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Antioxidant and antifungal properties of seed oils extracted from three leafy vegetables plants consumed in Côte d'Ivoire

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ABSTRACT

The aim of this study was to investigate the oils extracted from seeds of Amaranthus hybridus, Basella alba and Celosia argentea for their antioxidant and antifungal potential. The phytochemical characteristics and antioxidant compounds content of the plant oilseeds were determined using standard methods. Vitamin E content was analyzed by high performance liquid chromatography. The oxidative stability of these oils was determined by using the oven test method, and their antifungal effect was determined by agar dilution method. The studied seed oils highlighted the presence of tannins, steroids and terpenoids. Phospholipids, carotenoids, vitamin E and phenols contents of the studied seed oils were approximately 0.27 ± 0.01%, 1.00 ± 0.01 mg/g, 0.19 ± 0.01 mg/g and 6.70 ± 0.10 mg/g, respectively. Amaranthus hybridus seed oil showed more oxidative stability with peroxide value of 32.66 meq O_2/kg at 12th storage day in oven (60°C). Celosia argentea seed oil showed antifungal activity against Aspergillus fumigatus, Candida tropicalis and Trichophyton mentagrophytes with minimal inhibitory concentration (MIC) of 50%. A. hybridus, B. alba and C. argentea seed oils showed pronounced antioxidant and antifungal activities. These characteristics should be exploited for possible applications in the food supplement, pharmaceutical and cosmetic industries.

Key words: *Amaranthus hybridus, Basella alba, Celosia argentea,* seed oil, antioxidant and antifungal properties.

INTRODUCTION

Many oils for human consumption or for industrial purposes are derived from plants. The conventional plant oils are mostly represented by palm (*Elaeis guinensis*) oil, olive (*Olea europaea*) oil, soybean (*Glycine max*) oil and sunflower (*Helianthus annuus*) oil. These oils consist mainly of mono-, di- and tri-acylglycerols which act as solvent for minor constituents such as sterols, fat-soluble vitamins, pigments including chlorophylls and carotenoids, phenolic compounds, phospholipids and free fatty acids [1]. These minor constituents can have either pro-oxidative (e.g., free fatty acids and hydro-peroxides) or antioxidant (e.g., tocopherols, pigments, phenols and phospholipids) effects [2].

Antioxidant compounds are gaining in importance due to their dual role in food and pharmaceutical industries as lipid stabilizers [3]. Nutritionally important antioxidants such as α-tocopherol (vitamin E) improve the stability of oils [4]. Phenolic compounds may prevent deterioration through the quenching of reactions responsible for lipid rancidity [5]. Moreover, the higher antioxidant activity of some crude oils is partly due to polar lipids, especially phospholipids. Indeed, these compounds are usually considered free radical scavengers, antioxidant synergists and extenders for the action of primary antioxidants [6]. With regard carotenoids, it is accepted that they can act as primary antioxidants by trapping free radicals or as secondary antioxidants by quenching singlet oxygen [7].

Besides the antioxidant effect of these minor constituents of plant oils, another beneficial property is their antimicrobial activity. Indeed, this activity is due to the volatile and phytochemical components isolated from these oils [8]. Recent studies showed that some non-conventional seed oils such as *Nigella sativa*, *Coriandrum sativum* and *Guizotia abyssinica* seed oils contain essential fatty acids, antioxidants and antimicrobial constituents with high nutritional, pharmaceutical and cosmetic properties [9].

Amaranthus hybridus and Celosia argentea belong both to the family of *Amaranthaceae* while *Basella alba* belongs to the family of *Basellaceae*. In most countries of tropical Africa and particularly in Côte d'Ivoire, the leaves of these plants are widely consumed as green vegetables but their seeds are under-exploited. Indeed, some reports have revealed the antioxidant and antimicrobial activities of the leaves and flowers of these two tropical plants [10,11]. However, there is no report dealing with the antioxidant and antimicrobial properties of the seed oils of the three plants. Therefore, the aim of this work was to investigate the antioxidant and antimicrobial activities of these seed oils in order to explore their potential applications in food, pharmaceutical and cosmetic industries.

