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A Process of Electrohomeopathic remedy "SLASS" Preparation, Compared with Krauss Method By phytochemical Analysis, TLC and FTIR Studies

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Authors' contributions

This work was carried out in collaboration among all authors. Author PS designed the study, performed basic literature, conducted the experiment and wrote the first draft of the manuscript reedited and corresponding author. Author DCB wrote the protocol, conducted the experiment systematic way and recorded results. Author ES anaysed the phytochemical and IR spectral data and edited the article. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Electrohomeopathy medicines have been practicing since the 1850s in European countries and from the 1870s in India. There are various methods of preparation of Electrohomeopathic medicines that have been developed and practice by the different manufacturers. For the safety, efficacy, and stability of the medicines, there is a need for scientific research to standardize the procedure of medical preparations. SLASS is one of the commonly using remedy by the electro homeopathy practitioners for various disorders concern to the digestive system, excretory system and nervous system etc... In the present studies we attempted a comparative study of a Process of Electrohomeopathic remedy "SLASS" Preparation, Compared with the Krauss method by phytochemical analysis, TLC, and FTIR Studies. Our process and Krauss method showed significant differences in phytochemical profile TLC and FTIR spectral peaks. The yield of the

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extracts of *Aloe capensis* and *Gentiana lutea* are more in our method compared to Krauss method. Glycoside is present in Krauss method. These research outcomes with reproducibility will become standard markers or signatures to assess the quality, safety, and efficacy of the electro homeopathy medicine as well as reduce the risk of adulterations helps to develop specifications of the Electrohomeopathy remedies. Future work is essential to explore its therapeutic applications

Keywords: Electrohomeopathy; SLASS; remedy preparation; phytochemicals; FTIR; sureshbabu.

1. INTRODUCTION

Globally various herbal medical systems have developed in the different geographic regions based on observation, practice and experience on traditional usage of herbal medicine and medication over the time such as Ayurvedha, Siddha, Unani, Chinese, Homeopathy, Electro homeopathy and other traditional medical systems etc....

Electro-homeopathy or Electropathy as a medical system proposed by Italian Count Ceaser Mattie (1809-1896) during 1850's. The founder borrowed C.C.Mattei the concept from Paracelsus, the process of preparing the vegetable substances by means of a more or less complicated mode of separation, purification and cohobation [1,2]. Extraction of electrals or phytoconstituents from different plants and recombining in appropriate proportions as per the requirement of the patient's disease condition forms the core principle of the system [3].

During 1870s-1880s Count Ceaser Mattie's Electro homeopathy medical system became most popular across the globe. Practitioners, patient's beneficiaries & distributions of medicines spread about 44 countries with 297 medicine distribution depots all over the world. Father Augustus Muller a German Jesuit priest who studied in the USA and France. In the early 1870s Father Muller also became close contact with C.C. Mattie. When Father Muller reached Mangalore, Karnataka in south India on 31st December 1871 with other missionaries, he brought electro homeopathy remedies along with him. In around 1875 he started Leprosy treatment in Kankanady, Mangalore District, Karnataka, India. In 1879-80 Rev Fr Augustus Muller founded and registered Father Muller Charitable Institutions (FMCI) trust in Kankanady, Mangalore, Karnataka, India.

There were lot of documentary evidence available about the import of electro homeopathy remedies from Italy & other C.C. Mattie's electro homeopathy remedy depots from the European country by Fr., Muller for his successful treatment. From 1870's to until the death of C.C. Mattie, there were a lot of shreds of evidence available for the association, transactions & donations made by the C.C. Mattie to the Fr. Muller. During this period C.C. Mattie donated a huge amount to purchase the land for the construction of Fr Muller hospital in Kankanady, Mangalore District, Karnataka State. Dr. Fr Augustus Muller established an Electrohomeopathic leprosy hospital & asylum in Kankanady, Mangalore as far as before 1880. Fr Muller association & interaction with the C.C. Mattie, Electrohomeopathy medicine purchase & import from the C.C. Mattie, treatment record & their Clinical data are recorded from 1893, when the inpatient department (IPD) is opened in the Fr Muller' hospital establishment. These records are more evident for the efficacy of Electro Homeopathy remedies, which were documented up to the death of Fr Muller (1910) & postdeath period of Fr Muller up to 1940s.

