IDENTIFICATION OF ESSENTIAL OILS IN LIPPIA JAVANICA

Abstract

The herb Lippia Javanica bought from the Hilton's pharmacy Harare Zimbabwe were used for extracting the essential oils using the second method Russian Pharmacopoeian Article 1.5.3.0010.15 steam hydro-distillation. A strong aromatic, colourless, cloudy emulsion was obtained. For further analysis and identification of the essential oils present in L. javanica, a sensitive method of qualitative analysis was used. The results of qualitative analysis produced showed that L. javanica consists of more than 50 compounds from different chemical groups, like terpenes, ethers aldehydes etc. In this work only the results of wet method of qualitative analysis will be discussed.

Keywords

Essential Oils
Lippia Javanica
Phytochemicals
Pharmacognosy

Introduction

As part of the implementation of the State Strategy for the Development of the Pharmaceutical Industry of the Russian Federation for the period up to 2030, the priority direction is the production of medicines, including those of plant origin. Interest in phyto preparations remains relevant today due to their mild effect, low toxicity and the possibility of long-term use for the prevention and treatment of various diseases. With the development of science and medicine, more and more modern medicines are being created, but medicinal plants are still the main source for the future in medicine and health sphere.

L. javanica - Perennial shrub, herb which grows up to 4.5 m high with strong fragrant leaves that exude a lemon aroma when crushed [15-17]. It is naturally found in central, eastern and southern Africa. In Southern Afria, this species is native to Botswana, Malawi, Mozambique, South Africa, Swaziland, Zambia, Zanzibar and Zimbabwe [15–16].

The shrub has been used in medical practice in South Africa for many years because of its richness in chemical compositions, which means it has a wide range of pharmacological activity. The main pharmacological effects consist of anti-inflammation, antioxidant, antiplasmodial, antimicrobial, antituberculous, and antiviral, giving us the perspective to produce more quality and safe essential oils in the distant future [23–25].

This causes a diverse range of diseases, for the treatment of which the shrub is used [28-29]. According to the literature, it is used in the treatment of the following diseases and their symptoms (Table 1).

Table 1 List of diseases and method of application of Lippia Javanica in medical practice.

Diseases	Method of application of Verbena Javanese
Colds	Making a tincture from the leaves and shoots, or making an ointment based on the leaves
Cough	Preparation of tincture from leaves / stems / shoots, application of decoction in the form of inhalation
Sore throat	Preparation of decoction from leaves and rinsing based on leaf tincture
Tonsillitis	Making a tincture from the leaves
Fever	Making a decoction of the leaves
Infectious diseases of the lungs	Making a decoction of roots and leaves
Nasal congestion	Making a decoction of leaves with the addition of crushed compositions from other medicinal drugs
Bronchitis	Preparation of a tincture from the leaves or roots, or preparation of an ointment based on the leaves, followed by application under a gauze bandage in the throat area

Chest pain	Making juice from leaves and roots
Asthma	Making a decoction or inhalation from the leaves
Malaria	Making a decoction of leaves and roots

Nose bleeding	Making a decoction of the leaves
Migraine	Inhalations based on a decoction of leaves and stems
Anemia	Making decoctions based on roots

Based on the data in the table, we can conclude that Lippia javanica is most often used in the treatment of diseases of the otholaryngological and pulmonological profiles, which makes it possible for its long use in the medical field in the near future, production of new sprays, production of new lozenge to help the people in the future.

Separately, it is worth noting the importance of preparations based on Lippia Javanica in the complex treatment of coronavirus infection (COVID-19). This plant has been widely used in the treatment of infectious diseases for many years, so it is not surprising that the local population of Southern African region used essential oils, tinctures, inhalations and decoctions of Lippia Javanica for the prevention and then as part of the complex therapy of a novel COVID-19[25]. It simply implies that there is more to explore from this medicinal plant, so that the in the near future, there will be mass production of medicines against antimicrobials and viruses as the plant contains polyphenols and flavonoids which possess such effects.

