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Short Communication
Essentials Oils and the Phenolic Compositions of the Fruit Pulp of the Argan Fruit at Morocco

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Abstract

Background and Objective: The study of chemical compositions of the pulp of the argan tree at morocco aim at increasing the industrial and commercial value of the argan tree and avoid the extension of the argan tree. **Materials and Methods:** After the extraction and purification of the polyphenolic compounds and the essential oils of the argan fruit pulp, high performance liquid chromatography was used to couple with mass spectrometry and UV spectroscopy (LC/UV/MS), spectroscopic methods and $_{1}$H and $_{13}$C NMRs to identify the molecular structures of phenolic compounds and essential oils of the fruit pulp of the argan tree. Our work performed a more complete analysis of the phenolic fraction using the liquid chromatography/electrospray mass spectrometry (LC/MS-ESI) coupling. This technique allowed us to identify 16 phenolic compounds by comparing their LC/MS mass spectrum and their retention time with literature data. **Results:** The main phenolic compounds found in the fruit of the argan tree are phenolic acids, flavonoids-O-rhamnoglucosides, flavonoids-O-glycosides, flavan-3-ols and flavones. The results also show that the pulp contains a latex which is a polyisoprene whose structure has been elucidated. Furthermore, the GC/MS analysis of the chemical composition of the essential oils of the fruit pulp of the argan tree has identified several terpenes, the majority of which are camphor, 1,8-cineole and borneol. The presence of camphor in appreciable quantity in the fruit pulp of the argan tree is very interesting because it has an insecticidal activity, these activities could be valorized on the industrial level. **Conclusion:** The essential oil of the fruit pulp of the argan tree contains phenolic compounds with antioxidant effect and terpenes with insecticidal effect can lead to its use in phytotherapy.

Key words: Pulp, essential oils, polyphenols, mass spectrometry, retention time

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The argan tree (*Argania spinosa* (L). Skeels), described as an iron tree, is a forest fruit species with multiple uses constituting a characteristic forest region in southwest Morocco. Each part of the tree is usable and is a source of income or food for the user. Thanks to its deep roots, the argan tree represents an excellent anti-desertification barrier[1,2]. Under its shadow live a flora and fauna whose presence is decisive for the ecological balance of this region of southwest Morocco. The argan tree provides everything: wood, hanging pasture for goats and oil. The argan plantation, which has 20 million trees, plays an important role against unemployment, poverty, rural exodus, etc. The study of the biochemical composition of argan derivatives has revealed the presence of substances of great nutritional and health interest. Indeed, the secondary metabolites, although minority components are capable of providing desired properties, in particular in the pharmacological field. Argan oil is certainly the production on which a socio-economic development project could be based. The traditional system of production and marketing of argan oil has indeed evolved very rapidly in recent years, with the creation of cooperatives and private companies. This oil is in great demand and sought after in Morocco and abroad. Trade in argan products is thus a real economic paradigm these days due to the growing demand by the foreign market, especially by the European cosmetic industries. The development of the argan sector is a source of additional income for rural populations who, for the most part are engaged in agriculture and, in general, the different branches of activity of the argan tree have become an opportunity for development , especially for women. In other words, by promoting the marketing of argan oil to the international market, poor women will be able to increase not only their income but also their intellectual level by joining women's groups/cooperatives. They will thus be able to ensure the well-being of their children and their families. There is therefore no doubt that the argan tree is of potential interest, on the one hand as 2a source of natural compounds capable of finding lasting applications and on the other hand as a lever for socio-economic development in Morocco.

All the parts of the argan tree are used by the local populations: the wood and the woody shell of the fruit for heating, the almond of the fruit for the production of argan oil, the foliage, the pulp of the fruit and the oil cake (residue from the production of argan oil) for livestock[3,4].

The Argan tree is a fruit-forest tree whose main richness is the fruit, called "argan nut" and composed of a fleshy pulp and a very hard core containing the oil seed. The argan tree fruits from the age of 5 years; the fruits are ripe around June to August. Before the complete maturation, announcements are made in the weekly markets in order to prohibit the collective course in the Arganeraie and to fix the date of the harvest of the fruits.

In general, the holders of the right to harvest fruit often close the parties to protect animals. At ripening, canvases are placed under the trees and the fruits fall or rather are saplings. The fruits are then spread in the sun in mined layers in order to dry the pulp. They are then stored in a room.

