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Optimization of a dermatological cream process formulation from *Atractilys Gummifera* L roots

R. Larid* [1,2], H.Aksas[1,2] , S. Touzouirt[1,3] , I. Hocine[1], R. Kerbouche[1] ; M.E. Abbadi[1]

[1]Department of Process Engineering, Faculty of Technologies, M'Hamed Bougara University, Boumerdes, Algeria
[2] Research laboratory in food technology (LRTA), Faculty of Technologies, M'Hamed Bougara University, 35000 Boumerdes, Algeria
[3] Natural Resources Laboratory, Mouloud Mammeri University, Tizi-Ouzou, Algeria.

*Corresponding author: r.larid@univ-boumerdes.dz

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ABSTRACT/RESUME

Abstract: *Atractilys Gummifera L or glue thistle,is one of the medicinal plants of the Mediterranean area. Belonging to the Asteraceae family, it is used in traditional medicine in various ways (maceration, decoction and fumigation). The rhizome of this plant is used as a healing agent in traditional Algerian medicine: it helps to heal burns. The aim of our work is to optimize the formulation process of a cream for dermatological application based on the roots of Atractilys Gummifera L, by using the methodology of experimental design.*
The physicochemical characterization of the underground part of this plant reveals a richness in organic substances (sugars 8.5%, lipids 2.29%, Brix degrees 33.34%), and mineral substances (ashes 4.21%). The study of the various parameters considered influential on the formulation process revealed that the optimal conditions for a more stable cream are: the solid (rhizome) / liquid (water) ratio 0.4 (m / V), the stirring time for 15 minutes and the stirring speed 500 rpm.
The cream obtained has a rheofluidic (or pseudoplastic) behaviour, which can be described by the Casson model. This behaviour is suitable for a cream for dermatological application.

I. Introduction

According to the WHO [1], 80% of the African population still uses traditional medicine to treat themselves, in which most of the therapies involve the exploitation of the active principles of medicinal plants. Indeed, there are about 500,000 species of plants on earth, of which 80,000 have medicinal properties [2].
Due to its climate (Mediterranean, arid) and the nature of its soils, the Algerian flora abounds in several plant species that are still little or not studied, but have real pharmacological properties [3- 5].
The valorization of these plants is a particularly interesting field to develop for the creation of new products with high added value.
Among these medicinal plants, *Atractylis gummifera* L known as glue thistle, which belongs to the Asteraceae family. It is widely spread in the Mediterranean countries; all around Europe except the coast of France. In Algeria, this plant can be found [6] in the entire coastal region and the Tell, both in the plains and in the mountains [7].
Few studies were carried out on this plant, and it is in Algeria that the first botanical and chemical works were carried out by the military pharmacist and botanist Lefranc in 1866 [8], whose phytochemical study of the root revealed the presence of flavonoid heterosides (orientin, homoorientine, corymboside, neocorymboside) and diterpene heterosides. The research went on with the anatomical and pharmacochemical study [9-11], followed by the study of Khadhri .A and al (2013) [12], which showed that the root of *Atractylis gummifera* L has antioxidant activities due to its richness in flavonoids, anthocyanins and tannins.
Note that this plant - especially its rhizome- has been of more interest in recent years to researchers from North Africa, in Morocco [18,19], and Tunisia [12], because such a composition of bioactive substances

