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A new chemotype of *Cananga odorata* flowers for improved extraction of essential oils with bioeconomic perspectives in Eastern Africa

Léopold Havyarimana[1]* and Steve De Cliff[2]

[1,2] Department of Chemistry, Faculty of Sciences, University of Burundi, 2 Avenue de l'Unesco, P.O. Box 2700, Bujumbura, BURUNDI
**Corresponding Author: e-mail: leopold.havyarimana@ub.edu.bi - Tel: +257-79 90 88 73 or -69 72 04 62*
ORCIDs: https://orcid.org/0009-0008-7616-9247 (Havyarimana), https://orcid.org/0009-0003-6390-7152 (De Cliff)

Abstract

Due to its geographical position, Burundi benefits from several soil genesis factors and large variations in microclimates to which are added water resources, which give to Burundi a floral heritage of great diversity. This study therefore aimed to assess the physicochemical properties (density, refractive index, acid index and ester index) as well as the associated organoleptic characteristics (odor, color and appearance), of the essential oil extracted by hydrodistillation from fresh flowers of *Annonaceae* family tropical trees C. Odorata, a chemotype cultivated and grown in Bujumbura, Burundi. Analyses of physicochemical properties values were done and compared to reference values. It appears that for the refractive index, density and acid number, the findings of this study are within the pre-established standards. More interesting, the value of ester number was very beyond the norm by a factor of almost two, breaking the record already registered by AFNOR, therefore suggesting of an essential oil of exceptional quality. The findings presented in this study also highlight the importance of C. Odorata chemotype from the plains of Burundi as a promising potential candidate for bio-based products of public interests such as bio-based cosmetics and bio-based pharmaceuticals products, and therefore offer new insights bioeconomics in health and well-being. Finally, this plant can easily be cultivated, not only in Burundi, but also in countries of the Eastern Africa that share the same geoclimatic conditions as the Great African Rift.

Keywords: Oil Extraction; *Cananga odorata*; Hydrodistillation; Physico-chemical Properties; Burundi.

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1. Introduction

Cananga odorata (Lam.) Hook.f. & Thomson is a perennial tropical tree of the Annonaceae family. It exists in two forms, the genuine form and macrophylla form (Stashenko *et al*., 1996; Kristiawan *et al*., 2008), but only the genuine form is called ylang-ylang (Benini *et al*., 2010). It grows equally well in an equatorial climate as in a maritime subtropical climate. It is found in tropical rainforests and semi-dry forests. It is found at altitudes varying from sea level to 800 m and sometimes up to 1200 m near the Equator. Annual water requirements are 1500 to 2000mm, but the tree supports average annual precipitation ranging from 700 to 5000mm, although it tolerates short periods of drought (less than two months) (Chalot, 1928; Guenther, 1952; Manner *et al*., 2006). Cananga Odorata is one of the odoriferous plants for which more scientific research information is available on a range of its chemotypes. The data available on this plant indicate that the plant is native to the Moluccas Islands, an Indonesian archipelago. It was introduced to the western islands of the Indian Ocean in 1770. However, the time or how it was introduced to Burundi from the Southeast Asia region is unknown. C. Odorata has been exploited to produce ylang-ylang essential oils since the early 1900s. Currently, the main producers are the Union of the Comoros, Madagascar and Mayotte. Its essential oil is obtained by hydrodistillation or steam distillation from its fresh and mature flowers; it is mainly used in the cosmetics industry where it appears in the formulation of high-quality perfumes and major international brands. (Benini *et al*., 2010; Burdock *et al*., 2008; Guenter, 1952; Manner *et al*., 2011).