In the Electro Homeopathy medical system there are various remedies which are prescribed to patients in single or combinations of multiple remedies depending upon diagnosis, body temperament, disease severity, dosology and treatment. Each remedy is prepared by using multiple herbs. This is the only medical system in which medicines are prepared purely from plants and its parts. In this Electro Homeopathy medical system there is uniqueness in its principles of preparation of remedies, diagnosis, dosology, treatment and prevention of diseases comparable to other existing Medical Systems [4,5].

We have huge number of publications, books, literatures and other evidential documents of C.C. Mattie and his associated persons and followers like Zimple, Theodor Krauss, A. P. J. Gliddon, Father Muller, N. L. Sinha. Different methods of preparation had been evolving by modification and application by the different practitioners during the last 150 years in the Electro Homeopathy Medical system across the globe [6]. The Zimple and Krauss method as mentioned in German Pharmacopoeia [GHP].

Some visitors and Electrohomeopathic medicines manufacturers from India such as Dr. Sanjeev Sharma from RABISAN (Himachal Pradesh,) Dr. Achala from ELIXIR, Udaipur, Rajasthan, etc... Are preparing unique method of Electropathic remedy preparations. Other modified or some methods are applied by some Electro homeopathy pharma companies in India.

In the present investigation we attempted to prepare Electro homeopathy SLASS remedy in different methods and compared with a method of Theodor Krauss method 30 as mentioned in German Pharmacopoeia [GHP], and compared their quality by investigation of their qualitative analysis, phytochemical investigation and chromatography study such as TLC and FTIR spectra.

2. MATERIALS AND METHODS

2.1 Samples Collection

Aloe Capensis 1000 g is purchased form Indian Herbs New Delhi an authenticated by Dr. P. Suresh Babu, and voucher specimen is kept in our laboratory with the Electrohomeopathy plant code No. TDRF/EH/002/EHO10/ and *Gentiana Lutea* (Root) 1000 g purchased form Indian Herbs New Delhi, and authenticated by Dr. P. Suresh Babu, and voucher specimen is kept in our laboratory with the Electrohomeopathy plant code No. TDRF/EH/002/EHO46/.

2.2 Chemicals and Reagents

All chemicals including Ethanol (1000 ml), hexane (500 ml), Ethyl acetate (500 ml), Methanol (500 ml), Acetone (500 ml), Acetic anhydride (500 ml), Concentrated Sulfuric acid (500 ml), Chloroform (500 ml), Conc. Hydrochloric acid (500 ml), Copper(II) Sulphate (500 ml), Sodium hydroxide (500 mg), Potassium hydroxide (500 mg), Iodine (100 g), potassium iodide (100g), Glacial acetic acid (500 ml), Sodium acetate (500 ml), Concentrated Nitric acid (500 ml), Formic acid (500 ml), Sodium bicarbonate (100 ml) are purchased from COM BIOTECH private limited. Yeast purchased from local market.

2.3 A Process of SLASS Preparation by Plants Extraction

2.3.1 Part-A: Fermentation

Take coarsely powdered plant part of 100 parts, 400 parts of water, 40 parts of sucrose and 2

parts of yeast are added in a fermentation flask. Reaction carried out at room temperature 27°C up to 25 days with intermittent shaking. After this, filter out using filtering cloth and the filtrate was collected labeled as Part A and stored in airtight amber bottle at room temperature.

2.3.2 Part-B: Percolation

Take the powdered plant100 parts, 400 parts of alcohol 86% and allow it to percolate for 25 days. Regularly stir the solution up until extraction completes. Filter out using filtering cloth and the filtrate was collected, labeled as Part B and stored.

