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Original article

The effect of ^{60}Co-gamma radio-sterilization on *Boswellia carterii* essential oil composition

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Abstract

Background and objectives: Gamma-irradiation is used vastly for both decontamination and sterilization of natural products; but these high-energy rays can affect heat-sensitive essential oils. **Methods:** The oleo-gum-resin of *Boswellia carterii* (Burseraceae) was sterilized by γ-irradiation at dose 30 KGy. The essential oils obtained by hydrodistillation of non-irradiated *Boswellia* (NIB) and irradiated *Boswellia* (IB) were analyzed by GC/MS and the changes were compared. The structure of octyl acetate as the major component and marker of *B. carterii* was confirmed by MS/MS. **Results:** Twenty-five compounds comprising 99.55% of NIB oil and nineteen compounds comprising 98.61% of IB oil were identified. Major constituents which were common for both oils were octyl acetate (52.67 % in NIB, 76.51 % in IB), 1-octanol (6.37 % in NIB, 5.19 % in IB), duva-4, 8, 13-triene-1,3α diol (5.52 % in NIB, 3.94 % in IB), verticiol (13.63 % in NIB) and verticillane type diterpene (5.4 % in IB) they made up 78.19% and 91.04% of NIB and IB, respectively. **Conclusion:** Gamma irradiation was an efficient method for sterilization of *Boswellia carterii* oleo-gum resin, but it resulted in change in the essential oil composition particularly octyl acetate changed from 52.67% to 76.51%.

Keywords: *Boswellia carterii*, Burseraceae, essential oil, gamma sterilization, octyl acetate

Introduction

The genus *Boswellia* (Burseraceae), with 20 species is indigenous to dry regions of the world, from Ivory Coast to the Horn of Africa, Middle East and India [1]. Frankincense or olibanum, the oleo-gum-resin of *Boswellia*, is released after wounding the bark of the trees during dry seasons and occurs in more or less ovoid tears with dusty surface. Frankincense contains 3-9% volatile oil consisting of various monoterpenes (e.g. *α*-pinene and *α*-thujene) and sesquiterpenes, about

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60-70% resin and 27-35% gum [2,3]. Besides many non-pharmaceutical applications of *Boswellia* oleo-gum-resin in perfumes, toiletries and food supplements, its immune-modulatory, anti-inflammatory and cytotoxic properties have made it a highly-used herbal medicine [4,5]. Essential oils from *Boswellia* spp. have shown antioxidant, antimicrobial and anticancer activities alongside acetylcholinesterase inhibitory action and consequently an increasing worldwide attention is paid by pharmaceutical companies and researchers to this raw material [4,6].

Boswellia tears are highly prone to get contaminated with microorganisms during air-drying, collection, transportation, and storage. Therefore, using raw material without any suitable treatment could adversely affect consumers' health condition [7]. Enforcement of markets and industries to eliminate contamination from raw herbal materials seems compulsory to achieve satisfactory microbiological quality and public health safety [8]. Gamma sterilization is a feasible way to remove microbial contamination of dry medicinal herbs [9]. The major target of electromagnetic radiations of gamma is microbial DNA, being damaged as a consequence of ionization. Although γ-sterilization is less toxic and more environmental-friendly than other decontamination methods for dry medicinal herbs, its high doses may cause dramatic changes of flavors and heat-sensitive constituents like essential oils. So, GC/MS, as a leading technique, is a suitable analytical method for evaluation of γ-irradiation effects [10]. In this work, the changes of *B. carterii* volatile compounds after γ-sterilization at high dose (30 kGy) have been monitored by GC/MS. Major components of *B. carterii* essential oil have been identified by GC/MS/MS and the reliability of γ-sterilization method for a highly-contaminated sample of *B. carterii* oleo-gum-resin was evaluated.

Experimental

Plant material

The oleo-gum-resin of *Boswellia carterii* Birdw. (Burseraceae) was purchased from local market of Shiraz, Iran in January 2011. Samples were authenticated and deposited at the Herbarium of the Traditional Pharmacy Department, under code number of PM400-5.