The essential oil of ylang-ylang is divided into five fractions (or qualities) with different physicochemical properties: the Superior Extract (SE), the Extra (E), the First (I), the Second (II) and the Third (III). The SE fractions, E and I constitute the "top" fractions because they are the first to be obtained during distillation. Fraction II forms the "body" fraction and fraction III constitutes the "tail" fraction. The technique used to obtain these fractions is purely empirical. It depends on the elapsed distillation time, the volume of oil obtained and its density. The essences SE and E are distilled within the first two hours. Again, 2 to 3 hours later, fraction I is obtained. Fraction II is obtained between 1 to 2 hours. It is then necessary to open the cucurbit in order to add water and allow the distillation to continue. The essential oil then collected is fraction III, called the "tail" fraction. Generally, a distillation of ylang-ylang lasts from 20 to 24 hours. Fresh flowers contain 2 to 2.5% essential oil. It is therefore necessary to distill 4 to 5 kg of fresh flowers to obtain 100 mL of essential oil of ylang-ylang (all fractions combined). The essential oil obtained by unfractionated distillation (all fractions combined) is called "complete". This study concerns the "complete" fraction.

The trade in the ylang-ylang essential oil has long time been the primary source of income in the Union of Comoros with $7 913 000 in 2008, compared with $5 530 000 for cloves and $2 489 000 for vanilla (FAOstat, 2011). It has a great olfactory richness and is intended for the formulation of luxury perfumes, mass perfumery, the manufacture of cosmetic products, detergents, deodorants and soap (Florence, 2005; Raymond, 2009; Manner, 2006). However, despite its great economic importance, at least for these three producing countries, researchers like Benini *et al*. (2010) were quick to realize that the plant was in fact little known. This lack of investigations, in particular to evaluate the chemical polymorphism of the different production zones, therefore represented obstacles to resolving the problems of the sector, which put it in danger. Furthermore, this plant was not the subject of any improvement program, even though it generated a product with very high added value, probably due to the fact that the biology of its reproduction was little known. This is how an in-depth study of the plant and its essential oil made it possible to obtain the information that was then lacking in order to help the sector solve its problems, maintain itself, and even develop. Studies relating to the variation in the chemical composition of four fractions of essential oils extracted from C. Odorata, cultivated in four localities Grande Comore, Mayotte, Nossi-Bé and Ambanja, were studied and a total of 119 compounds, representing 85.7 to 96.4% of the total composition of essential oils were identified qualitatively and quantitatively by gas chromatography and mass spectrometry (Benini *et al*., 2012). The ISO 3063:2004(E) standard published by the Association Française de Normalisation and known as AFNOR 2005, is considered to be the reference by professionals in the fragrance industries (Surburg *et al*., 2006). It distinguishes two essential oil types based on origin: Madagascar essential oil and Comores/Mayotte essential oil.

A comprehensive literature review on the subject, as well as a concise summary providing comprehensive information on the traditional uses, phytochemistry and biological activities of C. Odorata, were presented by Bey Hing Goh *et al*. (2015), who demonstrated how the benefits of C. Odorata plant go far beyond a simple raw material for the perfume industry for which it is much better known, but also for medicine and, to some extent, agriculture (Goh *et al*., 2015). Indeed, the aromatic substances secreted by C. Odorata flowers contain interesting biological activities, ranging from antimicrobial, anti-inflammatory, hemostatic, healing activities to name just a few (Lis-Balchin *et al*, 2002; Christaki *et al*., 2012). Indeed, ylang-ylang essential oil has multiple medicinal properties (Christaki *et al*., 2012; Goh *et al*., 2015; Tan *et al*., 2015) some of which should be mentioned here: it would be an excellent reducer of hyperpnea and tachycardia. The essential oil of C. Odorata is also appreciated for its qualities of antidepressant, antibiofilm, antioxidant, antimelanogenesis, anti-inflammatory, hypotensive, sedative, antiseptic for the intestinal tract and its beneficial influence on blood circulation problems (Goh *et al*., 2015). It is a respiratory calming agent, asmatiform bronchitis, cardiac calming agent, antiarrhythmic, antispasmodic, extrasystolic palpitation, tonic, intellectual and sexual stimulant, sexual asthenia, impotence, frigidity, aphrodisiac, cell regenerator, skin and hair tonic of all types, eczema, asphyxiated or tired skin, dermatitis of psychosomatic origin, pruritus, prurigo, urticaria, pityriasis, scabies, radiotherapy, antiseptic, virucidal, action on intestinal parasites, seboregulatory, antidiabetic, contracture and muscle cramps, cystitis, urethritis, gynecological spasm, analgesic. Complete ylang ylang essential oil is particularly suitable for aromatherapy (Goh *et al*., 2015; Hongratanaworakit *at al*., 2004). All this range of biobased products and multiple uses of C. Odorata, which occur in different aspects of daily life, suggest the possibility that this plant could be part of a sustainable circular industry within the current concept of bioeconomy (EASTECO, 2022; Virgin *et al*., 2022). Burundi has an enormous bioeconomic potential favored by a climate and rainfall that are ready for all crops throughout the year. In Burundi, in addition to sugar cane, the other plant which has been regionally granted the status of bioeconomic plant is catnip (Nepeta cataria L., Lamiaceae) whose essential oils extracted from this plant also have multiple uses including that of having repellent activity against mosquitoes (Virgin *et al*., 2022).

