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Extraction of Aromatic Compounds from *Dorema Ammoniacum* and *Styrax Benzoin* Resins Using Maceration Method

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A B S T R A C T

This study aims to create a perfume using *Dorema ammoniacum*, *Styrax benzoin*, and α-pinene. The maceration method in mineral oil was used to extract the aromatic substances from the resins at a room temperature. Both resins were grounded and soaked in mineral oil for three months. The *Dorema ammoniacum* resin soluble in polar solvents, so aromatic substances were extracted by soaking it in 10% ethyl alcohol for 2-3 days. The top note, α-pinene oil, a monoterpene, was purchased in its pure form and used as is. The weight percentages of the components used to prepare the aromatic formulation containing the carbopol gel are as follows: *Dorema ammoniacum* (Solution, 10% EtOH) (20%), Benzoin oil extract (10%), *D. ammoniacum* oil extract (10%), α-pinene oil (5%), Glycerin (5%), α-pinene oil (5%), 2% Carbopol® 940 Solution (5%), Polyoxyethylene sorbitan 60 (6%), Glycerol monostearate (3%), Disodium EDTA (0.2%), Sodium Salicylate (1.5%), and then the completion of the percentage with distilled water. The formulation is prepared with a total weight of 20 grams. The perfume was evaluated for its organoleptic evaluation, such as color, scent, and shape, and was found to have a distinct aroma, liquid consistency, and acceptable shape.

Introduction

Resins are natural organic materials that are solid or semi-solid, amorphous, fusible, and flammable. They are typically transparent or semitransparent and yellowish to brown. Resins are formed in plant secretions and can be dissolved in organic solvents, but not in water. These secretions occur in specialized structures found either inside or on the surface of various parts of plants (Pilato, 2010). Resins are categorized into two types based on their origin: natural resins and synthetic resins. These are organic compounds and high molecular weight synthetic polymers. They are created by combining carbon, hydrogen, oxygen, and sulfur atoms through specific chemical bonds. The molecular structure of synthetic resins can be classified into three geometric shapes: linear, branched chain, and cross-linked. Examples of synthetic resins include rubber and polyethylene (plastic). Resins are commonly used in building decoration materials, natural fiber composites, adhesives, manufacturing fluorescent threads, water-resistant materials, and coating materials (Zhang, 2011).

Natural resins are derived from sticky substances secreted by the bark of trees and the stems of certain plants around the world. When exposed to air and sunlight, natural resin dries and hardens, often forming irregular masses or tear-shaped formations. Resins are collected directly from trees, such as frankincense, are considered modern resins. Some fossilized resins, like amber, are collected from the ground and are known as gemstones. Examples of natural fossilized resins include African copal gum and New Zealand gum. Resins produced in a different natural way with essential oils, containing a large amount of oils, are called oleoresins (Ali, 2020). There are various natural

resins, each derived from different sources. Examples of these resins include frankincense, balm, Gilead balm, amber, benzoin, mastic, and copal (Yogi *et al*., 2020).

Benzoin resin is a natural, complex balsamic gum resin obtained from the bark of the yan tree, also known as *Styrax benzoin*. The tree is native to Indonesia, Malaysia, and Thailand, and the resin is harvested by making incisions in its bark. The key compounds found in benzoin are cinnamic acid, benzaldehyde, vanillin, and benzyl benzoate. Benzoin gum is used in a wide range of cosmetics and aromatherapy products. It is a popular ingredient in perfumes as it helps slow the evaporation of fragrance and essential oils (Coppen, 1999). A previous study indicated that the chemical components of the benzoin plant are distinct from other traditional Chinese herbs. Benzoin contains various chemical components, including balsamic acid esters, peels, terpenoids, and sesquiterpenes, in addition to cinnamic acid, benzoic acid, and their derivatives, which are the most abundant components in benzoin (Chen et al., 2023).

In this study (Azzazy et al., 2022), the researchers extracted resin oil using hydrodistillation in a furnace equipped with two 950-watt magnetrons and an infrared temperature sensor. They placed the resin in distilled water at a ratio of 1:5 (weight/volume) and mixed it using an electromechanical motor at 100 degrees Celsius. The extraction process using the cooling water took 45 minutes, and the resulting liquid was collected in a 1 L bottle.