^{60}Co-gamma radio-sterilization

The oleo-gum-resin of *B. carterii* was cooled in a freezer (-20˚c) for 1-2 h and the pure resin material was powdered in an electrical grinder and packaged in double wrapped plastic bags for gamma sterilization according to the basis mentioned by Yordanov *et.al* [9]. Radiation dosimetery was conducted based on the initial microbial count. The powder was sterilized by gamma irradiation (irradiator: ^{60}Co, dose rate: 30 kGy). After irradiation, the samples were kept at room temperature in the darkness for 72 h to avoid any interference by radiation-induced paramagnetic effects.

Plate- count method for microbial enumeration

This method was performed in duplicate for each method and the mean count of the result was expressed [11]. *B. carterii* oleo-gum-resin was powdered and one g was aseptically transferred to sterile blender jars. Subsequently, each sample was blended in 9 mL of 0.1% peptone (saline) for 45 s. Because of possessing significant antimicrobial activity, serial dilutions were prepared so that the number of colony forming units (CFUs) in petri dishes would be less than 300 in the case of bacteria and less than 100 in the case of fungi. Duplicate one mL aliquots of each dilution sample were added to two separate sterile petri dishes 9 cm in diameter; 20 mL of liquid soybean-casein digest agar medium suitable for the cultivation of bacteria or sabouraud dextrose agar for the cultivation of fungi were added. After solidification of the soft agar, the petri plates were incubated at 35 °C for bacteria and at 25 °C for fungi for three and five days, respectively. The number of microorganisms in each sample was evaluated by multiplying the average number of colonies per plate by the dilution used. Colonies were counted

and the counts were expressed as colony forming units per gram (CFU/g).

Sterility test

The test for sterility was carried out under aseptic conditions according to USP [11]. Fluid thioglycollate medium was used for the culture of anaerobic bacteria. Soybean-casein digest medium was used for the culture of both fungi and aerobic bacteria and was incubated at 22.5±2.5 °C. For growth promotion and validation tests, each ready-prepared medium and each batch of medium prepared was tested. Portions of fluid thioglycollate medium were inoculated with a small number (not more than 100 CFU) of the following microorganisms using a separate portion of medium for each of the following species of microorganism: *Clostridium sporogenes* (for anaerobic bacteria), *Pseudomonas aeroginosa*, and *Staphylococcus aureus* (for aerobic bacteria). Portions of soybean-casein digest medium were inoculated with a small number (not more than 100 CFU) of the following microorganisms, using a separate portion of medium for each of the following species of microorganism: *Aspergillus niger*, *Bacillus subtilis*, and *Candida albicans*. They were incubated for at most 3 days in the case of bacteria 5 days in the case of fungi. The media were suitable if a clearly visible growth of the microorganisms occurred. The sterility of each sterilized batch of medium was confirmed by incubating a portion of the media at the specified incubation temperature for 14 days. After transferring the contents of the containers to the membrane, an inoculum of a small number of viable microorganisms (not more than 100 CFU) was added to the final portion of sterile diluent used to rinse the filter. Membrane filters (cellulose nitrate) having a nominal pore size not greater than 0.22 mm was used. Sample preparation for filter sterilization (*Boswellia* oleo-gum-resin) was diluted with sterilized isopropyl myristate (1:10).

Essential oils extraction

About 500 g dried oleo-gum-resin tears was powdered by grinder and submitted to hydro-distillation for 4 h using a Clevenger type apparatus. The obtained essential oil was dried with anhydrous sodium sulfate and stored at -20 °C before analysis.

GC/MS and GC/MS/MS analysis

GC/MS analysis was performed on a Agilent Technologies (USA) GC system: 7890A fitted with a DB-1 fused silica capillary column (30m×0.25 mm ID, film thickness 0.25µm) coupled with a 7000 triple Quadrupole mass detector under the following conditions: injection volume 1 µL (sample diluted 1/10 with *n*-hexane) splitless (n=3). Helium as the carrier gas at 1.2 mL/min constant flow mode, injector temperature 250 ºC, oven temperature 70 ºC for 0 min to 280 ºC for 4 min at 3 ºC/min (Total run time 74 min). Mass spectra: electron impact (EI+) mode, 70 ev and ion source temperature 250 ºC. Mass spectra were recorded over 50-500 amu range.