The present study started from the observation that the essential oils market in Burundi is a sector that has not yet been exploited. However, due to its geographical position, Burundi benefits from several soil genesis factors and large variations in microclimates to which are added water resources, which give to Burundi a floral heritage of great diversity (Figure 1). This study therefore aimed to assess the physicochemical properties (density, refractive index, acid index and ester index) as well as the associated organoleptic characteristics (odor, color and appearance), of the essential oil extracted by hydrodistillation from fresh flowers of *Annonaceae* family tropical trees C. Odorata, a chemotype cultivated and grown in Bujumbura, Burundi.

2. Materials and Methods

2.1. Sample Collection

In this study, the ylang-ylang essential oil has been distilled from the mature fresh flowers of the *Annonaceae* family tropical tree *C. Odorata*. Fresh flowers of C. Odorata used in this study were collected from two plants located in the lot of one of us, living down town in Bujumbura, the economical capital of Burundi. The flowers used for hydrodistillation were picked from these two trees between 6 a.m. and 7 a.m. Typical mature fresh flowers of C. Odorata are shown on Figure 2. The flowers were mixed and kept in a room at an ambient temperature of 22°C. The actual distillation process began always around 9am.

2.2. Distillation Process

The hydrodistillation of C. Odorata flowers was carried out in the CRUPHAMET laboratory of the Faculty of Sciences of the University of Burundi. Hydrodistillation products were classically obtained using equipment based on the circulatory distillation approach introduced by J.F. Clevenger in 1928 (Figure 3). Operation modes to obtain distillation are well established and are described in several pharmacopoeias (Eur. Pharm., 2008). Three distillations were carried out according to the process adapted and used by Benini *et al* (2012). One hundred grams (100 g) of ylang-ylang flowers were introduced into a 2000 mL flask to which 1000 mL of distilled water was added. The flowers were put in the water at 70 C and then bring to the boil. After the material to be hydrodistilled was introduced into the distillation flask, a heating system was turned on and a condenser was started by adjusting the water flow. With the tank heater and refrigerant on, the water flow was adjusted. The condensed steam thus obtained gives rise to an organic phase (essential oil) made of a mixture of the four fractions combined together and which is separated from the hydrosol by decantation. In principle, the essences start to be distilled within the first two hours, but water was added and the distillation was let to continue for at least 10 hours. The distillate thus obtained was introduced into a separator funnel into which 100 mL of salt water was added. The solubility of oil in salt water being very low, salt water was added to separate the oil and water. The essential oil thus found was dried over sodium sulfate for 24 hours.