MATERIALS AND METHODS

Plant materials

Mature seeds of *Amaranthus hybridus*, *Basella alba* and *Celosia argentea* were collected from market gardening of Abidjan district (Côte d'Ivoire) in June 2013. The plants were identified and authenticated by Professor Ake Assi (Botany Department of Félix Houphouët Boigny University – Abidjan). Voucher specimens (nos AMPT02, AMPT09 and ABAN10 respectively) of the plants were kept in the herbarium of National Center of Agronomic Research (CNRA) of Côte d'Ivoire. The seeds were washed thoroughly with distilled water to remove dirt and dried at 40°C for 24 h in an electric oven (Memmert, Germany).

Chemicals

Analytical grade solvents, standards, reagents and culture media were used to perform the study. Organic solvents and Folin-Ciocalteu's phenol reagent were obtained from Merck. All reference standards (α-tocopherol acetate, gallic acid, β-carotene) used were from Sigma-Aldrich. Culture media (Sabouraud agar) was manufactured by Bio-Merieux.

Extraction of oil from the seeds

Oils were extracted from 50 g crushed seeds (Laboratory crusher, Culatti, France) with 300 mL of n-hexane (40-60°C) in a Soxhlet extractor. Then the solvent was removed (vacuum-packed) at 40°C with a rotary evaporator (Heidolph, Hei-Vap, Germany). The extracted lipid was weighed to determine the oil content of the seed. Crude oils were stored at 4°C in air tight brown sterile glass bottles prior to analysis.

Phytochemical screening

The extracted oilseeds were tested for the presence of bioactive compounds such as tannins, saponins, flavonoids, steroids, terpenoids and glycosides by using standard methods [12, 13].

Test for tannins

Crude extract was mixed with 2 mL of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins.

Test for saponins

Crude extract was mixed with 5 mL of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for flavonoids

Crude extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet colour appeared after few minutes which indicated the presence of flavonoids.

Test for alkaloids

Crude extract was mixed with 2 mL of 1% HCl and heated gently. Mayer's And Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Test for steroid

Crude extract was mixed with 2 mL of chloroform. Then 2 mL of each of concentrated H_2SO_4 and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

Test for terpenoids
Crude extract was dissolved in 2 mL of chloroform and evaporated to dryness. To this, 2 mL of concentrated H_2SO_4 was added and heated for about 2 min. A grayish colour indicated the presence of terpenoids.

Determination of phospholipids content
Phospholipids content of oil samples was determined following a spectrophotometric method [14]. The test oil portion (5 g) was burned to ashes in the presence of magnesium oxide. The ashes obtained were dissolved in diluted nitric acid solution (65%). Absorbance was then measured at 460 nm using a spectrophotometer (T80+, PG instruments, England) after adding 10 mL of aqueous ammonium molybdate and 10 mL of aqueous ammonium metavanadate solutions. A standard curve of phosphorus (1 mg/mL) was used as reference.

Determination of carotenoids and total phenol contents
The carotenoids content of the oils was determined by measuring the absorbance of hexanic oil solution at 450 nm using a UV-Vis spectrophotometer (T80+, PG Instruments, UK). Different quantities (varying from 0 to 2 mg) of β-carotene were used as standards.

Total phenols were extracted four times with 10 mL of methanol-water in the proportion of 80:20 (v/v) by centrifugation at 1000 rpm for 10 min. Then, total phenols were quantified by Folin-Ciocalteu colorimetric method [15]. Briefly, 1 mL of methanolic extract was mixed with 1 mL of Folin-Ciocalteu reagent. After 3 min, 1 mL of saturated sodium carbonate solution (20%) was added to the mixture and adjusted to 10 mL with distilled water. The reaction mixture was kept in the dark for 1 h with intermittent shaking. The absorbance was measured at 725 nm using a spectrophotometer (T80+, PG Instruments, UK). Phenolic contents were calculated on the basis of the standard curve for gallic acid (1 mg/mL).