GC/MS/MS analysis for octyl acetate peak was performed on the same column and following conditions: collision gas N_2 with energy of 22, auxiliary temperature 280 °C, flow program 1.2 mL/min., mode splitless, heater-250°C, mode: product ion, precursor ion 121 MW, MS1 resolution: wide, MS2 from 40 to 240 MW, time 14 min. to 20 min., scan time 500 ms. Identification was based on comparison with NIST 08 and Wiley 275 GC/MS libraries, and comparison of mass spectra and/or retention indices (RI) with those published in literature [12-14]. Retention indices of the components were determined relative to the retention times of a series of *n*-alkanes (C_9-C_{30}).

Results and Discussion

In the present study, powdered *B. carterii* oleo-gum-resin was sterilized by ^{60}Co γ-irradiation and the components of both non-irradiated *Boswellia* (NIB) and irradiated *Boswellia* (IB) volatile oils were identified by GC/MS. Samples of powdered *B. carterii* oleo-gum-resin were examined for total microbial count before and after ^{60}Co γ-radiation.

Total colony forming units of pre-treated powder

were 78 CFU/g for bacteria and 11 CFU/g for fungi, while testing IB at dose 30 kGy showed no contamination. Therefore, this method evaluated as practical and reasonable one for microbial removal from highly-contaminated oleo-gum-resin samples.

The hydrodistillation of powdered *B. carterii* oleo-gum-resin before and after γ-ray treatment yielded 1.19 and 1.12 % (v/w) fragrant essential oils, respectively. In the present study, γ-irradiation was not found to affect the oil yield significantly. Similarly, Chatterjee *et al*. reported no changes in *Curcuma longa* volatile oil amount, in contrary, Seoa *et al*. reported 5-7% increase in *Angelica gigas* essential oil recovery yield after irradiation [15,16]. Twenty-five components in NIB and nineteen compounds in IB were identified (figure 1) amounted 99.55% and 98.61% of total constituents, respectively.

The results of GC/MS analysis have been summarized in table 1. Some chemical classes of essential oil such as oxygenated monoterpenes (2.62 to 0.28), monoterpene hydrocarbons (5.78 to 0.70 %), sesquiterpene hydrocarbons (0.26 to 0.09 %), oxygenated diterpenes (24.58 to 9.89 %), and diterpene hydrocarbons (5.95 to 2.73 %) decreased after γ-irradiation, while oxygenated hydrocarbons (59.94 to 84.10 %) increased. Qualitative composition of both oils proved that 18 compounds were similar. Major constituents which were similar in both oils: octyl acetate (52.67 % of NIB, 76.51 % of IB), 1-octanol (6.37 % of NIB, 5.19 % of IB), duva-4, 8, 13-triene-1,3α diol (5.52 % of NIB, 3.94 % of IB), verticiol (13.63 % of NIB) and verticillane type diterpene (5.4 % of IB). They made up 78.19% and 91.04% of NIB and IB, respectively. Identification of octyl acetate, as the major component, was confirmed by MS/MS analysis. Octyl acetate showed the most variation (about 25% increase) after γ-treatment. Octyl acetate is known as a marker and major component for *B. carterii* oil, existing approximately up to 60% in the oil. Basar *et al*. reported octyl acetate (39.3%), 1-octanol (11.9%) and α-pinene (10.9%) as the major components of *B. carterii* [14].