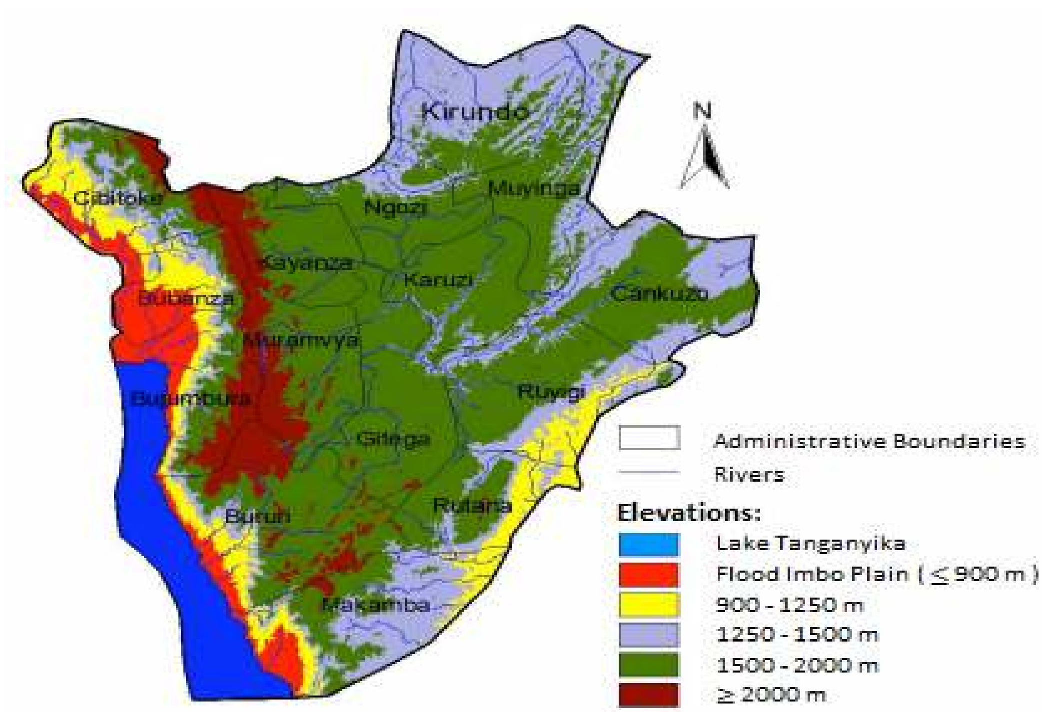

Figure 1: Map of the eco-climatic regions of Burundi, including the flood of Bujumbura in the plain of IMBO

Figure 2: Typical mature fresh flowers of C. Odorata

2.3. *Organoleptic and Physiochemical Properties Determination*

The analytical study of the essential oil obtained in this study concerned its organoleptic characteristics, namely the aspect, the color and the odor which were quite simply noted. Other determinations concerned the measurement of its physical quantity such as the yield (Eq. 1), the density (Eq. 2) and the refractive index (Eq. 3); and the calculation of the physicochemical properties such as the acid number (Eq. 4) and the ester number (Eq. 5).

2.3.1. The yield

The yield was obtained by considering the ratio of the quantity of oil collected after hydrodistillation to the quantity of biomass, expressed as a percentage.

$$\rho = \frac{m_{eo}}{m_{df}} \times 100 \quad (1)$$

where $\boldsymbol{\rho}$ is the yield, $\boldsymbol{m_{eo}}$ the mass of the essential oil, and $\boldsymbol{m_{df}}$ the mass of distilled flowers.

2.3.2. The density

The density of the essential oil was obtained as the ratio of the mass of a certain volume of the oil at 20 °C to the mass of an equal volume of distilled water at 20 °C (Dumortier, 2006). The density was corrected by taking the temperature into account according to the following formula (Fauconnier, 2006):

$$\mathrm{d_{20}} = \mathrm{d_{mes}} + (\mathrm{t_{samp}} - 20) \times 0.00073 \quad (2)$$

Where $\mathrm{d_{20}}$ is the desired density, $\mathrm{d_{mes}}$ the measured density and t_{samp} the temperature of the sample.