2.3.3 To potentisation of Expressed liquid

Take extracted liquids Part-A and Part-B 1 part each add 8 part of 30% alcohol to prepare Mother solution of 1st Decimals dilution (D1)and are Potentised2nd decimal dilutions (D2) and 3rd Decimal dilution mother tincture D3 as follows.

Take 1 part of D1 and add 9 parts of alcohol 30% which will yield D2 Mother Solution.

Take 1 part of D2 and add 9 parts of alcohol 30% which will yield D3 Mother Solution.

2.3.4 Labeling

Subsequent dilutions of the Mother tincture or solutions are produced accordingly, derived dosage forms are labeled as "EH/PSB/MT"/D1/D2/D3".

2.3.5 SLASS preparation

The electro homeopathic remedy SLASS is prepared by mixing 3^{rd} Decimal Dilution (D3) of *Aloe Capensis* and 3^{rd} Decimal dilution of *Gentiana Lutea* in 2: 1 ratio respectively.

The composition of an Electrohomeopathic remedy SLASS is as follows:

- 1. Aloe Capensis (D3) 20 part
- 2. Gentiana Lutea (D3) 10 part

2.3.6 Labeling of slass

SASS labeled as Electrohomeopathy Remedy, with indications, Contraindications, Dilution, Use as prescribed by Electrohomeopathic practitioners, Manufacturer Address, Manufacture Date, Batch No and Expiry Date.

2.7 Preparation of SLASS an Electrohomeopathy Remedy according to Krauss Method

We took 50 g of *Aloe capensis and 50g of Gentiana lutea* dried coarsely powdered plant materials and prepared the spagyric extracts and mother solutions as method explained by Theodor Krauss in German Homeopathy Pharmacopoeia (GHP) method 30. Extracts yield is recorded in the Table 3 and phytochemical analysis recorded in Table 4, Thin Layer Chromatography (TLC), and Fourier Transfer Infra-Red (FTIR) spectroscopy of the extracts, mother solutions and SLASS carried out and recorded.

3. RESULTS NAD DISCUSSION

The extraction of the A. capensis and G. lutea are done as explained in the methods. For each plant 50g quantity of the coarsely powdered plant material is to prepare Part A and Part B is taken. For the preparation of Part-A 50 g plant material, 200 ml of purified water, 20 g of sucrose and 1.0 g of S. cerviceae is added and allow it for fermentation. For part B to 50 g of plant material 200 ml of 86% ethanol is added and allows it for percolation. Both parts of each plant is kept for 25 days with regular stirring. While after 24 hour we noticed that fermentation part of the Gentiana Lutea found deficiency in liquid part to allow fermentation. So we added again 4 parts of purified water. After 25 days we have filtered liquid part and vield of the extracts Part A and Part B of both the plants is measured and recorded in the Table 1. Phytochemical screening of the Extracts, Mother Tincture (Mother solution) and SLASS remedy are evaluated and recorded as mentioned in the Table 2. In the Krauss method the yield of Part A and Part B of both the plants is measured and recorded in the Table 3. Phytochemical screening of the Extracts, Mother Tincture (Mother solution) and SLASS remedy are evaluated and recorded as mentioned in the Table 4. The yield of the extracts of Aloe capensis and Gentiana lutea are more in our method compared to Krauss method.

3.1 Phytochemical Investigations

Phytochemical investigations of the both *Aloe capensis and Gentiana lutea* all extracts of part A, part B, and mother solutions showed the presence of alkaloids, flavonoids, terpenes,

tannins and saponins but glycosides present in *Aloe capensis* but absent in all extracts and mother solution of *Gentiana lutea*. Where as in the Krauss method extracts alkaloids are present in Part A and mother solution of *A. capensis* but present in all extracts of *G. lutea*. Flavonoid group is present in all extracts of *G. lutea*. Terpenoids and tannins are present and fats and cholesterol is absent in all extracts of both plants and recorded in the Table 2 and Table 4.