The compositions of essential oils vary significantly with place of origin, harvest season, and weather. The complex molecular composition of many essential oils demands high resolution separation to characterize the range of compounds making up the oils. In addition, it is often necessary to study small differences between oils that correspond to variation in geographic or genetic origin of plant material.

Chromatography, especially gas chromatography (GC) and mass spectroscopy (MS) have been the most applied analytical techniques for essential oil analysis. The limitation of GC and GC-MS has pushed chromatographers to dig deep in search of better methods to analyze essential oil volatiles such as improvement in sample preparation prior to injection. Were tested such methods like steam distillation, dynamic headspace, static headspace and SPME (solid phase microextraction). As a result now advanced sample injection method is static headspace (HS-GC).

It is important to consider that the GC analysis of essential oils is commonly carried out to evaluate a particular plant or herb for its percentage oil content and constituents. The analytical values obtained in this way do not, however, refer directly to the volatile substances present in the herb itself and may not truly reflect the real quality of herb, depending on the isolation technique used. However, application of a method such as static headspace in GC, allows you to get only the profile of essential oils.

Mass spectrometry (MS) can be defined as the study of systems through the formation of gaseous ions, with or without fragmentation, which are then characterized by their mass-to-charge ratios (m/z) and relative abundances [41]. The analyte may be ionized thermally, by an electric field or by impacting energetic electrons, ions, or photons.

During the past decade, there has been a tremendous growth in popularity of mass spectrometers as a tool for both, routine analytical experiments and fundamental research. This is due to a number of features including relatively low cost, simplicity of design and extremely fast data acquisition rates. Although the sample is destroyed by the mass spectrometer, the technique is very sensitive and only low amounts of material are used in the analysis.

However, as is well known, compounds such as isomers, when analyzed by means of GC-MS, can be incorrectly identified; a drawback which is often observed in essential oil analysis. As is widely acknowledged, the composition of essential oils is mainly represented by terpenes, which generate very similar mass spectra; hence, a favorable match factor is not sufficient for identification and peak assignment becomes a difficult, if not impracticable, task.

From other side, provided data contained in mass spectral libraries have been recorded using authentic samples, it can be observed that the mass spectrum of a given some terpene is usually sufficient to ensure its identification when associated with its retention index obtained on methyl silicone stationary phases.

Materials and Methods.

The object of the study was herb Lippia Javanica. For determining the optimal results, pharmacognostic analysis, macroscopic analysis, microscopic analysis, quantitative analysis of essential oils and its qualitative analysis were carried out. Determination of the

content of essential oil was carried out by steam distillation of herb Verbena Officinalis and herb Lippia Javanica, followed by volume measurement. The mass fraction was expressed in mass-volume percent based on absolutely dry raw material or preparation. For the experiment, Method 2 of RF Pharmacopeia .1.5.3.0010.15 "Determination of the content of essential oil in medicinal plant raw materials and medicinal herbal preparations" was used. The material used: distilled water, 10% sodium hydroxide, glycerin, equipment to measure contents of essential oils, analytical balance, microscopy, and Petri dishes. Using an analytical balance, weigh 50 grams of chopped Verbena Officinalis herb and Lippia Javanica respectively. Place the sample in a round bottom flask. Add 500 ml of distilled water to a round bottom flask. The steam distillation process of essential oil took two hours.

All data were obtained using the following equipment:

- HS-autosampler TriPlus 500 (Thermo Scientific)
- Gas Chromatograph TRACE 1300 (Thermo Scientific)
- MS-detection with ISQ 7000 (Thermo Scientific)
- PC Workstation with Chromeleon CDS ver. 7.2.10
- MS Library NIST 16

Separation was providing with TraceGold 5 capillary column 30m x 0.32mm x 0.25mm (Thermo Scientific) in temperature programmable mode.