In other study (Susanti et al., 2023), an 80-mesh benzoin sample weighing 400 grams was extracted using the soaking method with ethanol at a 1:2 ratio. The extraction process involved stirring periodically for 3 x 24 hours at room temperature (RT). After filtering the solution, the resulting filtrate was washed with hexane to collect the polar part, followed by evaporation. Subsequently, the part was examined for the content of the secondary metabolite. The study results revealed that the ethanolic extract of incense contains 40% volatile compounds and 70% non-volatile compounds.

In 2023, Qingqin et al studied the effectiveness of benzoin oil for medical purposes, including resuscitation, regaining consciousness, stimulating blood circulation, alleviating pain, and reducing loss of consciousness resulting from stroke. According to modern pharmaceutical research, benzoin oil contains antimicrobial, anti-inflammatory, and anti-tumor activities. It has been found to be effective in treating various diseases and conditions such as fetal death, exposure to poison, cholera, and rheumatism. Additionally, it may help with psychological problems such as nightmares, insomnia, and fatigue. Benzoin oil is comprised of 95 herbal medicinal recipes; it contains many aromatic compounds (Fig. 1). Other study involved subjecting 300g of air-dried, finely ground oleoresin (80 mesh) to separate water and steam distillation processes, each lasting 3 hours. The resulting essential oils were dissolved over anhydrous sodium sulfate, filtered, and stored at 4 °C. To ensure consistent results, the extraction process was repeated five times, as described in the study by Ayub et al (2018).

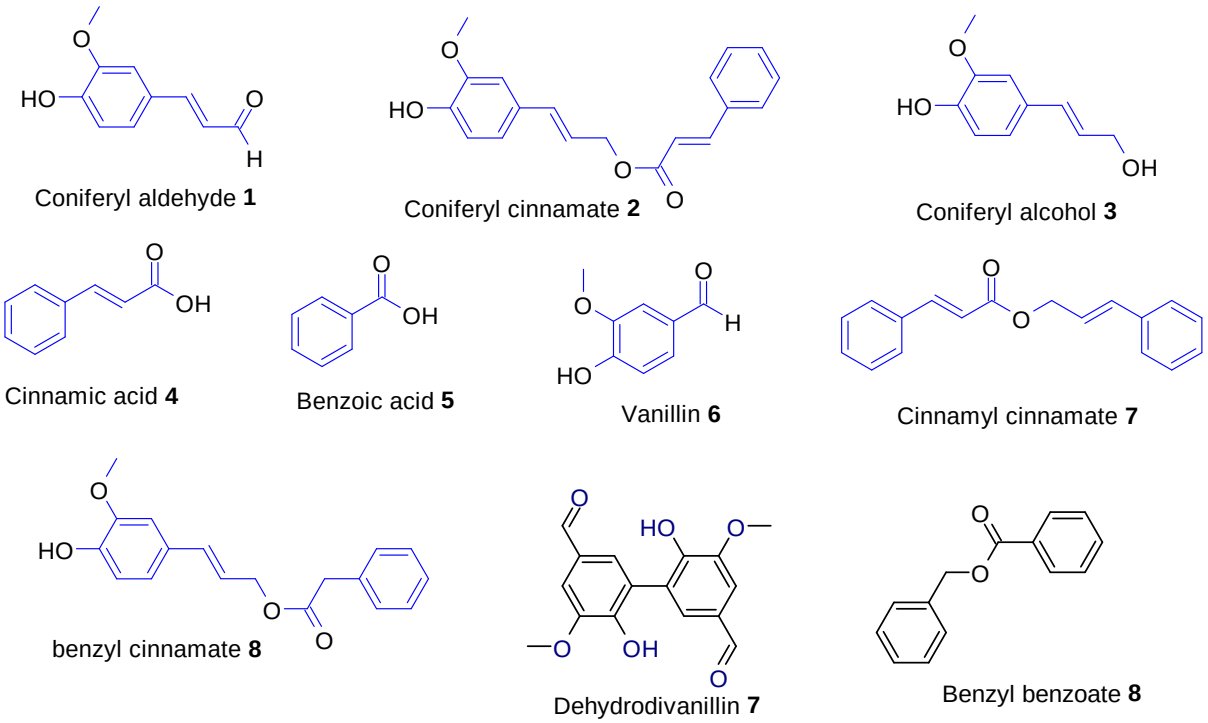


Figure 1: *Chemical structures of some active aromatic compounds that isolated from B. styrax*