To the best of our knowledge, the current report has presented the highest amount of octyl acetate (77%) in *Boswellia* oil after γ-irradiation. Octyl acetate was also reported as the major component of *Zosima absinthifolia* volatile oil (87.48%) with high antibacterial effect against *Bacillus subtilis* and *B. pumilus*, and strong activity against fungi [17]. Seven constituents δ-3-carene (2.04 %), linalool (0.98 %), borneol (1.07 %), citronellyl acetate (0.19 %), geranyl acetate (0.16 %), Isocembrene (0.13 %) and phenantrene derivative (7 ethenyl 1,2,3,4,4α,5,6,7,8,9,10,10αdodecahydro,1,1,4α, 7 tetramethyl) (0.17 %) that together amounted 4.74% were detected only in NIB. The amount of 14 constituents decreased after γ-irradiation. Our findings revealed that verticiol, a verticillane type diterpene in NIB changed to another verticillane type diterpene in IB. Further research is needed to identify the structure of this verticillane type diterpene. Duva-3, 9, 13 triene -1,5α diol 1-acetate showed the most decrease amount (about 4.5%) among other constituents, so it might have decomposed to form octyl acetate.

Correct and exact identification of *Boswellia* oleo-gum-resins from different parts of the world is one of the challenging issues in cosmeceutical and pharmaceutical industries. Most of the twenty five species of the genus *Boswellia* grow in Arabia, northeastern coast of Africa, and India. *B. sacra* grow in south Arabia. *B. carterii* and *B. frereana* are Somalian species, but another resin producing species, *B. serrata*, is found in the middle and northern parts of eastern India [18]. Because there is no microscopically index or simple identification method for this natural product, its adulteration which is mostly colophony or *Pinus* resins cannot be proved [19]. To authenticate the genus, existence of boswellic acids, the active constituents of *Boswellia* terpenoid, reportedly having anti-inflammatory properties, should be checked [20]. Then comparing GC/MS profile of essential oil with those in the literature is advisable. According to previous studies, the components of the species

vary greatly. For instance, the main components of *B.dioscorides* oil were reported to be α-thujen (9.3%) and α-pinene (8.3%). For *B. elongate*, incensol (14.8%), and for *B. socotrana*, ρ-cymen (13%) and camphor (11.6%) were identified [6]. Also, the amount of α-pinene in two African olibanum sold in Iranian local market was reported to be 34.8% and 48.0 %. [21]. Major chemical constituent of *B. sacra* have been also identified as α-pinene in a wide range of 65.49% to 78.45% for various samples [22].