Headspace parameters:

Incubator temperature (C⁰)	110
Incubation time (min)	45
Vial mixing mode	Fast
Vial pressurization mode	Pressure
Vial pressure (kPa) (aux. gas – nitrogen)	130
Pressure balance time (min)	1
Loop volume (ml)	1
Loop/interface temperature (C⁰)	120
Loading loop pressure (kPa)	74,2
Loop balance time (min)	1
Injection mode	Standard
Injection time (min)	1

GC parameters:

Sample Injection Unit	Pneumatic circuit Split/Splitless
Sample Injection Unit temperature (C⁰)	200
Split ratio	25:1
Carrier gas	Helium
Carrier gas flow (ml/min)	1,5 (constant flow mode)
Capillary column	TraceGold 5 capillary column 30m x 0.32mm x 0.25mm (Thermo Scientific)
Column temperature	Temperature programmable mode: initial 90⁰C (5 minutes) up to 260⁰C (10⁰/min) and 10 minutes hold.
Detector	MS

MS parameters

MS-system	MS-detector on the base of single quadrupole mass-analyzer
Interface/Ion source	Electron Impact Interface (EI) 70 eV
Interface/Ion source temperature (C⁰)	150
Manifold temperature (C⁰)	50
Quadrupole temperature (C⁰)	180
Scan mass ratio (Da)	35 - 450

Peaks identification was providing with NIST 16 MS Library.

Results and Discussions

The result of the pharmacognostic analysis was shown by microscopic features, including stomata, ethero vascular glands, and short simple unicellular hairs shown in figure 1-3.

As a result of the qualitative analysis, the presence of essential oil in the herb L. javanica was established. The volume collected reached about 20 ml. The volume collected didn't have a viscous and oily texture, most probably essential oils in Lippia Javanica are thermolabile. The liquid-watery with a characteristic strong aromatic odour and taste was obtained, figure 4.

Qualitative analysis for other active substances was conducted and it took 30 minutes. The following reagents were used ammonia 10%, sodium hydroxide 10%, ferric ammonium sulfate, Sodium molybdate, iron chloride

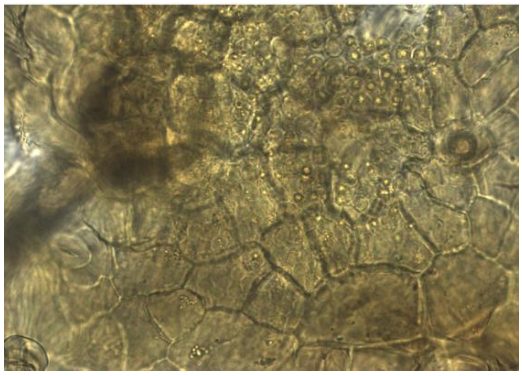

Figure 1 Microscope of the raw material of Herb Lippia Javanica (essential oil glands).

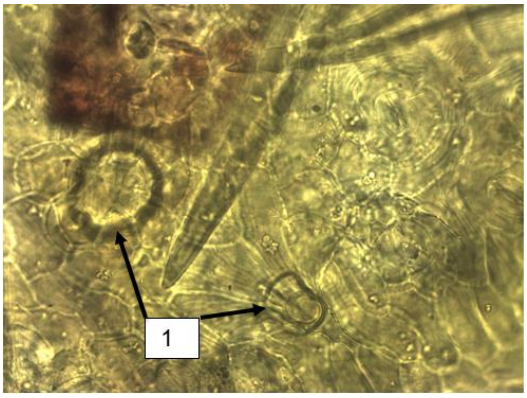

Figure 2 Microscope of the raw material of the Lippia Javanica 1) Essential oil receptacles

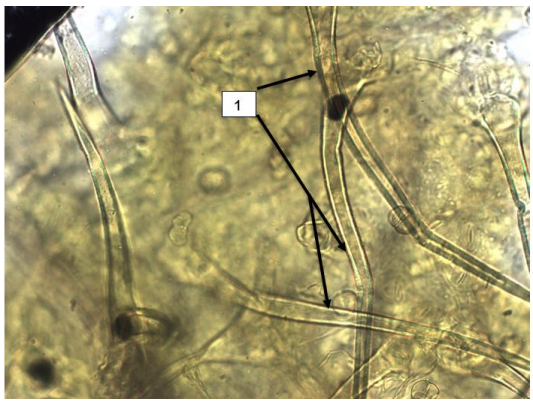

Figure 3 Microscope of the raw material of the Lippia Javanica (1- long glandular simple unicellular hair)

