L.) cultivated in Algeria: Peels and leaves, *Industrial Corps and prod.*, 2013, 50, 723- 730.