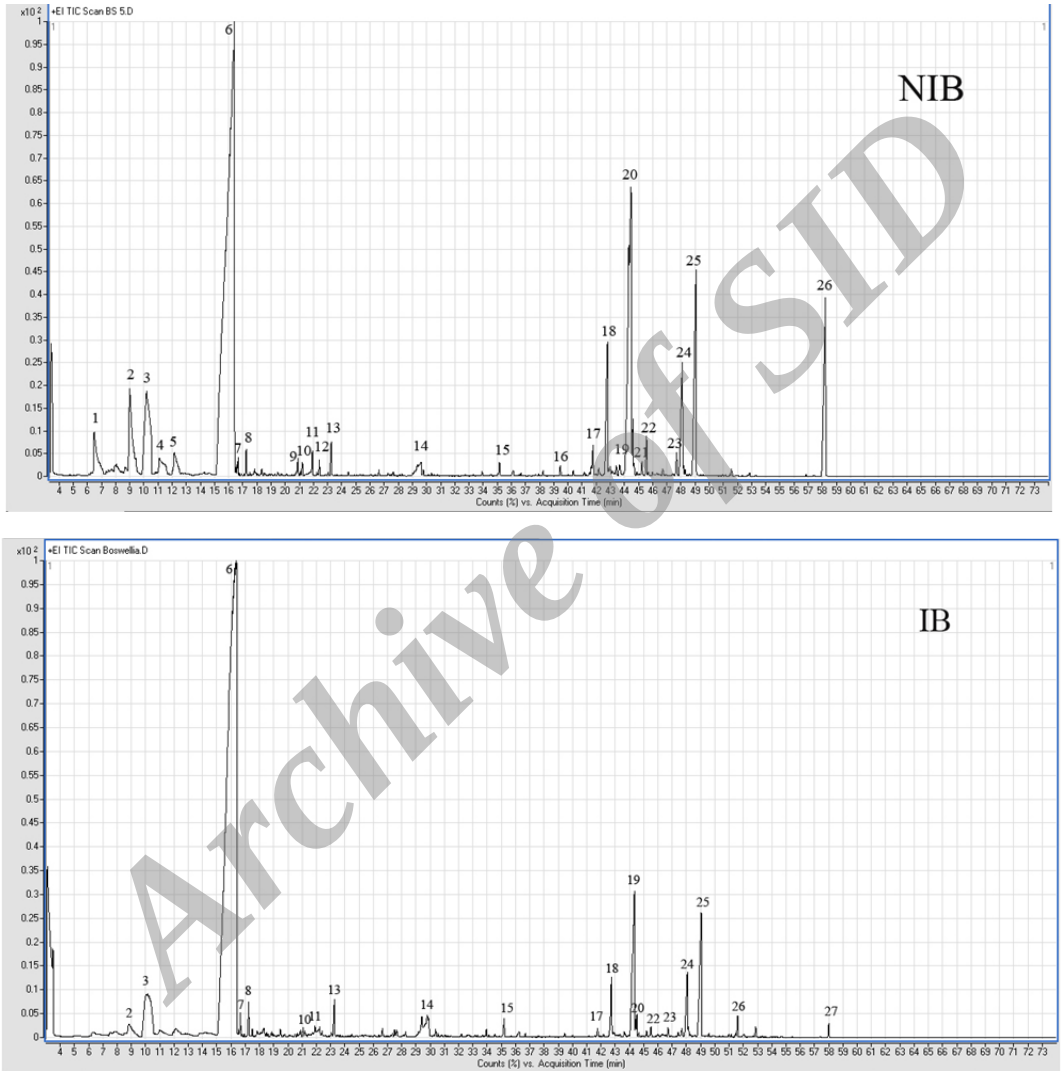

Figure 1. Typical gas chromatograms of the essential oil of *Boswellia carterii* without and with ^{60}Co-gamma Radio-sterilization (NIB and IB). The different compounds are shown by Arabic numerals; δ-3-Carene **1**; Limonene **2**; 1-Octanol **3**; Linalool **4**; Borneol **5**; Octyl acetate **6**; Carvone **7**; Dimethoxytoluene **8**; Citronellyl acetate **9**; Neryl acetate **10**; α- Copaene **11**; Geranyl acetate **12**; Decyl acetate **13**; Dodecanoic acid **14**; Benzyl benzoate **15**; Isocembrene **16**; Unknown **17**; Isophyllocladene **18**; Verticillane type diterpene **19**; Verticiol **20**; Phenantrene 7 ethenyl 1,2,3,4,4α,5,6,7,8,9,10,10α dodecahydro,1,1,4α, 7 tetramethyl **21**; 5β-Podocarp-12-ene-14-carboxylic acid 8,13-dimethyl, methyl ester **22**; Beyerene **23**; Sclarene **24**; duva-4,8,13-triene-1,3α diol **25**; Unknown **26**; Duva-3,9,13-triene-1,5α diol 1-acetate **27**

Table 1. Chemical composition of *Boswellia carterii* essential oils with and without ^{60}Co-gamma radio-sterilization