with proven therapeutic potential , will influence those of the various preparations obtained, particularly those of the food, cosmetic and pharmaceutical industry.
This plant, which enjoys great popularity throughout northwestern Africa, is used in traditional pharmacopoeia, especially for the treatment of various diseases such as psoriasis, syphilitic ulcers and epilepsy [12].
The root extract of *Atractylis gummifera* L can be recommended for local use on all infectious and inflammatory lesions of the skin [15]. In internal use, the plant defines a certain toxicity because of its narcotic properties, it is reputed to be purgative at low doses [15, 17], but it can be used in fumigations, for the treatment of colds, dizziness, headaches and paralysis [16].
In Algeria, the roots of *Atractilys Gumifera* L are used traditionally, as a healing agent contributing to cure burns in the form of powder in poultice or compresses, as evidenced by the study conducted by Karima Ounaissia et al 2019 [18], in the northeastern region of Algeria (El-Taref, Annaba, Skikda, Guelma and Souk Ahras).
Since there are no studies on the formulation of cream from the root of Atractylis gummifera L., this study is conducted to highlight the potentiality of this plant in traditional Algerian medicine, as a natural remedy that is declined from the know-how of our ancestors, so this work aims to develop a new process of formulation of a cream for external use using the methodology of experimental design.
In this work, we first characterize the root from the morphological and physicochemical point of view and then optimize the formulation protocol of the cream by design of experiments methodology. The optimal formulation was characterized from the physicochemical, organoleptic and rheological point of view.

II. Materials and methods

II.1 Plant material

Atractilys Gumifera L, is a herbaceous, spiny, fragrant, perennial plant with its highly developed underground parts (Figure 1).
It has two parts:

- An aerial part consisting of the stem, leaves, flowers and fruit.
- An underground part consisting of a voluminous rhizome, pivotal and fleshy (Figure 1)

Figure 1. *Rhizome of Atractylis gummifera L.*

Our study focuses on the underground part (the root) of the plant Atractilys Gumifera L, harvested during the month of April 2021 in the wilaya of Boumerdes (46 km east of Algiers), Algeria. The harvested roots were cleaned, peeled and crushed, then they are characterized from morphological and physicochemical point of view.
The physico-chemical characterization of the roots is based on the determination of:
The water content according to (NF T 60-305 June 1976);
The pH according to the method (NF V 05-108, 1970);
The titratable acidity according to the method (NF V 05-101, 1974);
Ash (NF V 05-113, 1972)
The rate of soluble solids (°Brix) (NF V 05-109, 1970)
Total sugars according to method of Nelson and Somogyi, 1952 [19]
Fat content (NF EN ISO 734-1, 2000)

II.2 Process for formulating cream

An ethnobotanical survey on the plant Atractylis gummifera L, was carried out in the conducted in the field during the year 2020. This survey is conducted among herbalists and inhabitants of the commune of Tamalous, in the wilaya of Skikda (eastern Algeria), in order to identify the therapeutic uses and habits of local populations.
The protocol adopted for the formulation of the cream is inspired by the results of the ethnobotanical survey. These results indicate that only the pulp of the root part of the plant is used in the preparation of the cream according to a mode of preparation either hot (by decoction), or cold, which is the most predominant method of preparation.
In this context, we opted for the hot method, where several formulations were carried out at a temperature of 100°C with different stirring speeds

and times in order to optimize the formulation process.

In a closed assembly (reflux heating), water is added to the plant material and then heated for the necessary time under agitation, the mixture is then wrung out in a sterile compress to obtain the maximum aqueous extract. The cream is obtained after the evaporation of the excess water, using a Heidolph Rotary evaporator and stored in a refrigerator at 6°C.

II.3. Optimization methodology

The optimization of the cream formulation process was studied using a methodology of experimental design using the full factorial design with 3 variable factors.

Table1 represents the various factors chosen (Stirring speed, Ratio: plant matter/water and Stirring time) as well as the optimized responses (cream yield and gelation). This study was conducted using Modde 6 software.

The cream yield is calculated using the following formula (1):

$$R\ (\%) = \frac{Mass\ of\ cream}{Mass\ of\ plant\ material} \times 100 \qquad (1)$$

The gelation of the product is assessed visually after evaporation using the rotary evaporator

Table 1. *Matrix of factorial design and recorded responses*

N°	Stirring speed (rpm)		Ratio : plant matter / water (%m/v)		Stirring time (min)		Cream yield (%)	Gélation (*)
	-1 : 250	+1 : 500	-1 :33%	+1 : 50%	-1 : 5	+1 : 15		
1	-1		-1		-1		39.27	2
2	-1		+1		-1		0	0
3	+1		-1		-1		0	0
4	+1		+1		- 1		0	0
5	-1		-1		+1		0	1
6	-1		+1		+1		0	1
7	+1		-1		+1		56.14	2
8	+1		+1		+1		43.5	2
9	0		0		0		14.25	1
10	0		0		0		23	1
11	0		0		0		21.7	1

