



Phenolic composition and antimicrobial activity of essential oils from the fruit pulp of the argan tree

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ABSTRACT: The study of the chemical composition of essential oils, the pulp of argan leachate, the phenolic compounds and the identification of the biological activity of secondary metabolites making it possible to increase the industrial and commercial value of the argan tree in the field of cosmetics, therapeutics and nutraceuticals and to allow the protection of the argan tree and the extension of the arganieriaie. After the extraction and purification of the polyphenolic compounds and the essential oils of the argan fruit pulp, we used high performance liquid chromatography coupled with mass spectrometry and UV spectroscopy (LC/UV/MS). This technique allowed us to identify 16 phenolic compounds by comparing their LC/MS mass spectrum and their retention time with literature data. 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. Our 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-cineode 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. 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: essential oils; bio-active components; polyphenols; pulp.

Composição fenólica e atividade antimicrobiológica de óleos essenciais da polpa do fruto da árvore de Argão

RESUMO: O estudo apresenta a composição química dos óleos essenciais, da polpa do lixiviado de argão, dos compostos fenólicos e a identificação da atividade biológica de metabólitos secundários, possibilitando assim aumentar o valor industrial e comercial da árvore de argão nas áreas da cosmética, terapêutica e nutracêuticos, e além disso, permitir a proteção das árvores de argão e a extensão da arganieriaie. Após a extração e purificação dos compostos polifenólicos e dos óleos essenciais da polpa do fruto de argão, utilizou-se a cromatografia líquida de alta eficiência acoplada à espectrometria de massas e espectroscopia UV (LC/UV/MS). Esta técnica permitiu identificar 16 compostos fenólicos comparando seu espectro de massa LC/MS e seu tempo de retenção com dados da literatura. Os principais compostos fenólicos encontrados no fruto da árvore de argão são ácidos fenólicos, flavonóides-O-ramnoglicosídeos, flavonóides-O-glicosídeos, flavan-3-ols e flavonas. Nossos resultados também mostram que a polpa contém um látex, que é um poliisopreno, cuja estrutura foi elucidada. Além disso, a análise GC/MS da composição química dos óleos essenciais da polpa do fruto da árvore de argão identificou vários terpenos, sendo a maioria cânfora, 1,8-cineode e borneol. A presença de cânfora em quantidade apreciável na polpa do fruto da árvore de argão é muito interessante porque tem atividade inseticida, que poderiam ser valorizadas a nível industrial. O óleo essencial da polpa do fruto da árvore de argão contém compostos fenólicos com efeito antioxidante e terpenos com efeito inseticida podendo levar ao seu uso na fitoterapia.

Palavras-chave: óleos essenciais; componentes bioativos; polifenóis; polpa.

1. INTRODUCTION

Argan oil is the main product of the argan tree, it is prepared from amandons of its fruit. The argan tree (*Argania spinosa* (L.) Skeels) is a tree that grows naturally in Morocco and other countries of region (CHARROUF, 2002).

One of the criteria for the production of high quality argan oil is the use of fruits; indeed, traditional pulping by the goats leads to a poor quality oil (HILALI et al., 2005; HARHAR et al., 2011). Commonly, all parts of the argan tree are used by the local people: the wood and the woody hull of

the fruit for heating, the amandon of the fruit for the production of argan oil, the foliage, the pulp of the fruit and the oil cake (residue of argan oil production) for livestock (LYBBERT et al., 2002; HILALI et al., 2020; IBOURKI et al., 2021).

The fruit pulp of the argan tree is naturally consumed in the argan plantations by goats (GUILLAUME et al., 2019). It represents 55 to 75% of the weight of the fruit (CHAHBOUN, 1993). It is light yellow-brown when it is fresh and turns dark brown during its desiccation after harvest or fall of the fruit.

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 (CHAHBOUN, 1993). The importance of the argan tree in the rural economy of this semi-arid region is therefore considerable.

In novel studied on local products of argan three, antibacterial effect of Moroccan argan oil has been documented by NAFIS et al. (2022). Also, similar report has been published for Algerian argan oil (BENABDESLEM et al., 2022). Whereas phytochemical components of different part of argan tree has been identified (Idrissi et al., 2021), the antimicrobial effects of argan pulp is almost unknown.

The study of the chemical composition of argan fruit pulp derivatives has been undertaken with the aim of identifying new metabolites allowing to increase the industrial value of the fruit pulp of the argan then commercial of the argan tree.

2. MATERIALS AND METHODS

2.1. 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.

2.2. 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.

2.3. Chemical compound identification

We used high performance liquid chromatography coupled 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.

3. RESULTS

3.1. 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 to 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% (FELLAT-ZARROUK et al., 1987)

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

Tabela 1. Composição da polpa do fruto da árvore de argão.

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

3.2. 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) (TAHROUCH et al., 2000) 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 (OULAD-ALI et al., 1996).

In our study, we relied on the retention time of the peaks of the phenolic compounds in our sample and compared to those of the control peaks of the reference compounds.

We identified 16 phenolic compounds in the fruit pulp of the argan tree (Table 2) (Charrouf et al., 2007). The results are grouped in Table 2.

3.3. 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 argan is very interesting (Table 3). Indeed, in combination these two compounds have an insect repellent effect or an insecticidal activity (HARHAH et al., 2011). These activities could be valued on an industrial scale.

3.4. 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) (REID et al., 2006).

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) (REID et al., 2006).

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

Tabela 2. Compostos fenólicos da polpa do fruto da árvore de argão.