2.3.3. The refractive index

The refractive index is the ratio between the sine of the angles of incidence and of refraction of a light ray of determined wavelength, passing from the air in the essential oil maintained at a constant temperature (AFNOR, 2005). This is carried out by using a refractometer, an instrument designed to measure an optical constant, which is a characteristic of the material being examined. This optical constant is known as "Refractive Index" and can be used to give valuable information about the material being tested. In this study, the refractive index was measured by using the ABBE refractometer, Bellingham-Stanley model. The fundamental definition of a refractive index is based on the speed of light. Light travels at a constant speed in a vacuum, but the speed is reduced when the light passes through any other medium. The ratio of these two speeds is the refractive index of the medium:

$$RI = \frac{S_V}{S_S} \quad (3)$$

where **RI** is the refractive index, $\mathbf{S_v}$ the speed of light in vacuum, and $\mathbf{S_s}$ the speed of the light in the substance, here the essential oil of C. Odorata. The acid number is a very good index of preservation of essential oil, and corresponds here to the ratio between the mass of potassium hydroxide necessary to react with all the acids initially present in the oil and the mass of the sample of oil used for the experiment.

2.3.4. The acid numbers

The acid number of the essential oil is the number of milligrams of potassium hydroxide required to neutralize the free acids present in 1 gram of oil (OSMA, 1994; AFNOR, 2005). To determine the acid number, the ethanol was first neutralized with the potassium hydroxide solution, to make a 0.1M ethanolic potassium hydroxide solution. Then the ethanol was dosed with the potash until the solution turns green (qualitative characteristic of neutral pH). During the dosage of the acid by potash, ethanolic potassium hydroxide (neutralized with potassium hydroxide) plays the role of solvent for the fatty acids contained in the essential oil and the potassium hydroxide contained in the aqueous phase. In this study, 2g of essential oil was weighed in a beaker using the analytical balance. With a 10 mL graduated cylinder, 5 mL of neutralized ethanol was put in the beaker containing the oil and 3 drops of colored indicator was added to the reaction mixture. Then the reaction mixture with potassium hydroxide was dosed. The volume from the change in the titrated solution (normally blue) indicating the change in reagent was noted and the acid number was determined with the following formula:

$$A_n = \frac{56.11MV}{m_{eo}} \tag{4}$$

where A_n is the acid number, m_{eo} the mass in grams of essential oil, V the volume in millilitres of KOH required to neutralize the acid (mL), 56.11 the molar mass of KOH and M the molarity of the ethanolic KOH.

2.3.5. The ester numbers

Finally, the last property that has been determined was the ester number, which is defined as the number of mg of KOH necessary for the neutralization of the acids released by the hydrolysis of the esters contained in 1 g of essential oil. Hydrolysis of the esters present in the essential oil was done by heating the later in the presence of an EtOH solution previously titrated with KOH and followed by the dosing in return of the excess of alkali with a standardized 0.5 M HCl solution. The amount of KOH used to saponify the esters contained in C. Odorata essential oil was determined by the difference between the sample and the blank (a mixture containing all reagents except the analyte). The ester number of the essential oil is obtained using the following formula:

$$E_n = \frac{56.11M(B_{ac} - S_{ac})}{m_{eo}} \tag{5}$$

where E_n is the ester number; B_{ac} the volume in mL of the acid required for the blank; S_{ac} the volume in mL of the acid required for the sample; M the molarity of HCl solution and 56.11 the molar mass of KOH.

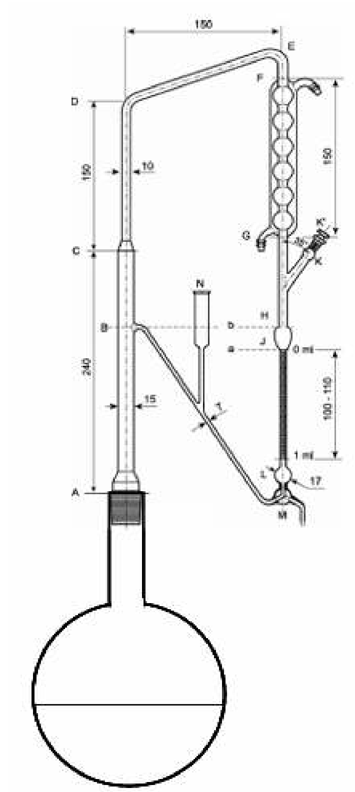

Figure 3: A Clevenger-type circulatory distillation apparatus as reported in the European Pharmacopoeias (Clevenger, 1928; European Pharmacopoeia, 2008).