According to literature survey main constituents of Aloe are aloe-emodin-anthrone 10-Cglucoside type phytochemicals [7]. Jannathul et al., in 2019 reported that Aloe extracts confirmed presence of alkaloids, flavonoids. the carbohydrates, proteins, saponins, phenols, terpenoids and phytosterols by Gas Chromatography studies Literature shown the presence of hydroxyanthracene, anthraguinone and glycosides aloin A and B, 2-acetonyl-5-(aloeresins) [8]. methyl-chromones Sterol includes campesterol, *β*-sitosterol, lupeol, and cholesterol, and the elements such as Al, B, Ba, Ca, Fe, Mg, Na, P, Si etc has also been reported to be present in the Aloe extract.

According to literature survey main constituents of G. lutea bitter glycosides, alkaloids, yellow colouring matters, sugars, pectin, amarogentin and fixed oil [9,10]. The bitter glycosides mainly contain gentiopicrin (also called gentiopicroside) which is water soluble crystalline compound. Another bitter principle was identified as loganic acid in Gentiana lutea L. for the first time [11]. The yellow colour of fermented gentian root is due to xanthones and gentisin (also known as gentiamarin) isogentisin and gentioside. Gentian also contains gentisic acid and about 0.03 gentianine and percent of the alkaloids gentialutine. Gentian is rich in sugars which include the trisaccharide gentianose, the disaccharide gentiabiose and sucrose which on fermentation can convert on glucose and fructose and for very long fermentation can convert into alcohol and CO2. Gentian root extracts showed no toxicity and were generally well tolerated [12].

Pharmacologically Aloe has wide variety of properties. It was used as treatment of seborrhoeic dermatitis, peptic ulcers, tuberculosis, fungal infections and for reduction of blood sugar (glucose) levels [13]. In preliminary human clinical studies, the gel has shown significant results in the treatment of asthma, peptic ulcers, and diabetes mellitus [14,15]. The gel has been sold in the health food market as a tonic, as well as for "supporting the immune system" and "supporting healthy breathing".

A large number of pharmacological investigations of *G. lutea* have been carried out based on the phytoconstituents present in it but a lot more can still be explored and utilized in a therapeutic manner. *G. lutea* is useful as Neuritogenic, Choleretic, Antioxidant, CNS stumulant, Antiatherosclerotic, Gastro protective and Antimicrobial Activity [16].

3.2 Thin Layer Chromatography (TLC) analysis

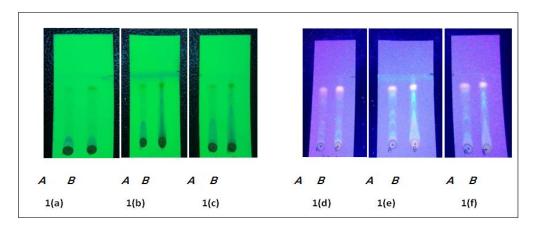
3.2.1 Thin Layer Chromatography (TLC) analysis for both extract of *Aloe capensis*

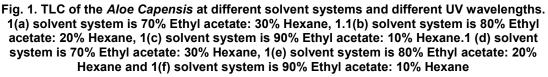
Thin Layer Chromatography (TLC) analysis for both extract of *Aloe Capensis* Part A (First spot loaded) and Part B (Second spot loaded) on TLC plate was analyzed and images are captured and noted in Fig 1. The solvent system uses 70% Ethyl acetate: 30% Hexane (a), 80% Ethyl

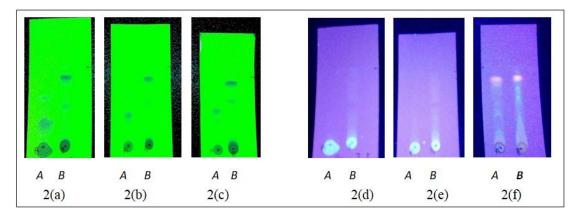
Yield	Part A	Part B	Moth. Sol. D1	D2	D3
Aloe capensis (50 g)	180 ml	180 ml	1,800ml	18,000ml	1,80,000ml
Gentiana lutea (50) g	180ml	180 ml	1,800ml	18000ml	1,80,000ml