In a study (Rajani et al., 2002), the ripe fruits of *D. ammoniacum* collected at deciduous time were subjected to hydrodistillation to produce essential oil which was subsequently analyzed by GC/MS. The oil was tested against cancer cells, and based on these results, *D. ammoniacum* essential oil has antimicrobial activity and low cytotoxic activity providing a scientific basis for its traditional use. In a study (Norani, et al., 2023), *D. ammoniacum* was collected from the southwestern part of Iran and the content of essential oils (EOs) and their compositions were examined using gas chromatography techniques. The main compounds of the volatile oils in the resin of *D. ammoniacum* as major compounds are (Fig. 2): (2E, 6E)-farnesol **9** (12.2%), cuparene **10** (11.5%), *β*-bisabolene **11** (6.1%), and (2Z,6Z)-farnesol **12** (8.7%).

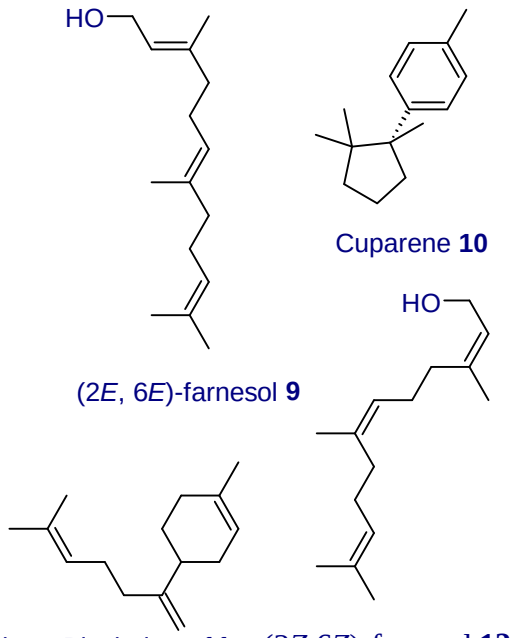


Figure 2: *Chemical structures of some active aromatic compounds isolated from D. ammoniacum (Norani et al., 2023).*

The maceration method is a simple and cost-effective traditional extraction method. It requires only a basic container for extraction, but it does take a long time (Tambun et al., 2021). This process involves soaking plant material in a closed container with a solvent at RT for 2-3 days, with regular stirring to obtain plant extracts. The goal of this method is to soften and break down plant cell walls to release soluble plant components. After a specific period of time, the mixture is pressed or filtered using filtration or decanting (Stéphane et al., 2021). Maceration is the simplest and most widely used procedure, working on the principle of molecular diffusion, which is time-consuming (Fig. 3). This method ensures that the concentrated solution accumulates around the surface of the particles for further extraction (Stéphane et al., 2021).