(0 : No gelling, 1 : Average gelling, 2 : Good gelling)

II.4. Characterization of the cream

A cream was formulated under the optimal conditions given by the Modde software (Stirring speed: 500 (rpm), Ratio : plant matter / water : 50% and Stirring time: 15mn). An organoleptic and physicochemical characterization and a rheological study of this cream was carried out.

II.4.1. Organoleptic and physico-chemical characterization

The organoleptic characteristics of the cream were evaluated in terms of color, smell, appearance and homogeneity.

For the homogeneity, 0.2 g of the cream was spread in a thin layer between slide and coverslip. The absence of lumps was checked with the naked eye [20].

The physic-chemical characteristics of the cream (pH, acidity, moisture and ash) were determined according to the same protocol given in section 1.1.

II.4.2. Rheological behavior of cream

The rheological study is carried out using a ViscoTester viscometer (VT550) provided with the RheoWin Data Manager software to control the device and data processing. The geometry used is cone/plate, with an inclination of 1° and the speed gradient interval [1-500 1/s]. All measurements were carried out at 20°C.

III. Results and discussion

III.1. Characterization of the raw material

- **Morphological characterization**

The macroscopic examination of the root of Atractylis gummifera L, allowed us to identify the following characteristics:

- A swivelling and very voluminous rhizome, of irregular conical shape, with the presence of fine and tight transverse striations (figure 1).
- A thin, yellowish-brown epidermis (Figure 2.a).
- The presence of numerous superficial creeping ramifications (Figure 2.b).

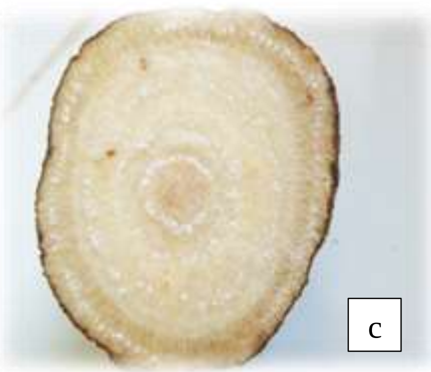

Figure 2. *Morphological characterization of Atractylis gummifera L*
(a): epidermis, (b) : Creeping branches; (c): Latex flow from the rhizome

- A brittle fibrous pulp of pale yellow color, characterized by the flow of a whitish latex (Figure 2.c).

The underground organs, fleshy in the fresh state, include a large turnip-shaped main rhizome, with 53, 5 cm long and 16 cm of diameter, having fine roots and creeping rhizomes, with a very hard, fibrous, yellowish section, revealing concentric striations. These results are in concordance with those reported by other auteurs [16, 17, 21].

- **Physicochemical characterization**

The results of physicochemical analysis of the rhizome are shown in the following table.

Table 2. *Physico-chemical characterization of Atractylis gummifera* L *root*

Parameters	*Average content*
pH	*6,5 ± 0,2*
Titratable acidity (% citric acid)	*4,66 ± 0,95*
Humidity (%)	*65 ± 0,67*
Degrees Brix (°Bx)	*33,34 ± 0,0*
Sugar (%)	*8,5 ± 0,09*
Fat (%)	*2,29*
Ash (%)	*4,21 ± 0,17*

The chemical composition of the plant drug varies with the vegetative cycle of the plant. These variations can be qualitative: appearance of an active principle and disappearance of another, quantitative: the content of active principles can pass by a maximum and then decrease quickly [22].

The chemical composition of the root of Atractylis gummifera L (table N°2), is characterized by its richness in total sugars with a content of 8,5 g/100g, and a considerable rate of degrees Brix **(**33,34). These contents integrate free sugars and water-soluble polysaccharides.