Determination of vitamin E content
The oil sample (1 g) was diluted in 10 mL of hexane. Thereafter, 200 µL of this mixture was transferred into a screw-capped tube where 800 µL of methanol were added. After being vortex-mixed and centrifuged (3000 rpm for 5 min), the samples were filtered through a 0.45 µm pore size filter and the overlay was used for high performance liquid chromatography (HPLC) analysis [16]. Separation by HPLC was carried out using an Acquity liquid chromatography system (Waters, USA) equipped with an optical detector TUV system and a BEH C_{18} column (150 X 0.25 mm i.d., 1.7 µm particle size) (Waters, USA). The injection volume was 10 µL. The mobile phase was methanol-water (98:2, v/v) and the elution was performed at a flow rate of 2 mL/min. The analytical column was kept at 45 °C. Vitamin E of oil sample was detected at 292 nm and identified by comparing its retention time with this of authentic standard. Quantification of vitamin E identified in oil sample was done by using a standard curve (concentration versus peak area) of α-tocopherol acetate. All the data obtained were stored and processed by Empower software (Waters, USA).

Determination of oxidative stability
Oxidative stability of oil samples was determined by using an electric oven test [17]. Oil sample (20 g) were poured into a 50 mL open beaker and then, kept in the darkness of an oven (Memmert, Germany) at 60 ± 1°C for up to 21 days. At 3 days interval, samples were withdrawn, cooled at room temperature (25°C) for 15 min and then analyzed for peroxide value determination.

Fungal cultures
Standard fungal species were obtained from the Biotechnology Laboratory, UFR Biosciences, University Félix Houphouët Boigny-Abidjan (Côte d'Ivoire). Six fungal species, namely, *Aspergillus fumigatus* (896/AB), *Trichophyton mentagrophytes* (13801/D), *Trichophyton rubrum* (14301/D), *Candida albicans* (3076/PV), *Candida tropicalis* (13763/D) and *Cryptococcus neoformans* (3812/B), were used.

Determination of antifungal activity
Antifungal activity was determined using the agar dilution method. First, oil samples were dissolved ethylene glycol [18]. Series of culture media at different oil concentrations (varying from 0 to 100%) were prepared. The Sabouraud plate's agar surfaces were inoculated with 10 µL of microbial inoculum (10^5 cells/mL). An inoculated and incubated agar culture medium without the test oils was used as positive growth control for each assay. The plates were incubated at 30°C for 72 h. In each case, the colonies were counted by using a colony counter (JP Selecta, Spain) and the minimum inhibitory concentration (MIC) of the oils was determined. The MICs were determined as the lowest concentration of oil that produced no visible microbial growth after incubation period.

Statistical analysis

Each sample was analyzed in triplicate and data are reported as mean ± standard deviation (SD). Analysis of variance (ANOVA) was performed using StatPlus 2009 (Analystsoft Inc) software. Statistical significance was set at $p < 0.05$.

RESULTS

The phytochemical analysis of *A. hybridus*, *B. alba* and *C. argentea* seed oils is shown in Table 1. The screening of phytochemical compounds revealed the presence of tannins, steroids and terpenoids in the three oilseeds analyzed. Only *C. argentea* seed oil was characterized by the presence of flavonoids and alkaloids. The studied oilseeds were saponins free.

Table 1: Phytochemical analysis of *A. hybridus*, *B. alba* and *C. argentea* oilseeds

Phytoconstituents	Seed oil		
	A hybridus	***B alba***	***C argentea***
Tannins	+	+	+
Saponins	-	-	-
Flavonoids	-	-	+
Alkaloids	-	-	+
Steroids	+	+	+
Terpenoids	+	+	+

-: absence; +: presence

The oil yield and antioxidant constituents of *A. hybridus*, *B. alba* and *C. argentea* oilseeds are shown in Table 2. The chromatographic profile of vitamin E is depicted in Figure 1. The oil yields of *A. hybridus*, *B. alba* and *C. argentea* seeds were 9.76 ± 0.74, 25.46 ± 0.67 and 7.82 ± 0.48 %. Parameters such as carotenoids and vitamin E contents showed significant difference ($p < 0.05$) for the studied oilseeds. The contents of these constituents were (1.02 ± 0.01, 0.72 ± 0.01, 1.27 ± 0.01 mg/g) and (0.25 ± 0.01, 0.20 ± 0.01, 0.12 ± 0.01 mg/g), respectively. There was no significant difference ($p > 0.05$) concerning the phospholipids content (mean value of 0.29 ± 0.01%) of *B. alba* and *C. argentea* oilseeds. The same observation was noted for the total phenols (mean value of 6.40 ± 0.1 mg/100g) of *A. hybridus* and *B. alba* oilseeds.