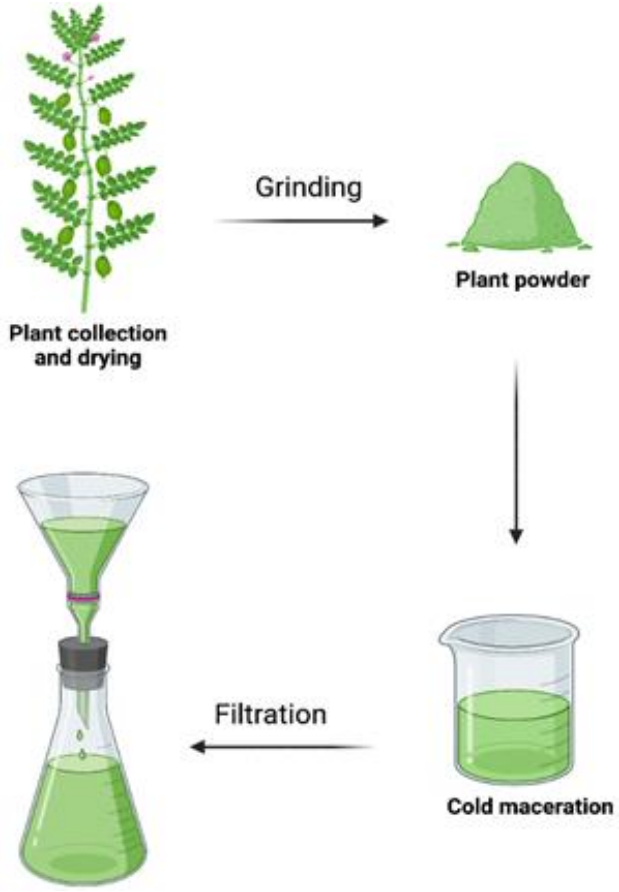


Figure 2**:** *maceration method of extraction*

Materials and Methods

2.1. Chemicals and equipment:

Glycerine (98%) was bought from Fluka chemika. (1*S*)-(-)-α-Pinene (99%), Polyoxyethylene sorbitan 60, Sodium salicylate, and Carbopol® 940 were purchased from ACROS ORGANICS. Disodium EDTA and KOH were bought from Merk. Ethanol (96%) was bought from Freeman's (FreemanSupply.com). All used natural resins; frankincense, benzoin and mastic were purchased from local distributors in Libya (Fig. 4). Mineral oil was purchased from Sigma. Samples

mixing were carried out using Vortex mixer (Bio Cote). PH measurements were performed using a pH Benchtop meter (Orion 2 star, Thermo Scientific).

Figure 3: *Photos of the resins used.*

2.2. Preparation of potassium hydroxide solution (0. 0.5 M KOH):

A precise amount of 7.01 g (0.125 moL) of KOH was weighed in a glass beaker and dissolved in 50 ml of distilled water, then transferred to a clean 250 ml volumetric flask, where the volume was adjusted to the calibration mark with additional distilled water. The compound was allowed to completely dissolve in distilled water with gentle shaking, and stored at RT for future use.

2.3. Preparation of 2% Carbopol® 940 polymer gel:

The gel was prepared by dissolving 2.0 g of the Carbopol® 940 in 50 ml of distilled water and shaking it thoroughly. Next, 20 ml of a potassium hydroxide solution (0.5 M KOH) was added to adjust the pH to 7.4. Finally, the solution was topped up to 100 ml with continuous stirring to ensure a homogeneous gel was obtained (Fig. 5).

Figure 4: *2% of the prepared Carbopol 940 polymer gel.*

2.4. Extraction of resinous aromatic content by cold maceration in mineral oil:

To obtain the aromatic extract using the maceration method, both *S. benzoin* and *D. ammoniacum* resins were finely ground. Separately, 500 mg of each resin was weighed and transferred to a 50 ml volumetric flask. The volume was then filled to the mark with mineral oil. This mixture was left to infuse at RT for approximately three months. Finally, it was filtered through filter paper to remove any unwanted residues. Note that two types of *D. ammoniacum* resins, red and gray, were used in this process (Fig. 6).

Figure 5: *Maceration of aromatic extracts in mineral oil.*

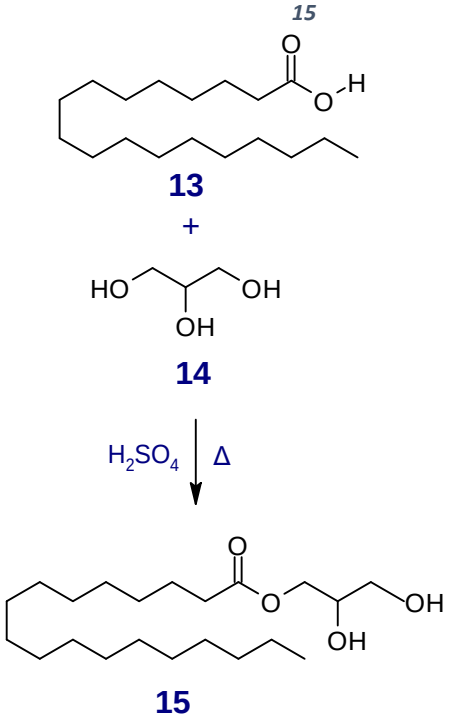
2.5. Extraction of resinous aromatic content by maceration in 10% EtOH:

To obtain the aromatic extract of *D. ammoniacum* through maceration, the resin was finely ground, and then 500 mg of the ground resin was weighed, and transferred to a 50 ml volumetric flask. The volume was then filled to the mark with 10% ethanol. The mixture was left to infuse at RT for approximately 3 days. Subsequently, it was filtered using filter paper to remove any unwanted residues.

2.6. Fisher esterification to prepare glycerol monostearate 15

Stearic acid **13** (15 g, 0.053 moL) and glycerol **14** (4.9 g, 0.053 moL) were added to a 250-mL two-necked round bottom flask equipped with a magnetic stirrer and a reflux condenser. After that, chloroform (10 mL) was added as a solvent, and then drops of sulfuric acid were added to them as a catalyst. The reaction flask is heated for 2 hours (start timing when the reaction mixture started to boil) under refluxing. The mixture was cooled, washed with 5% sodium bicarbonate (20 ml) (2 x) to neutralize the acid from the reaction medium. The participant is filtered and left to dry in vacuum to give the desired product **15** (17.0 g, 89.9%) as white solid: Mp 66-68 °C, lit. value 57-66 °C (Averill et al., 1929).

***Equation 1**: practical preparation of glycerol monostearate 15*



2.7. Preparation of the aromatic composition:

The weight ratios of the components of the aqueous and oily phases were calculated and summarized in Table 1.

***Table 1:** shows the weight ratios of the materials used in preparing the composition with the consistency of the aromatic gel (o/w).*

Phase	Ingredient Name	%W/W
A (aqueous phase)	Distilled water	36.8
	Glycerin	5.0
	Dorema ammoniacum (Solution, 10% EtOH)	20.0
	Disodium EDTA	0.20
	Sodium salicylate	2.0
B (oil phase)	α-pinene oil	5.0
	Benzoin oil extract	10.0
	D. ammoniacum oil extract	10.0
	Polyoxyethylene sorbitan 60	6
	GMS	3
C (gel)	2% Carbopol® 940 gel solution (aq.) (Carbopol, KOH)	5

The formulation was performed according to the following steps:

i. The gel emulsion was prepared by distributing Carbopol 940 polymer (2% of Carbopol polymer gel) in distilled water by stirring until homogeneous.
ii. The contents of the aqueous phase were weighed and then added to the components of step 1 and then mixed well with the addition of Sodium salicylate and Disodium EDTA to the aqueous phase.
iii. The blend of emulsifying agents (Polyoxyethylene sorbitan 60 and GMS) were weighed and mixed well.
iv. The contents of the oil phase and the emulsifying agents were weighed and mixed well.
v. The components of the aqueous phase were heated in beaker 1 and the components of the oil phase were also heated in beaker 2 and both were heated in a water bath to a temperature of about (75 °C) . The components of the aqueous phase were slowly added to the components of the oil phase and the mixture was stirred well until it was mixed together on the hot and a shaker was used for this purpose.
vi. The resulting perfume was stored in a tightly closed container at RT.

3. Discussions:

3.1. Components of the aromatic composition

The perfume consists of scents that are aromatic compounds, essential oils, solvents and fixatives. The components were chosen to include the volatile oil with a terpene structure (α-pinene), which represents the top notes as well as the aromatic resins. Summary of the components of the aromatic composition is shown in Fig 7.