No.	Components[a]	% Composition		RI[b]	RI[c]	Methods of identification
		NIB	IB			
1	δ-3-Carene	2.04	-	935	1008	MS,L
2	Limonene	3.74	0.70	1021	1024	MS
3	1-Octanol	6.37	5.19	1063	1068	MS, L
4	Linalool	0.98	-	1089	1097	MS
5	Borneol	1.07	-	1118	1169	MS,L
6	Octyl acetate	52.67	76.51	1225	1214	MS, MS/MS
7	Carvone	0.08	0.17	1233	1243	MS
8	Dimethoxytoluene	0.28	0.49	1247	-	MS
9	Citronellyl acetate	0.19	-	1337	1353	MS
10	Neryl acetate	0.16	0.11	1344	1362	MS
11	*α*- Copaene	0.26	0.09	1362	1377	MS,L
12	Geranyl acetate	0.16	-	1374	1381	MS,L
13	Decyl acetate	0.40	0.60	1393	1408	MS
14	Dodecanoic acid	0.50	1.80	1567	1567	MS
15	Benzyl benzoate	0.12	0.33	1717	1760	MS,L
16	Isocembrene	0.13	-	1848	-	L
17	Unknown	0.28	0.10	1918	-	-
18	Isophyllocladene (Kaur-15-ene)	2.77	1.17	1950	-	L
19	Verticillane type diterpene	0.21	5.4	1978	-	MS
20	Verticiol	13.63	0.19	2007	-	MS
21	Phenantrene 7 ethenyl 1,2,3,4,4α,5,6,7,8,9,10,10α dodecahydro,1,1,4α, 7 tetramethyl	0.17	-	2032	-	L
22	5β-Podocarp-12-ene-14-carboxylic acid 8,13-dimethyl, methyl ester	0.51	0.16	2042	-	MS
23	Beyerene	0.35	0.14	2115	1932	L
24	Sclarene	2.53	1.42	2130	1975	L
25	Duva-4,8,13-triene-1,3α diol	5.52	3.94	2161	-	L
26	Unknown	-	0.20	2254	-	-
27	Duva-3,9,13-triene-1,5α diol 1-acetate	4.71	0.20	2500	-	L
	Total identified	99.55%	98.61%			
	Oxygenated hydrocarbons	59.94	84.10			
	Monoterpene hydrocarbons	5.78	0.70			
	Oxygenated monoterpenes	2.64	0.28			
	Sesquiterpene hydrocarbons	0.26	0.09			
	Oxygenated diterpenes	24.58	9.89			
	Diterpene hydrocarbons	5.95	2.73			
	Others	0.40	0.82			

[a] Components are listed in order of their elution from DB1 column.
[b] Linear retention indices were calculated using a homologous series C_9-C_{30} n-alkanes;
[c] Retention indices according to the literature [12]
MS and MS/MS: by comparison of the fragmentation patterns with those of the computer mass libraries NIST 08 library, Wiley 275 and Adams 2007 [12]; L: by comparison of mass spectra and/or retention indices with literature [13,14]; NIB: non-irradiated *Boswellia*; IB: irradiated *Boswellia*.

The essential oil of *Boswellia carterii* which has been studied intensively was reported to have octyl acetate as its major constituent by approximately 60%. The amount of 39.4% octyl acetate in *B. carterii* essential oil was reported by Basar [23].

Additionally, another research carried out by Marongiu *et al.* has supported the idea of predominant existence of mentioned compound in *B. carterii* essential oil. They reported a wide range of 25% to 45% of octyl acetate in the volatile oil of this species extracted by supercritical carbon dioxide [24]. The present sample contained 52.67% of octyl acetate which increased to 76.51% after gamma irradiation. Oleo-gum-resins are highly prone to microbial contamination, and gamma irradiation can be a suitable choice for decontamination. In the current study, microbial assays showed that γ-irradiation (30 kGy) was a practical method for sterilization of *Boswellia* oleo-gum-resin. While γ-rays increase temperature, the irradiation has caused noticeable changes in the essential oil. In another study, six different parts of medicinal herbs containing licorice radix, cinnamon bark, poncirin immature fruit, citrus unshiu peel, coptis rhizome, and apricot kernel had been γ-irradiated at 5, 10, 25, and 50 kGy, and the changes were monitored through measuring the active components of each herb, The results showed increase in hesperidin, poncirin and cinnamic acid, but the amounts of glycyrrhizin, berberine and amygdalin didn't change significantly in different radiation doses [10]. Finally, this method of sterilization could be suggested for other oleo-gum-resins; however, assessing the changes of active compounds is strongly advisable and has to be taken into consideration.

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Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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