The pulp of argan fruit is naturally consumed in argan groves by goats. It represents 55-75% of the weight of the fruit[5]. It is light yellow-brown in color when fresh and turns dark brown during drying after harvesting or dropping the fruit.

The pulp is essentially characterized by the presence of numerous laticiferous canals, encountered both on the periphery of the fruit and under the thickened epidermis [5]. The importance of the argan tree in the rural economy of this semi-arid region is therefore considerable. The study of the chemical composition of the derivatives of the pulp of the argan fruit was undertaken with the aim of identifying new metabolites making it possible to increase the industrial value of the pulp of the argan fruit then commercial of the argan tree.

The pulp is essentially characterized by the presence of numerous laticiferous channels, found both on the periphery of the fruit and under the thickened epidermis[6]. The importance of the argan tree in the rural economy of this semi-arid region is therefore considerable. The study of the chemical composition of argan fruit pulp derivatives has been undertaken with the aim of identifying new metabolites allowing increasing the industrial value of the fruit pulp of the argan then commercial use of the argan tree.

MATERIALS AND METHODS

Plant material: The pulp of the argan was brought from the region the province Essaouira namely in Tidzi village in 2007on August.

The study Was carried out at laboratory of Plant Chemistry and Organic and Bioorganic Synthesis, Faculty of Science, University Mohamed-V, Morocco from 2007-2012.

Study of the phenolic composition of the fruit pulp of the argan tree: To make the separation and characterization of the main phenolic compounds present in the fruit pulp of the argan tree, we took 20 g of the fruit pulp of the argan tree and

then 150 mL of methanol/water mixture (4/1), after stirring for 30 min, we evaporated the liquid phase (hydroacoolic extract) to dryness under vacuum at 35°C and then 3 times 100 mL of hexane was added. The insoluble part with hexane is extracted with 100 mL of ethyl acetate (twice) and then the liquid phase (ethyl acetate extract) is evaporated to dryness under vacuum at 35°C. and finally 5 mL of methanol are added.

Extraction of essential oils from the fruit pulp of the argan tree: Concerning the extraction of essential oils from the pulp and the chemical composition of the essential oil extracted from the pulp of the fruit of the argan tree we used the hydro-distillation, the training with the steam to extract the essential oils.

Chemical compound identification: High performance liquid chromatography was used to couple with mass spectrometry (LC-ESI-MS/MS) to separate and identify phenolic compounds and essential oil compounds from the fruit pulp of the argan tree.

RESULTS

Composition of the pulp of the fruit of the argan tree: The pulp, which accounts for at least 50% of fresh fruit, consists of almost 5% fat and about 10% protein. The pulp is also rich in polyphenols and saponosides and contains a latex Cellulose and carbohydrates account for 28-34% of the wet matter. The lipid extract of the pulp consists of 33.3% glycerides, 3.3% unsaponifiable matter and a latex (rubber and percha) 63.4%.

Study of the phenolic composition of the fruit pulp of the argan tree: The separation and characterization of the main phenolic compounds present in the fruit pulp of the argan was performed using high performance liquid chromatography-mass spectrometry (LC-ESI-MS/MS) techniques. This method already applied to other plants (cocoa, lepechinia graveolens) is important for the study of polyphenols. It makes it possible to determine the molecular weight and to give certain structural information of the molecules.

The study, relie on the retention time of the peaks of the phenolic compounds in the sample and compared to those of the control peaks of the reference compounds.

The identification of the 16 phenolic compounds in the fruit pulp of the argan tree (Table 1). The results are grouped in Table 2.

Study of the composition of the essential oils of the fruit pulp of the argan tree: Terpenic oxygenated derivatives (OTDs) are the main constituents of the essential oil of argan fruit pulp. Camphor is the main compound with (35.5%). 1,8-Cineole is present in appreciable percentage (16.0%). Endobornol and 2-(4-methylcyclohex-3-enyl)-propan-2-ol were found in similar percentages with 11.8 and 11.1%, respectively. The presence of camphor and 1,8-cineole in appreciable quantities in the fruit pulp of argane is very interesting (Table 3). Indeed,in combination these two compounds have an insect repellent effect or an insecticidal activity. These activities could be valued on an industrial scale.