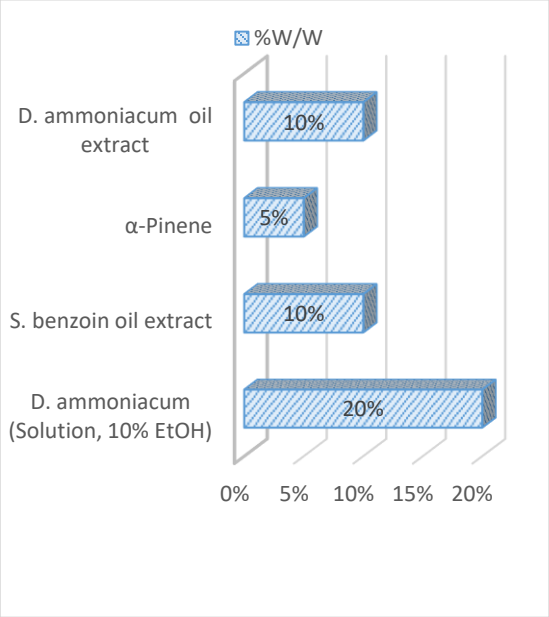


Figure 7: *shows the proportions of the aromatic components used in preparing the composition*

These resins were chosen because they are one of the medicinal and aromatic resins that are widely available in the local market and have been used since ancient times due to their distinctive scents and because they are rich in many aromatic compounds that can be used in preparing many preparations. The aromatic composition consists of the materials shown in Table 2.

Table 2: *The materials included in the aromatic composition with a mention of the function of each.*

Component Name	Function
Distilled water	Solvent
Glycerin	Non-ionic humectant
S. benzoin	Aromatic resin polymer
D. ammoniacum	Aromatic resin polymer
α-pinene oil	Oily fragrance
Ethyl alcohol	Solvent
Polyoxyethylene sorbitan 60	Non-ionic surfactant
Disodium EDTA	Chelating agent Contributes to stabilization of different phases
Sodium salicylate	Preservative
Mineral oil	Emollient
Carbopol® 940 gel	Gelizing agent

The chemical structures of some of the ingredients in the formulation are shown in Fig. 8

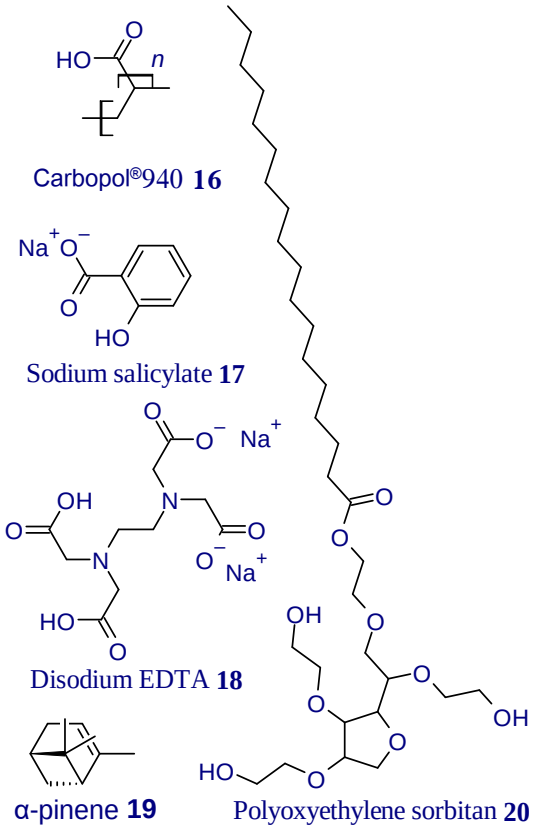


Figure 8*: Chemical structures of the ingredients in the aromatic formulation*

3.2. Calculations of required hydrophilic-lipophilic balance (RHLB) values

In this study, the RHLB of each component of the oil phase was calculated as a percentage of the total components of the oil phase to determine the required HLB value for the formulation (Jessicawieland 2023; Leal et al., 2023). The total percentage of the components of the oil phase in the formulation was calculated as follows: 10 + 10 + 5 = 25.0%.