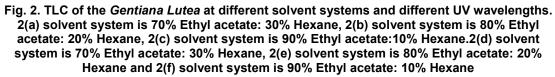
SL NO	PHYTOCHEMICAL GROUP Test	1. Aloe capensis TDRF/EH/002/EH010/K/01/01			2. Gentiana lutea TDRF/EH/002/EH046/K/01/01			
		Part A	Part B	Mother tincture	Part A	Part B	Mother tincture	SLASS
1	ALKALOIDS	+	+	+	+	+	+	+
2	FLAVONOIDS	+	+	+	+	+	+	+
		+	+	+	+	+	+	+
3	TERPENOIDS	+	+	+	+	+	+	+
4	FATS	-	-	-	-	-	-	-
5	CHOLESTEROL	-	-	-	-	-	-	-
6	TANNINS	+	+	+	+	+	+	+
7	SAPONINS	+	+	+	+	+	+	+
8	GLYCOSIDES	+	+	+	-	-	-	-

*+ Indicate Presence: - Indicate Absence





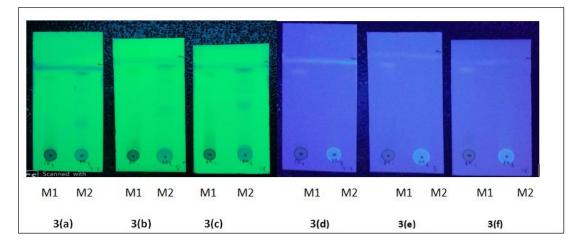




acetate: 20% Hexane 1(b), 90% Ethyl acetate: 10% Hexane(c). Images (a), (b), (c) are seen in UV 254 nm (Short wave), and (d), (e) (f) are seen in UV 365nm (Long wave).

3.2.2 Thin Layer Chromatography (TLC) analysis for both extract of *Gentiana lutea*

Thin Layer Chromatography (TLC) analysis for both extract of *Gentiana Lutea* Part A (First spot loaded) and Part B (Second spot loaded) on TLC analyzed and images plate was are captured and noted in Fig 2. The solvent system uses 70% Ethyl acetate: 30% Hexane (a), 80% Ethyl acetate: 20% Hexane 1(b), 90% Ethyl acetate: 10% Hexane(c). Images (a), (b), (c) are seen in UV 254 nm (Short wave), and (d), (e) (f) are seen in UV 365nm (Long wave).



3.2.3 Thin Layer Chromatography (TLC) analysis for mother solution (Tincture) of Aloe Capensis & Gentiana lutea

Fig. 3. TLC of the Mother Solution (Tincture) of Aloe Capensis (M1) and Gentiana Lutea (M2) at different solvent systems and different UV wavelengths; 3(a) solvent system is 70% Ethyl acetate: 30% Hexane, 3(b) solvent system is 80% Ethyl acetate: 20% Hexane, 3(c) solvent system is 90% Ethyl acetate: 10% Hexane. 3(d) solvent system is 70% Ethyl acetate: 30% Hexane, 3(e) solvent system is 80% Ethyl acetate: 20% Hexane and 3(f) solvent system is 90% Ethyl acetate: 10% Hexane

3.3 FTIR Chromatography Analysis

For FTIR analysis of samples obtained using Perkin Elmer Spectrum Two FT-IR Spectrometer (Part No: L10552211) is used. Before analysis, system calibrated and instrument verification Conducted checked humidity as well. Background analysis should be performed before sample analysis. Sample using NaCl Window and recorded the peaks.

FTIR spectra of the each parts of the plant extracts, mother solution of *A. capensis* an G. lutea and SLASS an that o Krauss method were compared and shown significant differences.