Table 2: Extraction yield and antioxidant constituents contents of *A. hybridus*, *B. alba* and *C. argentea* oilseeds

Parameters	Seed oil		
	A hybridus	***B alba***	***C argentea***
Extraction yield (%)	9.76 ± 0.74^{a}	25.46 ± 0.67^{b}	7.82 ± 0.48^{c}
Phospholipids (%)	0.44 ± 0.01^{a}	0.50 ± 0.01^{bc}	0.48 ± 0.01^{c}
Carotenoids (mg/g)	1.02 ± 0.01^{a}	0.72 ± 0.01^{b}	1.27 ± 0.01^{c}
Vitamin E (mg/g)	0.25 ± 0.01^{a}	0.20 ± 0.01^{b}	0.12 ± 0.01^{c}
Total phenols (mg/100g)	6.30 ± 0.10^{a}	6.50 ± 0.10^{ab}	7.20 ± 0.10^{c}

Means in the lines with no common superscript differ significantly ($p < 0.05$).

The antifungal activity of *C. argentea* seed oil is presented in Figure 3. In the concentration range of 0 – 100%, *C. argentea* seed oil totally inhibited the growth of *A. fumigatus*, *C. tropicalis* and *T. mentagrophytes* (MIC, 50%) All the other tested fungi (*Trichophyton rubrum*, *Candida albicans* and *Cryptococcus neoformans*) were resistant to the three oils in the concentration range of 0 to 100% (data not shown). *B. alba* and *A. hybridus* oilseeds did not showed antifungal activity on all selected fungal species.

DISCUSSION

In terms of their oil content, the seeds of *B. alba* are richer in lipids than most of most well known seed oils such as cotton (13%), soybean (14%), and palm fruit (20%) [19]. Therefore, the tests seeds could be explored as alternative sources of oils for industrial purposes.

The phospholipids content of *A. hybridus*, *B. alba*, and *C. argentea* oilseeds is lower than that (15 to 25 mg/g) of soybean oil but higher than that (0.3 mg/g) of rapeseed oil which is often used in cosmetic formulations [14, 20]. This level of phospholipids may contribute to the stability and antioxidant activity of the oils. Indeed, phospholipids are usually considered free radical scavengers, antioxidant synergists and extenders for the action of primary antioxidants [6]. Test oils are richer in carotenoids than expensive oils such as niger (*Guizotia abyssinica*) seed oil (70.2 ± 0.03 mg β-carotene/100 g) and coriander (*Coriandrum sativum*) seed oil (89.2 ± 0.05 mg β-carotene/100 g)

[21]. Carotenoids are important ingredients in cosmetic industries due to their antioxidant activity and protective effect on the skin [22]. Thus, in this respect, the test oils could find use in the cosmetic industry.