Table 1: Composition of the fruit pulp of the argan tree

Organic material (%)	Fiber ADF (%)	Crude protein (%)	Extract ethereal (%)	Extractable non-nitrogenous (%)
92.7	34.5	8.7	6.6	42.9

Table 2: Phenolic compounds of the fruit pulp of the argan tree

Compounds (%)	T.R	[M-H]	Fragments	MS/MS exper. Neutral loss scan	MS/MS exper. precursor ion scan	MS/MS exper. product ion
Galic acid (5.0)	0.82	169	125			
Protocatechuic acid (21.8)	1.44	153	109			
Catechine (2.8)	4.06	289			289	245
Isorhoifoline (7.2)	7.13	577				
Epicatechin (14.7)	7.65	289			289	245
Procyanidin (2.7)	7.67	579			579	289,245
Rutin (0.1)	10.87	609		308	609	301
Hesperidin (4.5)	11.19ou 11.43	609	463, 301	308	609	301
Hyperoside (13.4)	11.46	463		162	463	301
Isoquercitrine (10)	11.70	463		162	463	301
Quercetin-o-pentose	12.33	433		132	433	301
Naringenin-7-o-glucoside	12.69	433		162	433	271
Rhamnetine-o-rutinoside (0.5)	13.37	623		308	623	315
Quercetin (1.6)	17.83	301			301	151, 121, 107
Luteolin (0.2)	17.94	285				
Naringenin (0.07)	18.51	271			271	119,109

Table 3: Chemical composition of the essential oil of the fruit pulp of the argan tree

Kovats Retention Index (KRI)	Compounds	Percentage
830	Furan-2-carbaldehyde	2.19
956	2-Methylbutanoic acid	4.95
1033	1,8- Cineole	16.02
1042	Toluene	0.47
1098	Linalool	1.63
1108	2-Phenylethanol	0.79
1131	3,5-Dimethyl-4-ethylidene- Cyclohex-2-ene-1-one	1.45
1143	Camphor	35.53
1165	Endo-borneol	11.81
1173	2,6,6-Trimethylbicyclo [3.1.1] Heptan-3-one.	0.39
1177	4-Terpineol	3.81
1185	1-Isopropyl-4-methylcyclohex-3-en-ol	11.15
1238	1-Phenylethane-1,2-diol	2.06
1294	2,4-Decadienal	1.23
1463	5-Hexyl-dihydrofuran-2 (3H) -one	2.38
1535	2-Pentadecyn-1-ol	0.74
1681	6-Heptyl-tetrahydropyran-2-one	0.91

Table 4: Percentage of fatty acid composition of argan fruit pulp

Fatty acids	Percentage
Myristic C14: 0	14
Pentadecanoic C15: 0	-
Palmitic C16: 0	27
Heptadecanoic C17: 0	-
Palmitoleic C16: 1	1
Stearic C18: 0	6
Oleic C18: 1	15
Linoleic C18: 2	20
Linolenic C18: 3	4
Nonadecenoic C19: 1	-
Groundnut C20: 0	1
Gadoleic C20: 1	1

Study of the chemical composition of the fruit pulp of the argan tree: The fruit pulp of the argan tree is characterized by its low fat content (2%). However, it is richer in carbohydrates (20%), cellulose (13%) and protein (6%). It has a guttoide latex (4%) corresponding to a polyisoprene 86% cis (rubber) and 14% trans (gutta-percha).

The fatty acid composition of the fat of the fruit pulp of the argan tree is close to that of the argan oil. The predominance of myristic acid (C14: 0 = 4.3%), palmitic acid (C16: 0 = 18.4%), linolenic acid (C18: 0 = 6.3%) is noted. , oleic acid C18: 1 = 42%), linoleic acid (C18: 2 = 18.8%), arachidic acid (C20: 0 = 1%), gadoleic acid (C20: 1 = 1%) and a relatively higher level of linolenic acid (C18: 3 = 4.6%) (Table 4).

Study of the unsaponifiable pulp of the fruit of the argan tree: The study of secondary metabolites of the argan tree was undertaken with the aim of identifying new compounds allowing to increase the industrial and commercial value of the argan tree. If successful, the protection of the argan tree and an extension of the argan tree would be strongly stimulated. From the pulp of the argan fruit, (+) - catechin, (-) - epicatechin, rutin, p-hydroxybenzoic acid, hydroxycinnamic derivatives and resorcinol have been isolated. Erythrodiol, lupeol, α- and β-amyrin, other triterpenes have been found in the unsaponifiable pulp; these are taraxasterol, Ψ-taraxasterol, betulinaldehyde and betulin. The sterols identified in the fruit pulp of the argan tree are schottenol and spinasterol, their content in unsaponifiable matter is less than 0.4%.