The sugar content is comparable to that obtained by Edmond. L, 1866 (8%) [8] on the same plant from the region of Mostaganem, and it is high compared to the results of Hamadi. F and al (2014) (3.6%) [23] dealing with the plant of the same family (*Carthamus caeruleus* L).

These results suggest that polysaccharides are responsible for the functional properties developed by the roots of *Atractylis gummifera* L.

The roots of this plant has low lipid (2.29%) and ash (4.21%) contents compared to those reported by Hamadi. F and al 2014 [23] , for the *Carthamus caeruleus* L roots.

Water-soluble polysaccharides extracted from plants are interesting sources of additives in industrial applications, especially food, pharmaceutical and cosmetic applications because of their ability to modify the functional properties of the products in which they are incorporated [24].

The results obtained indicate an average pH of 6.5, so the fresh rhizome is slightly acidic. This pH is included in the range of the optimum pH for the growth of most plants which is between 6.0 and 7.0 as most of the mineral elements needed by plants are available [25].

The ash or mineral salts represent an average content of 4.21%. This result is close to that reported by Edmond. L, 1866 (4.5% /MF), [8] for the same plant.

III.2. Statistical study of the results

III.2.1. Model validation

The quality of the fitted polynomial model was assessed by the coefficient of multiple determination (R2) and Fisher's test and approved by the residuals study.

To have a good fit, R2 must be greater than 0.8. The Fisher F-value is the ratio of the mean square of the model to the mean square of the residuals. This value is used to calculate the probability that these two squares are not equal. If the experimental Fisher's F (F_{Obs}) is greater than the Fisher's F given by the tables (F_{Crit}), the variations in the measured responses are indeed due to variations in the factors[40]. The results are summarized in Table 3.

Table 3. *Values of R^2, p-value as well as Fisher's test for the two responses studied.*

	R^2	P-VALUE	F_{CRIT}	F_{OBS}
PERFORMANCE	0.907	0.046	4.534	6.51
FREEZING	0.917	0.037	4.534	7.33

Models with p-value values (the probability that the model's coefficient is zero or insignificant) close to zero are statistically significant and considered appropriate to describe the corresponding response under study.

Figures 3 and 4 show the evolution of the residues for yield and gelation as a function of the factors studied. They show that the two latter are normally distributed and that their dispersion is due to random causes.

According to Table 3 and Figures 3 and 4, the conditions are respected for the two responses studied, from which we conclude that our model is statistically valid. This allows us to proceed to the optimization step of the formula.