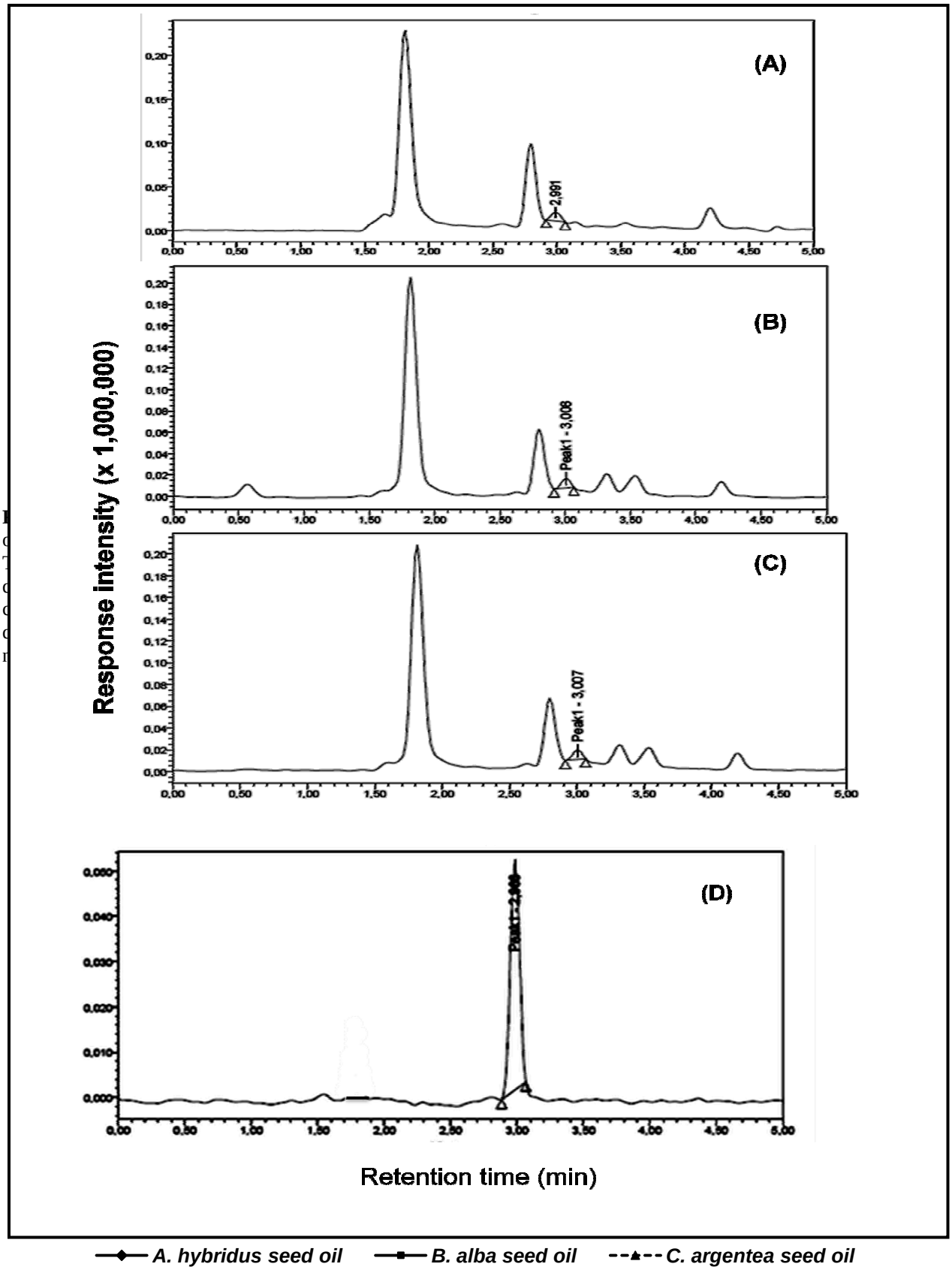

Fig 2: Variation of peroxide value of *A. hybridus*, *B. alba*, and *C. argentea* oilseeds, during storage at 60°C.