Volatile substances in the fruit pulp of the argan tree were analyzed and resorcinol was identified as the major component (73.5%).

DISCUSSION

The separation and identification of the main phenolic compounds present in the argan fruit pulp were performed using high performance liquid chromatography coupled with mass spectrometry (LC-ESI-MS/MS)[7]. In the study, the retention times of the peaks of the phenolic compounds of our sample compared to those of the controls (reference compounds) and completed by an analysis of the fragmentation of the molecules by mass spectrometry.

The study of the phenolic composition of the fruit pulp of the argan tree has already been studied by Charrouf[7], who was able to identify only four phenolic compounds (catechin, epicatechin, rutin and p-hydroxybenzoic acid)[7]. This study completed the separation and identification of the majority of the phenolic compounds in the fruit pulp of the argan tree, which allowed us to identify 16 phenolic compounds [8]. This study was approached for the first time as a biochemical approach to establish a polyphenolic identity card and allows to highlight a fairly important structural diversity encompassing four main groups of phenolic compounds:

- Phenolic acids consisting of gallic acid (5%) and protocatechuic acid (21.1%). These compounds are more dominant than flavonoids. The study the p-hydroxybenzoic acid wasn't found among the phenolic acids of the pulp of the argan tree[9,10]
- The flavonoids-O-rhamnoglucosides the most dominant compounds is isorhoifoline (7.2%) and hesperidin (4.5%) against rutin (0.1%) and rhamnetin-O-rutinoside (0.5%) are less dominant[9,10]
- Flavonoids-O-glycosides: The major compounds are hyperoside (13.4%) and isoquercetine (10%). On the other hand, naringenin-7-O-glucoside was the most minor component of this type of flavonoid in the fruit pulp of the argan tree (the percentage of naringenin-7-O-glucoside and quercetin-3-O-arabinose is 15.3%), (compounds 11 and 12)[9,10]
- There are other phenolic compounds in the argan pulp ie catechin (2.8%), epicatechin (14.7%), procyanidin (2.7%), quercetin (1.6%), luteolin (0.2%) and naringenine (0.07%). It is noted that epicatechin was the most sensitive compound in the fruit pulp of the argan tree after protocatechuic acid (21.1%)[9,10]

The fruit pulp of the argan tree has been found to be rich in epicatechin and other catechin derivatives whose natural antioxidant power is important according to many studies. However, such combinations of natural phenolic compounds could be used for better preservation of argan oil. Terpenic[11] oxygenated derivatives (OTDs) are the main constituents of the essential oil of the argan fruit pulp. Camphor is the main compound with (35.5%). 1,8-Cineole is present as a percentage appreciable (16.0%). Endo-borneol and 2-(4-methylcyclohex-3-enyl)-propan-2-ol have been found in similar percentages with 11.8 and 11.1%, respectively. The presence of camphor and 1,8-cineole in appreciable quantity in the fruit pulp of arganeis very interesting[12]. Indeed, in combination these two compounds have an insect repellent effect or Terpenic oxygenated derivatives (OTDs)[13] are the main constituents of the essential oil of argan fruit pulp. Camphor is the main compound with (35.5%)[14,15]. 1,8-Cineole is present in appreciable percentage (16.0%). Endo-borneol and 2- (4-methylcyclohex-3-enyl) -propan-2-ol were found in similar percentages with 11.8 and 11.1%, respectively[15].

CONCLUSION

This study makes it possible to identify 16 phenolic compounds in the pulp of argan fruit which makes the pulp of the argan fruit very important in the area of cosmetics or industry. The presence of camphor and 1,8-cineole in appreciable quantity in the pulp of the argan fruits is very interesting. Indeed, in combination these two compounds have an insect repellent effect or an insecticidal activity. These activities could be valued on an industrial scale.

SIGNIFICANCE STATEMENT

This study was gap roached for the first time as a biochemical approached to identify the polyphenol composition of the oil of the fruit pulp of argan. Moreover, the study of the oil composition of the pulp showed that here are 17 compounds. The most important are camphre and 1,8 cineole witch can be used in field of industry or cosmetic.

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