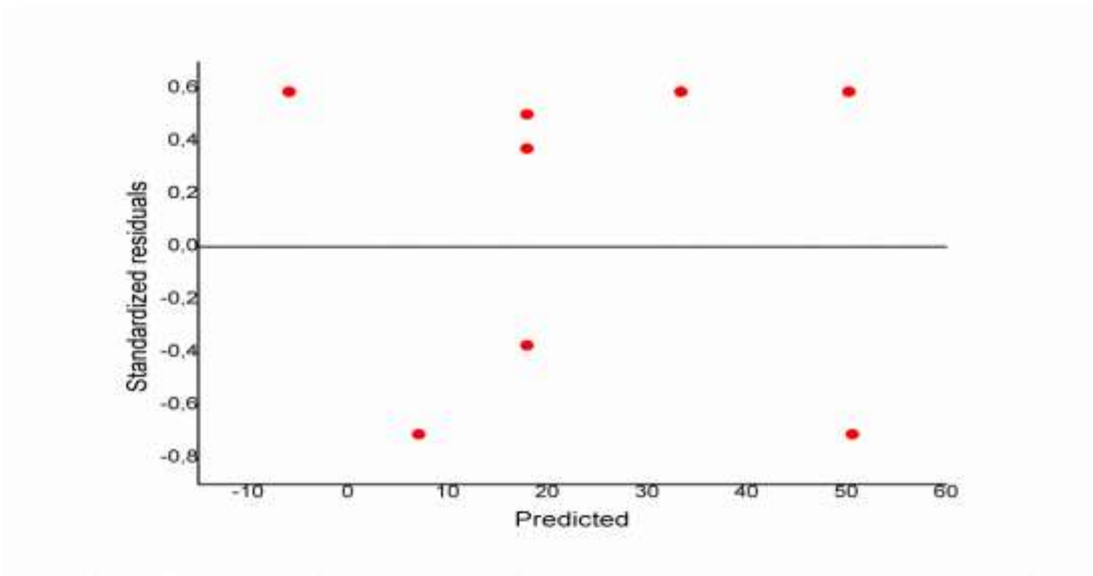

Figure 3. *Residue evaluation based on predicted yield values*

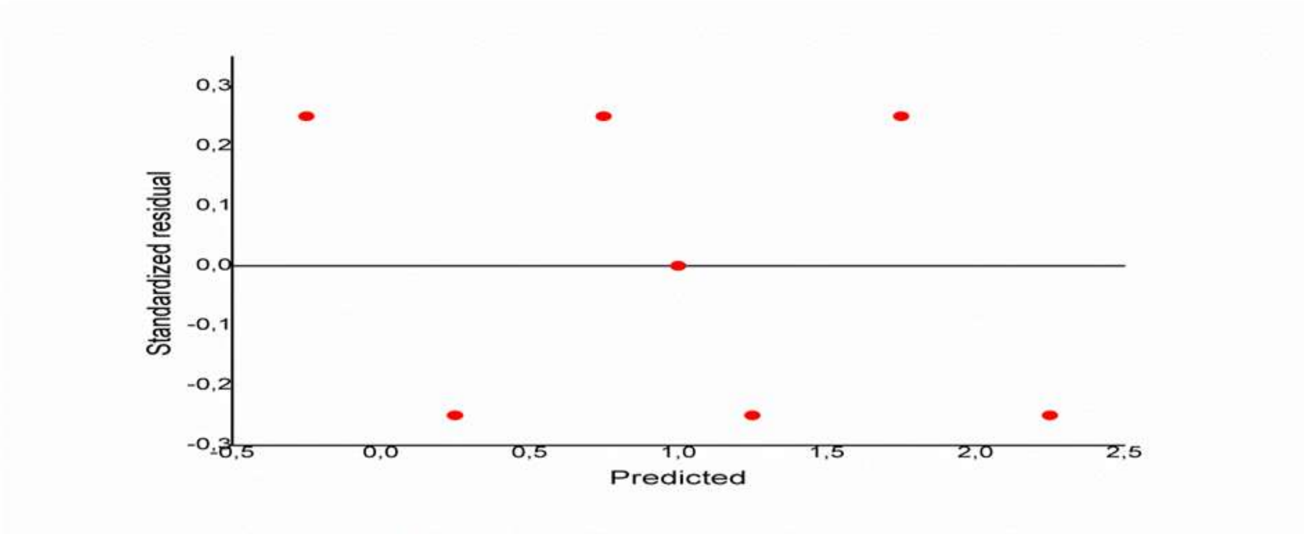

Figure 4. *Evaluation of residues versus predicted values for gelation*

The statistical analysis of the results shows that at the 5% significance level, only the stirring time factor has an influence on the two responses studied. It has a positive influence on the gelling response and the interaction of this factor with the stirring speed factor has a positive influence on both the yield and gelling responses.

According to Edmond Lefranc, 1866 [8], the major compound of the aqueous extract of *Atractylis gummifera* L root is inulin, it represents 46.5% of the mixture. This compound has very important gelling properties.

From this, we assume that the yield as well as the gelation are directly related to the presence of inulin in the extracts.

The solid/water ratio factor does not affect the responses in the ranges studied. Because the range of variation of this ratio is the most favorable for the extraction of polysaccharides responsible for gelation. Inulin is soluble in water only from the conditions of temperature and mass/volume ratio of 90°C and 35% [27].