Figure 4. Organoleptic Testing of Lippia Javanica Essential Oils

Qualitative analysis of possible active compounds was also carried out. L. javanica showed the presence of flavonoids with the reaction 10% ammonia, yellow colour and pale yellow colour with 10% sodium hydroxide. Yellow colour with ferric ammonium sulfate showed the presence of Tannins. A dark-green staining with iron chloride showed the presence of Phenol and Flavonoids in both Plants. Lippia Javanica gave a pale-red colour with sodium molybdate showing the presence of phenols.

Figure 5 results of flavonoids identification with 10% ammonia

1) Control Lippia Javanica, 2) Experiment Lippia Javanica,

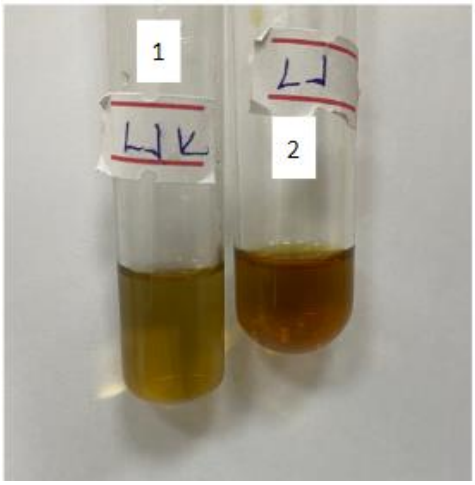

Figure 6 results of flavonoids identification with 10% sodium hydroxide
1) Control Lippia Javanica, 2) Experiment Lippia Javanica,

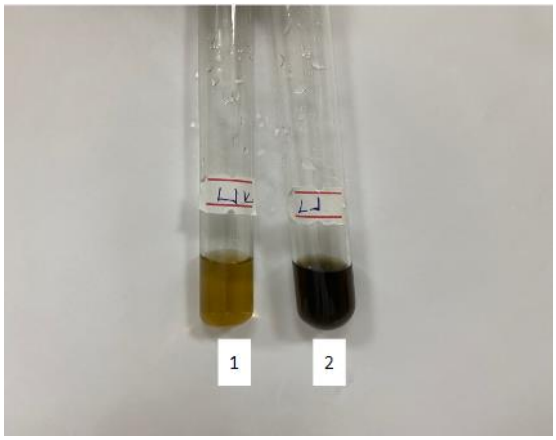

Figure 7 Tannin identification with ferric ammonium sulfate $NH_4Fe(SO_4)_2$
1) Control Lippia Javanica, 2) Experiment Lippia Javanica,

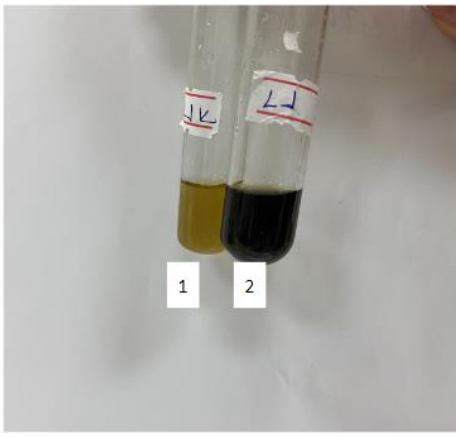

Figure 8 Phenol identification with iron chloride FeCl 3
1) Control Lippia Javanica, 2) Experiment Lippia Javanica,

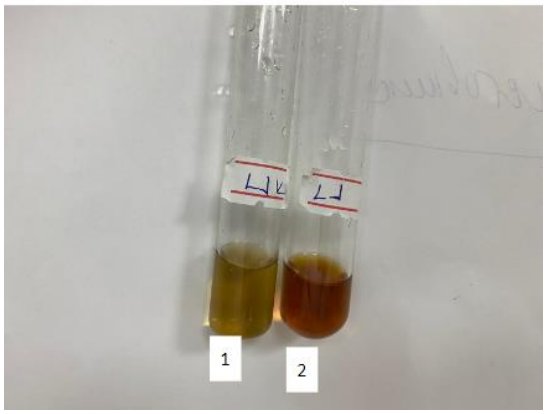

Figure 9 Phenol identification with Sodium molybdate - Na2MoO4
1) Control Lippia Javanica, 2) Experiment Lippia Javanica,

Raw essential oil from Lippia Javanica was extracted with steam distillation. Row oil is it was placed in 20 ml vial and sealed.