3. Results and Discussion

3.1. Quality Values Within and Beyond Pre-established Standards

The essential oil obtained in this study was collected, put and sealed in 50 mL vials. It had a strong oily texture, a yellowish amber color (between yellow and orange), as well as a powerful exotic floral scent with a note of jasmine. By simply opening/closing the vial for few seconds, the smell quickly invades the surroundings space up to tens of meters. Table 1 presents the results of physical and physicochemical properties which play a prior role in qualification of the quality of an essential oil, compared to ISO 3063:2004(E) and other available reference values (AFNOR, 2005; Dumortier, 2006; Benini *et al*., 2006).

Table 1. Physico-chemical properties of the complete essential oil of fresh flowers of C. Odorata cultivated in Bujumbura, Burundi

#	Physico-chemical properties	This study	Standards	References
1	Yield (%)	1.050	[2 - 2.25]	Benini *et al*., 2006
2	Density (g/l)	0.940	[0.906 - 0.990]	ISO 3063:2004(E)
3	Refractive index	1.502	[1.495 - 1.513]	ISO 3063:2004(E)
4	Acid number	0.420	< 2	ISO 3063:2004(E)
5	Ester number	350.6	[80 - 180] Mayotte	ISO 3063:2004(E), Dumortier, D., 2006
			[40 - 185] Madagascar	ISO 3063:2004(E), Dumortier, D., 2006
			[45 - 200] Comoros	ISO 3063:2004(E), Dumortier, D., 2006

The yield was low, but this characteristic has nothing to do with the quality of the essential oil. And as a result, it is not even part of the standard characteristics. It does not affect the quality of the oil and can be improved without affecting the quality of the essential oil. The refractive index of an essential oil is a unique number that designates how the oil responds to and bends light. Essentially, it is a measurement that tests how the speed of light is altered when passing through the oil. Indeed, the AFNOR 2005 standards provide for the essential oil a refractive index between 1.495 (for high quality essential oils) and 1.513 (for lower quality essential oils). The refractive index found in this study is 1.502, which indicates an essential oil of high purity. This small refractive index is an indication that Canaga Odorata essential oil of the present study has low light refraction, which could favor its use in cosmetic products.

The AFNOR standard (AFNOR, 2005) recommends a density between 0.906 for low quality oils and 0.990 for very high-quality oils. The AFNOR standards set at 0.925 a density below which the oil is considered to be of quality III. The density of the complete ylang-ylang essential oil presented in this study is 0.940, suggesting that the analyzed oil meets the standards of a very good quality essential oil.

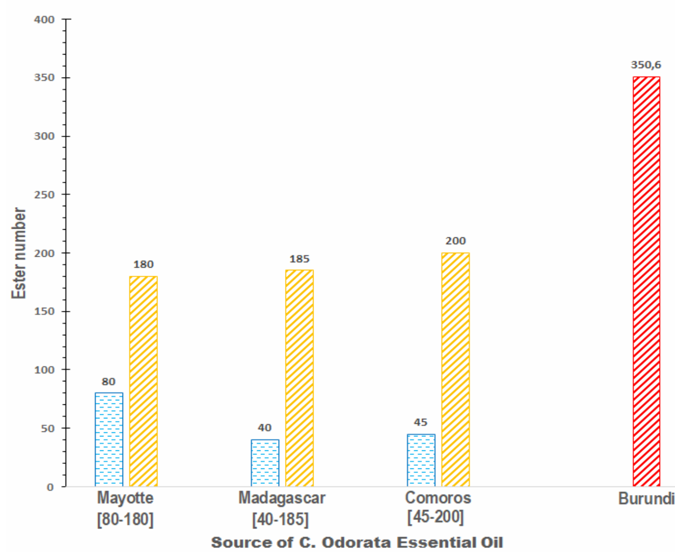

Figure 4: Highlight of the gap between the ester number of C. Odorata essential oil from Burundi in comparison with the ester numbers of essential oils from Mayotte, Madagascar and Comoros, taken as international standards