From the surfaces of the responses as a function of the significant factors Figure 5 and Figure 6, it can be seen that the best results (better cream yield: 50,61% and good gelation: 2.25) are obtained at high levels in terms of stirring speed and duration, which is explained by the influence of these two factors on the extraction yield of inulin [28].

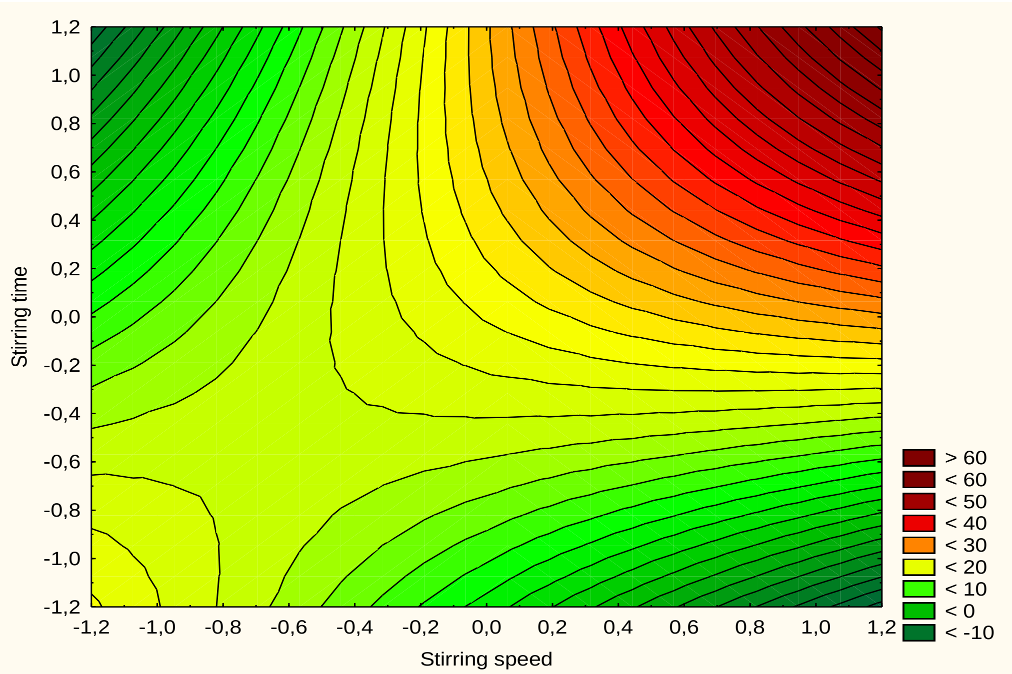

Figure 5. *Iso-response of yield as a function of agitation speed and duration. The plant material/water ratio is maintained at the average level.*

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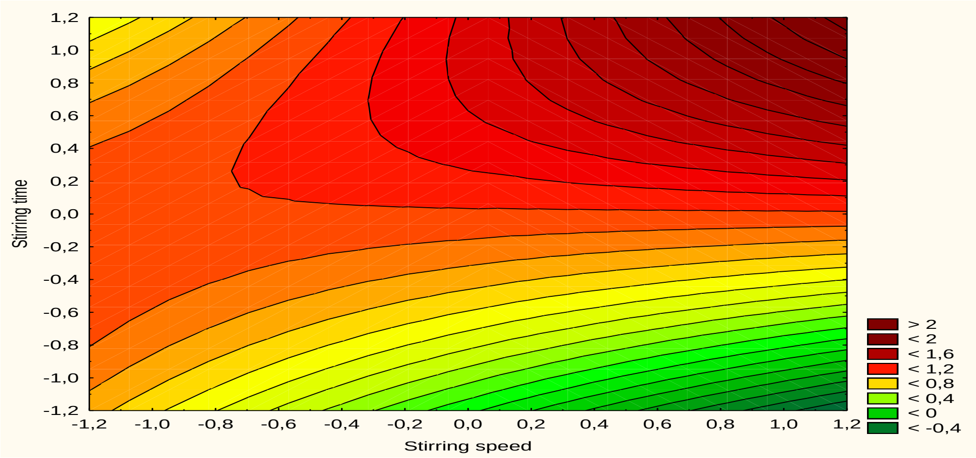

Figure 6. *Iso-response of gelation as a function of stirring speed and duration. The plant material/water ratio is maintained at the medium level.*

III.2.2. Optimization phase

The optimization of the cream formulation was done with Modde 6 software using the "optimizer" module after having validated the mathematical model.