3.2.4 Thin Layer Chromatography (TLC) analysis for Mother Solution (Tincture) of SLASS remedy

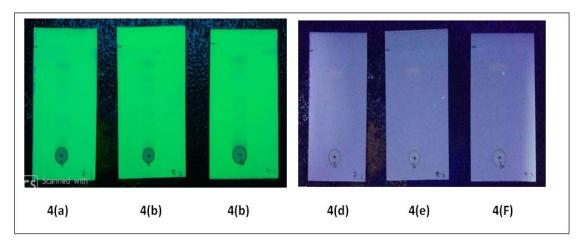


Fig. 4. TLC of the *Gentiana Lutea* at different solvent systems and different UV wavelengths; 4(a) solvent system is 70% Ethyl acetate: 30% Hexane, 4(b) solvent system is 80% Ethyl acetate: 20% Hexane, 4(c) solvent system is 90% Ethyl acetate:10% Hexane. 4(d) solvent system is 70% Ethyl acetate: 30% Hexane, 4(e) solvent system is 80% Ethyl acetate: 20% Hexane and 4(f) solvent system is 90% Ethyl acetate: 10% Hexane



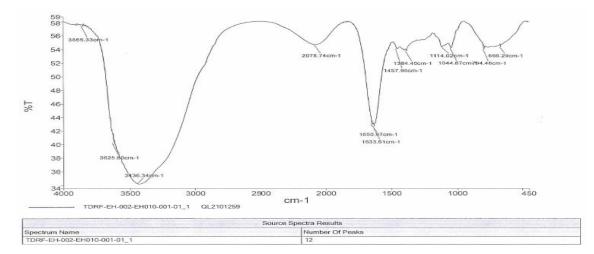


Fig. 5 (a). Infra-Red Spectroscopy of Aloe Capensis (Part A). Total 12 peaks obtained

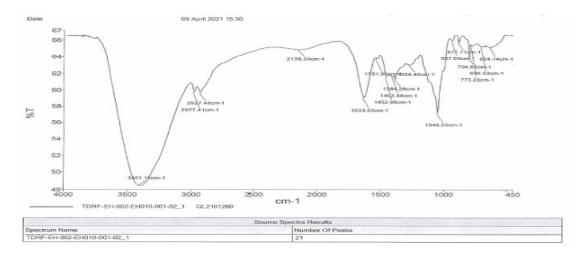


Fig. 5 (b). Infra-Red Spectroscopy of Aloe Capensis (Part B). Total 21 peaks obtained

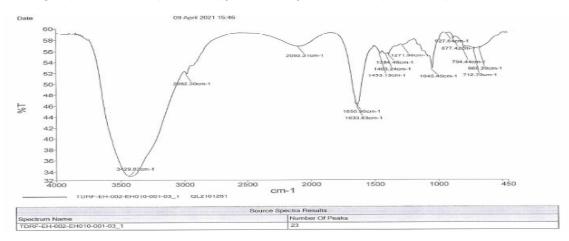
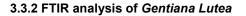


Fig. 5 (c). Infra-Red Spectroscopy of Aloe Capensis (mother solution); Total 23 peaks obtained



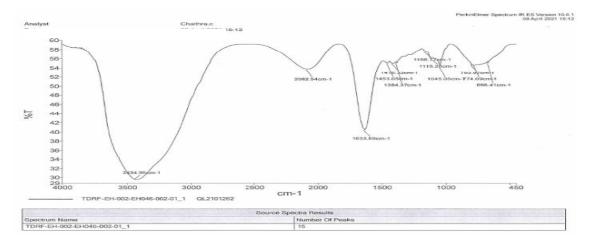
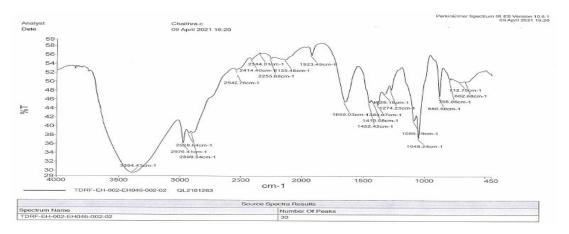
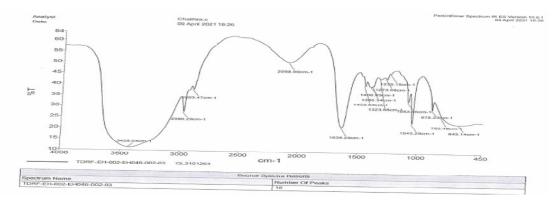