Mineral oil extract of *D. ammoniacum* 10/25 x 100 = 40.0%

Mineral oil extract of *S. benzoin* 10/25 x 100 = 40.0%

α-Pinene oil 5/25 x 100 = 20.0%

The required RHLB for each component in the oil phase was calculated using the following equation:

$$RHLB_i = \mathrm{HLB}_i \times f_i$$

Where fi is the mass fraction of the oil i; HLBi is the previously calculated and referenced value for the fatty substance. For example, the RHLB values were calculated for the mineral oil extract:

$$\mathbf{RHLB_{mineral\ oil\ extract} = (40.0/100)\ x\ 10.5 = 4.20}$$

And so on for the remaining components. The calculations are summarized in Table 3.

Table 3: *Table (3) shows the calculation of the required hydrolipid balance (RHLB) value for oil phase materials in the preparation of gel-like formulation of fat-in-water emulsion (o/w).*

Oil-phase ingredients	%W/W	HLB of the ingredient	RHLB contribution to the formulation	Reference for HLB_i
Mineral oil extract of *D. ammoniacum*	10.0	10.5	4.2	Kruglyakov, 2000
Mineral oil extract of *S. benzoin*	10.0	10.5	4.2	Kruglyakov, 2000
α-Pinene	5.0	13.0	2.6	(Brookhaven Instruments, 2023)

The required RHLB for blended oils was calculated using the following equation (Pasquali, et al., 2008):

$$\mathrm{HLB}_{required} = \sum \mathrm{HLB}_i \times f_i$$

Where fi is the mass fraction of the oil i.

$$\mathrm{HLB}_{required} = 4.2 + 4.2 + 2.6 = 11.0$$

3.3. Selection of surfactant combination for a target HLB value

Often, a surfactant is used in combination with two or more other surfactants (usually of different weights rather than a single surfactant itself). Combining surfactants results in better interfacial mixing and better physical stability of emulsions. The surfactant must be chosen to match the target RHLB value calculated above. Among the surfactants available to us with an HLB value of (14.3) are: Polyoxyethylene sorbitan 60 (Fig. 8), and its HLB value is 14.9 (Table 4), which is close to the calculated value.

Table 4: *Properties of the surfactant for the fat emulsion in water (o/w).*

Oil-phase ingredients	%W/W	HLB of the ingredient	Reference for HLB value	RHLB contribution to the formulation
Polyoxyethylene sorbitan 60	6.0	14.9	Kruglyakov, 2000	9.93
GMS	3.0	3.8	Lipophilic emulsifying agent	1.27

$$\mathrm{HLB}_{required} = 9.93 + 1.27 = 11.2$$

3.4. Study of the solubility of aromatic resins

The solvents used to test the solubility of aromatic resins, after they were finely ground, included water, alcohol, and a glycerol-water mixture. The results indicated that *D. ammoniacum* exhibited the highest solubility, while *S. Benzoin* showed the lowest solubility. These findings are summarized in Table 5.

Table 5*: Testing the solubility of aromatic resins and the surfactant.*

Solvent / Component	EtOH (RT)	Warm EtOH	H_2O (RT)	Warm H_2O	10% Glycerol (RT)
S. Benzoin	low solubility	low solubility	low solubility	low solubility	low solubility
D. ammoniacum	soluble	soluble	soluble	soluble	soluble

3.5. Preparation of the aromatic composition:

In order for these solutions to be safe for the skin, a diluted solution was chosen from them to match the amounts of aqueous extracts that used in the manufacture of aromatic materials. All extracts from F_1 to F_3 showed distinct aromatic odors (Table 6).

***Table 6**: shows the weight ratios of the samples under study (F_1-F_3).*

		F_1	F_2	F_3
Phase	**Ingredient Name**			
A (aqueous phase)	Distilled water	to 100	to 100	to 100
	Glycerin	5.0	5.0	5.0
	D. ammoniacum (Solution, 10% EtOH)	.....	20.0	20.0
	D. ammoniacum resin	5	……	……
	Disodium EDTA	0.1	0.20	0.20
	Sodium salicylate	2.0	2.0	2.0
B (oil phase)	α-pinene oil	5.0	5.0	5.0
	Benzoin oil extract	10.0	10.0	10.0
	D. ammoniacum oil extract	10.0	10.0	10.0
	Polyoxyethylene sorbitan 60	5.0	6	6
C (gel)	2% Carbopol® 940 gel solution (aq.) (Carbopol, KOH)	…..	…..	5

3.6. Determination of pH

500 mg of the sample were accurately weighed and dispersed in 50 mL of water. The pH of the mixture was adjusted to 6.9 at 25°C using a digital pH meter.