The software suggests several solutions to meet the criterion of maximizing responses (yield and gelation).

The proposed solutions indicate high values for stirring time and speed and medium values for the plant material/water ratio. We have chosen the values of 500 rpm for the stirring speed and 15min for stirring time with a solid/liquid ratio of 0.4.

A cream was formulated under the chosen optimal conditions (optimal formulation) for physicochemical and rheological characterization.

III.3. Physicochemical and organoleptic characteristics of the cream

III.3.1. Physicochemical characteristics

The physicochemical characteristics of the cream are summarized in Table 4.

Table 4. *Physicochemical characteristics of cream*

Physicochemical parameters	
pH	5.22±0.2
Titratable acidity (% citric acid)	5.5±0.00
Humidity (%)	75±0.5
Ash (%)	0.8±0.2

From the physicochemical characteristics, we see that the formulated cream keeps the characteristics of the raw material (root pulp).

The chemical composition of the cream obtained from the rhizome of *Atractylis Gummifera* L, is not studied yet, only a few studies have been carried out on their aqueous extracts, and have focused on the chemical composition and toxicological effect. However, some work [23] has been done on other species of the same family (Asteraceae), namely *crathamus ceaurulus* L, which whose cream has the same traditional uses as those of *Atractylis Gummifera* L.

The formulated cream is slightly acidic, which is very suitable for a dermatological product. Indeed, the pH of normal skin varies between 4.0 and 7.0 [29]. The skin's surface is slightly acidic (pH 6 approximately), which plays a role in the defense of the epidermis against bacterial aggression attack [30]. This acidic pH gives them a bacteriostatic action by inhibiting the development of pathogens that can harm the tissues, thus accelerating healing by preventing the onset of a concomitant infection [31].

The pH of the formulated cream is in agreement with the results found in other studies [20, 32], for healing ointments and creams.

III.3.2.Organoleptic characteristics

The examination of organoleptic characteristics constitutes the first approach to the quality of a preparation, and must be thoroughly investigated by the manufacturer in order to observe any anomalies. The organoleptic characteristics focused on the determination of appearance, color, odor and homogeneity.

The results show that the formulated cream has the following characteristics:
Appearance: shiny and homogeneous, thick with no lumps when spread;
Color: light beige;
Odour: balsamic.
According to Lefranc E, 1866 [8] , the underground parts give off a slight balsamic odor, somewhat nauseating; the flavour, initially sweet and mild, then hot and bitter, leaves a very persistent sensation of pungency in the aftertaste.

Homogeneity: macroscopically, we checked the homogeneity of the cream by spreading a thin layer between glass blade and slat. We check with the naked eye the absence of aggregates and the good distribution of the cream. Our cream has no visible particles or lumps, so it is perfectly homogeneous.

III.4. Rheological study of the cream

Rheology is the science that studies and describes the flow, deformation, and rupture of matter under stress [33].

The study of stability and behavior of the creams during application are important factors for the acceptance by the subjects. The ingredients and the production process influence the viscosity of the cream. It is measured to check the product appearance and its behaviour on the skin [34,35].

The rheogram in Figure 7 indicates that the cream has a rheofluidic behaviour. This behaviour is very adapted to the Casson model (R 2= 0.9989)., which is described by the following equation (2): [33]

$$\eta = \eta_\infty + \frac{\eta_0 - \eta_\infty}{1+(\lambda\dot{\gamma})^n} \qquad (2)$$

Where: λ and n are constants; λ is a characteristic relaxation time, η0 and η∞ are the viscosities respective at zero and infinite shear.