A low acid number is an indicator of good preservation of the oil. The higher the ester index, the better the olfactory quality of an essential oil. The acid number should be as small as possible, basically less than 2. The essential oil analyzed in this study gave an acid number of 0.420, which is low enough. Finally, among all the physicochemical properties evaluated, the most determining factor in the choice of a quality essential oil is the ester number. A high ester number indicates the presence of a large number of esters responsible for varied scents. The ester number must be higher as possible for an essential oil of good quality, combining varied scents from varied esters. The essential oil analyzed in this study reveals an ester number, fortunately very atypical, of 350.6. This ester number is very high and even outside the standards known until now. This difference is even more visible in Figure 4 which attempts to highlight this important gap between the ester number of C. Odorata essential oil from Burundi in comparison with the ester numbers of the three niches known worldwide for being privileged producers of quality C. Odorata essential oil. These niches are Mayotte, Madagascar and Comoros, respectively. It appears that for the refractive index, density and acid number, the results of this study are within the pre-established standards, and well beyond for the ester number.

3.2. Thoughts on Economic Benefits

Until now, AFNOR recognizes the existence of two niches of C. Odorata essential oil. The essential oil from Comoros and Mayotte, and the essential oil from Madagascar. These are two niches of differentiated quality that are not comparable, but neither of these niches has an ester index as high as the one found in this study for a variety of C. Odorata cultivated in Burundi. This exceptional ester number is the main uniqueness of C. Odorata essential oil extracted from the ylang-ylang of the Imbo plain in Burundi. The essential oil of ylang-ylang from Bujumbura in Burundi, extracted by hydrodistillation, has organoleptic properties already much appreciated in perfumery and could be very coveted in health and aromatherapy. This preliminary study, which was limited to a characterization based solely on the physicochemical properties, opens up interesting prospects for research on this plant which could become another sector of industrial plant in Burundi. For this, it will be necessary to make a much more detailed study which will have to include the characteristics of the soils of possible culture. On the other hand, it would be desirable for biologists and agronomists to look into aspects of improvement of the plant itself. Thus, the biology of reproduction, a prerequisite for any varietal improvement program, remains little known. It is always difficult to know with certainty when pollination takes place, who is the pollinating agent; and if there is one in the production area, what is the type of fertilization, etc. In addition to these shortcomings, we see that the abscission of flowers at each stage of their development is important and that this plant produces very little fruit. This also represents an obstacle with a view to varietal improvement. Speaking precisely of varietal improvement, research should focus on the vegetative capacities which are also not known. Indeed, in addition to the coppicing which seems to be widely practiced in the Comoros, no study has been conducted on the possibilities of cuttings, layering, in vitro multiplication, etc. All this information is however essential for a varietal improvement of the plant, and consequently, of its essential oil.

4. Conclusion

In the present study, key physicochemical properties used to qualify a quality of C. Odorata essential oils were investigated. These properties are the refractive index, the density, the acid number and the ester number. The value obtained were compared with refence values established for three most producing countries, namely Madagascar, Mayotte and Comoros. More interesting, the value of ester number was very beyond the norm by a factor of almost two, breaking the record already registered by AFNOR, therefore suggesting of an essential oil of exceptional quality. The findings presented in this study highlight the importance of essential oil obtained by hydrodistallation of fresh flowers of C. Odorata threes cultivated and grown in Bujumbura, Burundi, as a promising potential candidate for bio-based products of public interests such as bio-based cosmetics and bio-based pharmaceuticals products, and therefore offer new insights bioeconomics in health and well-being. Indeed, this plant can easily be cultivated, not only in Burundi, but also in countries of the Eastern Africa that share the same geoclimatic conditions as the Great African Rift.

Acknowledgements

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Biographical notes

Leopold Havyarimana and **Steve De Cliff** are both part of the Department of Chemistry, University of Burundi, Bujumbura, Burundi. Leopold Havyarimana is specialized in organic chemistry. Steve De Cliff is specialized in physical chemistry.