Total ion chromatogram (TIC) obtained under the above conditions is shown in fig. 1.

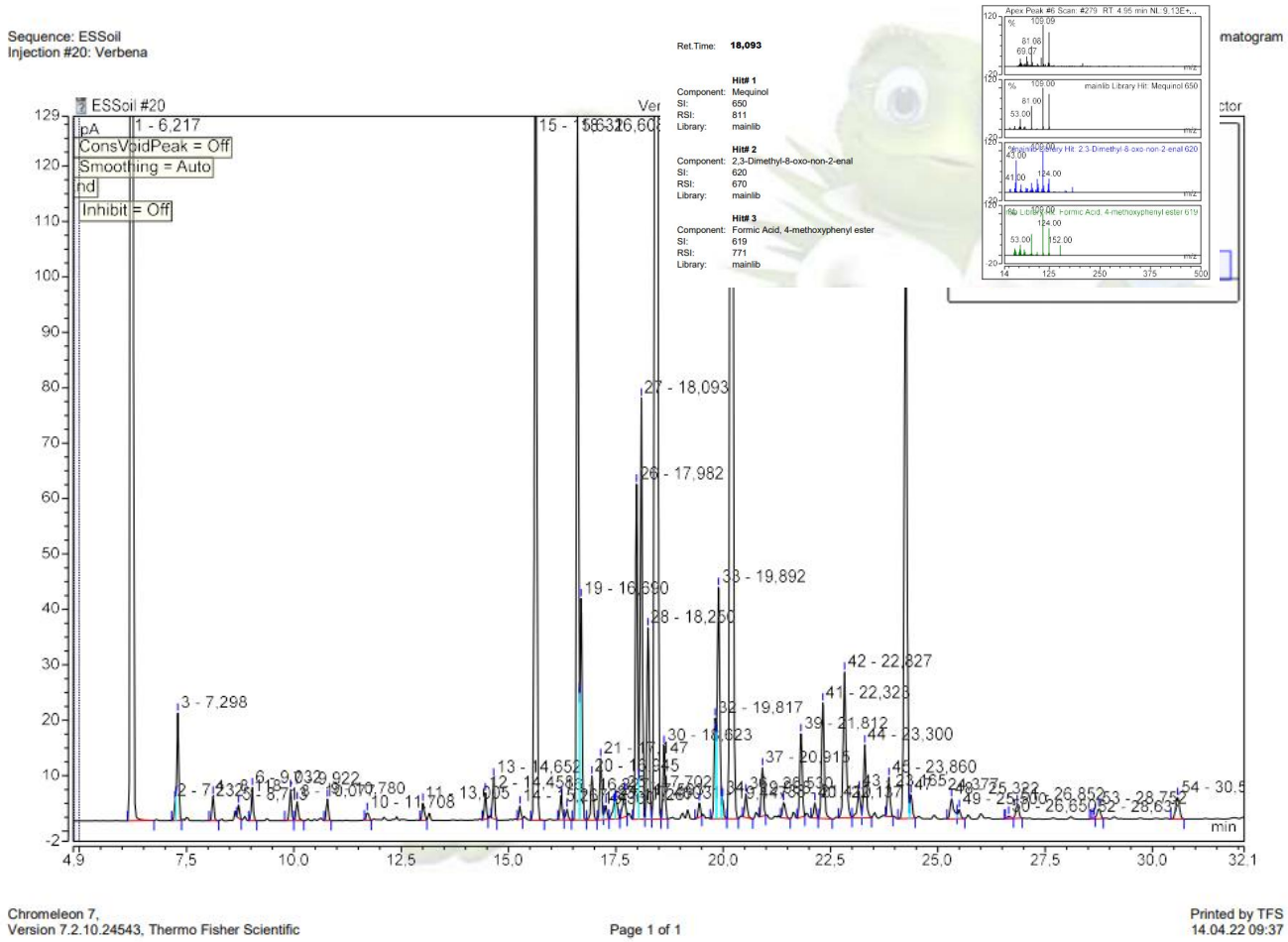

Figure 1. TIC of essential oil from Lippia Javanica

The signals of 54 components were registered on the chromatogram. Part of the peaks, especially at the end of the chromatogram, were identified as components of the stationary liquid phase.

Most of the minor components were identified as esters of C4-C10 alcohols and C2-C5 acids, as well as benzoic acid. Several minor peaks were identified as simple terpens like geraniol, pinen and cineol. The major components were identified as benzoic acid (peak at 7.298), phenylmethyl benzoate (peak at 15.671), ethyllinolaol (peak at 16.581) and its cis-isomer (peak at 16.690), mequinol (peak at 18.093), piperonal (peak at 18.442), isocitroneol and corimbolone (peaks at 22,827 and 23,300 respectivelly). MS library search results are showing on fig. 2.

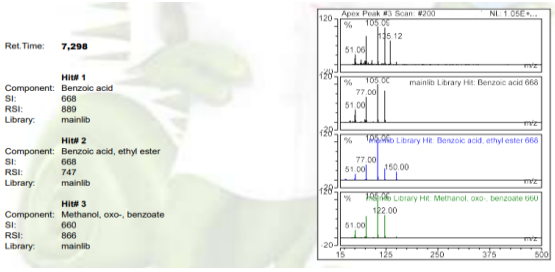

Figure 2. Library search results for benzoic acid, ethillinolaol and mequinol

Conclusions.

A chemical classification of essential oils was carried out. The features of the chemical structure, physical and chemical properties of essential oils were described.

Literature reviewed that Lippia Javanica was being used as an additive to other medication to fight against Coronavirus (COVID-19) in the southern African region.

This scientific research presents a method for determining the content of essential oil, describes its quantitative assessment and assessment of the quality of raw materials. The research is ideal to be put into practice and help the medicinal and pharmaceutical sphere to expand in the near future, due to introduction of such essential oils extracted by cheapest and efficient methods.