Figure 7 shows the log-log rheogram of viscosity versus shear rate for the studied formulation.

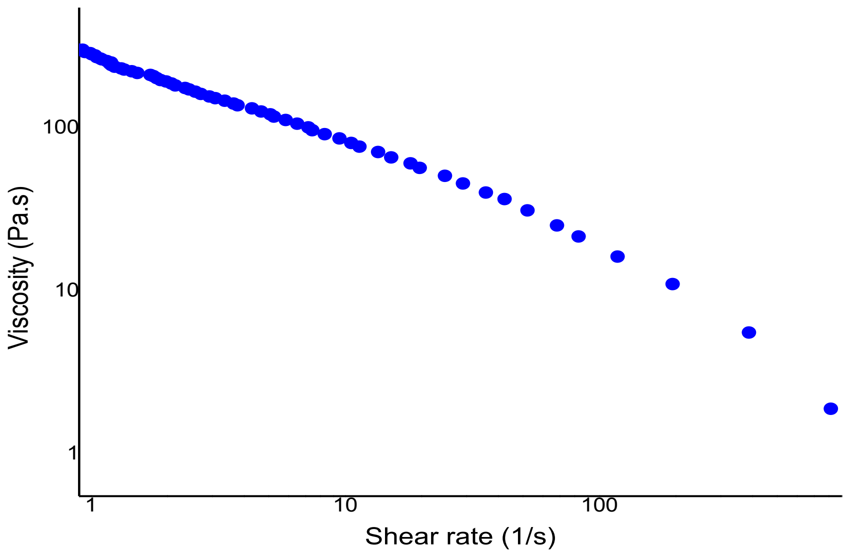

Figure 7. *Viscosity variation of the cream as a function of the shear rate*

At rest, the cream has a high viscosity (600Pa.s), which indicates its stability. According to these characteristics, our product remains stable at rest (in storage); at the same time it is easy to spread on the skin.
The formulated optimal cream has a high viscosity compared to that reported in other studies [32,36], where the viscosity of the creams varies from 360 to 450 cps and 290-480 cps respectively. This variation can be explained by the ingredients added to the formulation and the production process [34,35].

IV .Conclusion

Recalling that our work is part of the search for new formulations of natural origin in this case medicinal plants.
Thus, we are interested in studying the plant Atractilys Gummifera L, which is a plant species very well known for its various therapeutic virtues.

In this work, we have opted for the optimization of a cream formulation for dermatological application, obtained from the rhizome of *Atractilys Gummifera* L.; with the purpose of making available to the population, a new natural phytocream without any added additives.

The physicochemical characterization allowed us to determine the content of different components of the root namely: acidity (4.66%), water (65%), ash (4.21%), °Brix (33.34%), sugar (8.5%) and lipids (2.29%).

The optimization of the cream formulation was done with Modde 6 software. According to the results, it appears that the factors influencing the formulation of cream are: the speed and duration of agitation.
The physicochemical characterization reveals that the formulated cream has an acidity (4.00%) and a pH of 5.2 with a water content of (75%) and ash (0.8%).
The cream obtained has a rheofluidic (or pseudoplastic) behaviour, which can be described by the Casson model. This behaviour is suitable for the cream since it is easy to spread on the skin.
The study of the various parameters considered influential on the formulation process of cream, revealed that the optimal conditions for a more stable cream are: the solid (rhizome) / liquid (water) ratio 0.4, the stirring time (extraction) for 15 minutes and the stirring speed 500 rpm.
This study introduces a strategy for the development of a natural pharmaceutical formulation, which can potentially be used as an alternative to that of chemical synthesis.
As it happens in some African countries, our ambition is to develop an improved traditional Algerian medicine (**ITAM)**, as well as to valorize and promote the use of medicinal plants due to their richness in natural active biomolecules having made the object of clinical investigation.

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