N°	Component (%)	T.R	[M-H]	Fragments	MS/MS exper. Neutral loss scan	MS/MS Precursor scan	exper. ion	MS/MS Product ion	exper.
1	Acide galique (5.0)	0.82	169	125					
2	Acide protocatechique (21.8)	1.44	153	109					
3	Catechine (2.8)	4.06	289			289		245	
4	Isorhoifoline (7.2)	7.13	577						
5	Epicatechine (14.7)	7.65	289			289		245	
6	Procyanidine (2.7)	7.67	579			579		289,245	
7	Rutine (0.1)	10.87	609		308	609		301	
8	Hesperidine (4.5)	11.19ou 11.43	609	463,301	308	609		301	
9	Hyperoside (13.4)	11.46	463		162	463		301	
10	Isoquercitrine (10)	11.70	463		162	463		301	
11	Quercetine-o-pentose	12.33	433		132	433		301	
12	Naringenine-7-o-glucoside	12.69	433		162	433		271	
13	Rhamnetine-o-rutinoside (0.5)	13.37	623		308	623		315	
14	Quercetine (1.6)	17.83	301			301		151, 121, 107	
15	Luteoline (0.2)	17.94	285						
16	Naringenine (0.07)	18.51	271			271		119,109	

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

Tabela 3. Composição química do óleo essencial da polpa do fruto da árvore de argão.

N°	IK	Constituant %	%
1	830	furane-2-carbaldéhyde	2.19
2	956	Acide 2-méthylbutanoïque	4.95
3	1033	1,8- cinéole	16.02
4	1098	Toluène	0.47
5	1108	Linalool	1.63
6	1131	2-phényléthanol	0.79
7	1143	3,5-Diméthyl-4-éthylidène- cyclohex-2-ène-1-one	1.45
8	1165	Camphre	35.53
9	1173	Endo-bornéol	11.81
10	1177	2,6,6-triméthylbicyclo[3.1.1]heptan-3-one, (1a,2a,5a)	0.39
11	1177	4-Terpinéol	3.81
12	1185	2-(4-méthylcyclohex-3-enyl)propan-2-ol	11.15
13	1238	1-phényléthane-1,2-diol	2.06
14	1294	2,4 Décadiénal	1.23
15	1463	5-hexyl-dihydrofuran-2(3H)-one	2.38
16	1535	2-Pentadécyn-1-ol	0.74
17	1681	6-heptyl-tétrahydropyran-2-one	0.91
Total			97.49

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

Tabela 4. Porcentagem da composição de ácidos graxos da polpa da fruta argão.

Acides gras	Pourcentage %
Myristique C14 : 0	14
Pentadécanoïque C15 : 0	-
Palmitique C16 : 0	27
Heptadécanoïque C17 : 0	-
Palmitoléique C16 : 1	1
Stéarique C18 : 0	6
Oléique C18 : 1	15
Linoléique C18 : 2	20
Linoléénique C18 : 3	4
Nonadécénoïque C19 : 1	-
Arachidique C20 : 0	1
Gadoléique C20 : 1	1

3.5. 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, betulinolaldehyde 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% (HILALI et al., 2007). Volatile substances in the fruit pulp of the argan tree were analyzed and resorcinol was identified as the major component (73.5%; KARLESKIND, 1992).

3.6. Study of the composition of the saponosides of the pulp of the fruit of the argan tree

The methanolic extract of the pulp has revealed the presence of three saponosides including a new natural substance: arganine K (Figure 1), this extract represents remarkable antioxidant properties and inhibits the proliferation of human cancer cell lines of thymic origin (HPB-HALL - CONSEIL OLEICOLE INTERNATIONAL, 2001).

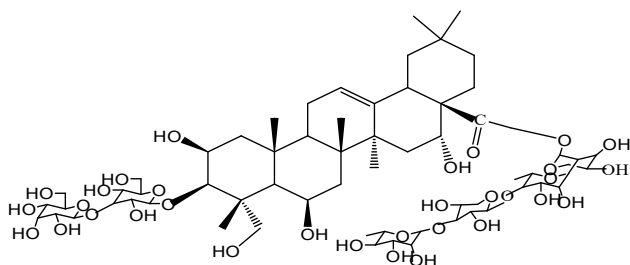


Figure 1. Saponin extracted from the pulp of the argan fruit (ArganineK).

Figura 1. Saponina extraída da polpa do fruto do argan (ArganineK).

4. 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). In present study, the retention times of the peaks of the phenolic compounds of sample were used 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; Guillaume (2007), who was able to identify only four phenolic compounds (catechin, epicatechin, rutin, and p-hydroxybenzoic acid). This work 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. 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. In our study we did not find p-hydroxybenzoic acid among the phenolic acids of the pulp of the argan tree.

- 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.

- Flavonoids-O-glycosides: The major compounds are hyperoside (13.4%) and isoquercetin (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).

- 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%).

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 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 argan is very interesting. Indeed, in combination these two compounds have an insect repellent effect or 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%). Endo-borneol 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 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 (HARHAR et al., 2011; LIU et al., 2021). These activities could be valued on an industrial scale for antimicrobial uses.

5. CONCLUSIONS

This study was approached for the first time as a biochemical approach to establish a polyphenolic identity card, chemical composition of the essential oil of the fruit pulp of the argan tree and allows to highlight a rather important structural diversity including four main groups of phenolic compounds in the pulp of the argan fruit and also this study shows that camphor is more important in the essential oil these metabolites make the pulp more important at the industrial level. These activities could be valued on an industrial scale for antimicrobial uses.

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