The research considered the external features, geographical distribution and habitats of Lippia Javanica, which contain essential oils. The vision is to import the medicinal plant from the Southern African Countries at a cheaper price and produce more medicines here in the Russian Federation, or building a pharmaceutical manufacturing company in Zimbabwe to produce essentials oils of high quality and safety to the local people. It was found that L. javanica contains essential oils, of higher quality which makes it a positive outcome for the vision of producing medicinal, cosmetical essential oils of higher quality and safety to the population to use .

The literature review and the practical part showed that in L. javanica there are thermolabile, they lacked the viscosity and oily texture, and research work will be carried out with another method of extracting thermolabile essential oils. An alternative method can be used so that the vision of producing essential oils from Lippia javanica can still be fulfilled, individually or with the help of grants.

In Addition macro and microscopic features were considered, all possible diagnostic features on Lippia Javanica were confirmed. Qualitative analysis showed that in L. javanica contain flavonoids, tannins with Lippia Javanica containing phenols in addition shown by a pale-red colour with sodium molybdate.

Therefore, the composition of the essential oil L. javanica was studied by HS-GS-MS method. Was showing that essential oil consists more than 50 compounds from terpenes, ethers, aldehydes and aromatic acids.

Thus, Lippia Javanica can be introduced into medical practice as a source of essential oils and as source of other phytochemicals, because of its medicinal value. The research need to be realized so that it can be patented or sold to big pharmaceutical companies so that they can make more essentials oils and other medicines like NSAIDs from the basis of Lippia javanica.

Supplementary materials

No supplementary materials are available.

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Author contributions

Investigation: Nyamukondiwa Malachi

Conflict of interest

The authors declare no conflict of interest.

Additional information

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