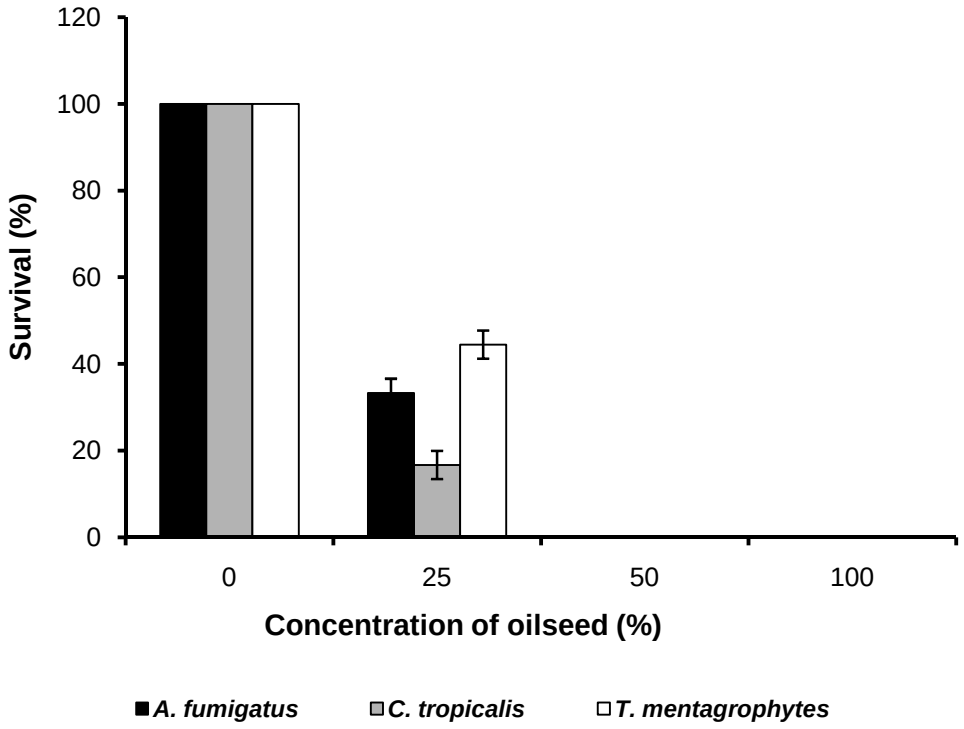

Fig 3: Antifungal activity of *C. argentea* seed oil.

Total phenol content of the test oils is lower than that reported for olive oil which is in the range of 10 to 30 mg/100 g [20]. Nevertheless, the oils are richer in phenolic compounds than other oils such as that of pumpkin seeds (3.9 ± 2.6 mg/100g) [23]. These compounds have previously been correlated with the oxidative stability of vegetable oils. As a result, the relatively high concentration in phenolic compounds of the studied seed oils could be a great asset for their use as stabilizers in the food and cosmetic industries.

Oxidative stability decreased in the order: *A. hybridus* seed oil > *B. alba* seed oil > *C. argentea* seed oil. The initial increase in peroxide values may be due to the induction of its production by elevation in temperature [24]. In contrast, the decrease in peroxide value may suggest the weak stability of hydroperoxides at high temperatures [17]. The oxidative stability of *A. hybridus* is an essential parameter for its sensorial and nutritional qualities [25]. This oxidative stability may be explained by the relatively high content of vitamin E which is a strong antioxidant compound. Indeed, the main biological function of vitamin E is the protection of the polyunsaturated fatty acids of cell membranes from free-radical damage in the oxidative stress [26]. Therefore, *A. hybridus* seed oil could be suitable as antioxidant additive to preserve fatty foods and skin care products against oxidative deterioration [3].

C. argentea seed oil showed antifungal activities against *A. fumigatus*, *C. tropicalis* and *T. mentagrophytes*. The antifungal activity of this oil is worthy of note because fixed oils which are mainly used as diluents for essential oils, usually do not show significant antimicrobial activity [27]. The antimicrobial activity of this oilseed may probably be due to the presence of flavonoids and alkaloids which were reported to possess biological activity against microorganisms [28]. Consequently, *C. argentea* seed oil could be used as antifungal agents for skin care products since *A. fumigatus*, *C. tropicalis* and *T. mentagrophytes* are mostly involved in superficial infections of the skin.

CONCLUSION

The relatively high content of antioxidant compounds (carotenoids, phospholipids, vitamin E and total phenol) and the oxidative stability, confer on *A. hybridus*, *B. alba*, and *C. argentea* seed oils higher antioxidant activity than other well-known and high-value oils. Furthermore, *C. argentea* seed oil has the potential to be used in antimicrobial therapy against *A. fumigatus*, *C. tropicalis* and *T. mentagrophytes* which are usually involved in skin mycosis. Consequently, seeds of *A. hybridus*, *B. alba*, and *C. argentea* are potential new sources of high-value oils with pharmaceutical, cosmetic and nutritional applications.

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