3.7. Formulation

The fragrance ratios used in the aromatic composition are 45% as shown in Table 7. So the perfume can be classified as a type of perfume extract.

***Table 7**: Components of the perfumery formulation.*

Phase	Ingredients	%W/W
A (Water phase)	Distilled water	36.5
	Glycerin	5
	D. ammoniacum (Solution, 10% EtOH)	20
	Disodium EDTA	0.2
B (Oil phase)	α-pinene oil	5.0
	Benzoin oil extract	10.0
	D. ammoniacum oil extract	10.0
C (Emulsifying blend)	GMS	3.8
	Polyoxyethylene sorbitan 60 (tween 60)	6.0
D	Sodium salicylate	1.5

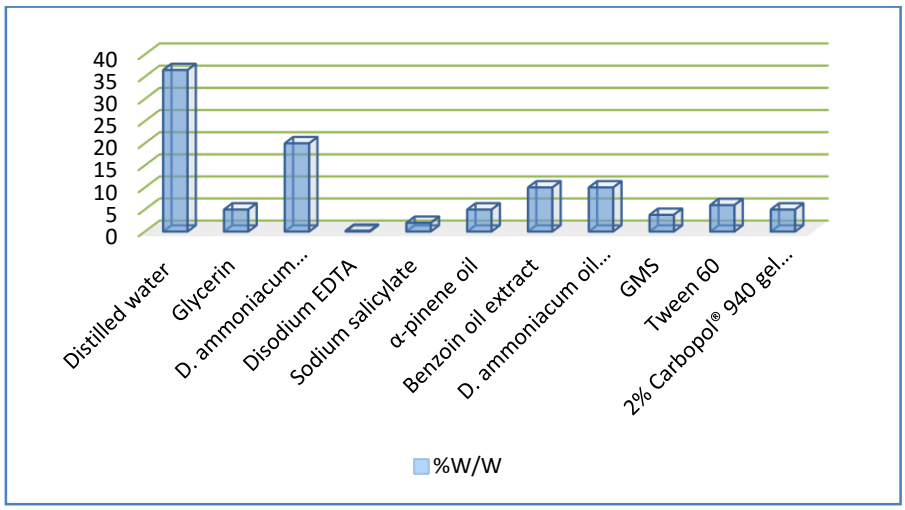


***Figure 9**: the weight ratios of the ingredients involved in the preparation of the perfumery formula.*

3.8. Organoleptic Evaluation

The perfume was evaluated for its sensory properties such as color, smell and flow. The results are listed in Table 8. The smell was beautiful and concentrated, dominated by the smell of *D. ammoniacum* and α-pinene oil.

Table 8: shows the summary of the organoleptic evaluation.

Property	Characteristic
Shape	Medium consistency liquid
Color	White
Smell	Pungent and distinctive
Structure	White liquid

4. Conclusions

The results indicate that the concentrations of both resins in the oil medium produced distinct fragrances, making them suitable for use as perfumes. The reason may be attributed to the fact that the solubility of the aromatic materials in the oil was more soluble, noting that the method used depends entirely on the smells of *D. ammoniacum* resin, benzoin, and α-pinene oil as top and middle notes. The advantages of the infusion method used in this study are that it is simple, inexpensive and easy to apply, so it can be used as a method for preparing perfumes.

5. Recommendations

It is recommended to continue research in the field of testing the effectiveness of aromatic resins as top, middle and base notes because they are rich in aromatic materials, as well as extracting volatile oils from them and studying them.

Conflict of Interests:

The authors declare that there is no conflict of interests regarding the publication of this paper.

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