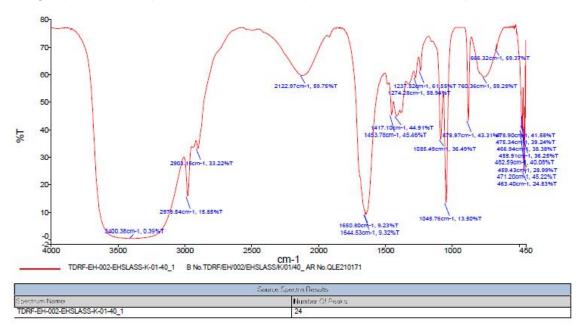
Fig. 6 (a). Infra-Red Spectra of Gentiana Lutea (Part A); Total 15 peaks obtained

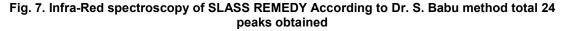












3.4 Preparation of SLASS an Electrohomeopathy Remedy according to Krauss Method

We took 50g of *Aloe capensis and 50g of Gentiana lutea* dried coarsely powdered plant materials and prepared the spagyric extrat mother solutions as method explained by Theodor Krauss in German Homeopathy Pharmacopoeia (GHP) method 30. Phytochemical analysis, Thin Layer Chromatography (TLC), and Fourier Transfer Infra-Red (FTIR) spectroscopy of the extracts, mother solutions and SLASS carried out and recorded.

Table 3. Yield of the extracts part A, part B and mother solutions

Yield	Part A. D1	Part B. D1	Part A D2	Part B D2	Moth. Sol. D3
Aloe Capensis (50 g)	250 ml	11 ml	2500ml	110ml	1100 ml
Gentiana Lutea (50) g	155ml	125 ml	1550ml	1250ml	12500ml

Table 4. Phytochemical screening of the extracts, mother solution and SLASS; Prepared according to the krauss method

SL NO	PHYTOCHEMICAL GROUP Test	1. Aloe capensis TDRF/EH/002/EH010/K/02/45			2. Gentiana lutea TDRF/EH/002/EH046/K/02/46			
		Part A	Part B	Mother tincture	Part A	Part B	Mother tincture	SLASS
1	ALKALOIDS	+	-	+	+	+	+	+
2	FLAVONOIDS	-	-	-	+	+	+	+
3	TERPENOIDS	+	+	+	+	+	+	+
4	FATS	-	-	-	-	-	-	-
5	CHOLESTEROL	-	-	-	-	-	-	-
6	TANNINS	+	+	+	+	+	+	+
7	SAPONINS	+	-	+	+	-	+	+
8	GLYCOSIDES	+	+	+	-	-	-	+

*+ Indicate Presence: - Indicate Absence

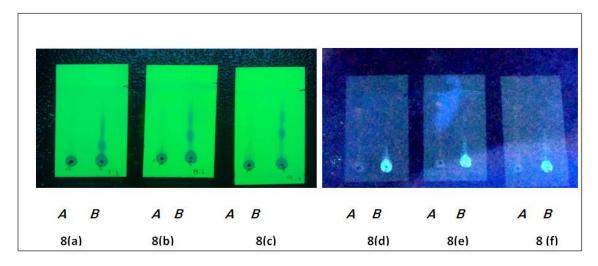


Fig. 8. TLC of the *Aloe Capensis* at different solvent systems and different UV wavelengths; 8(a) solvent system is 70% Ethyl acetate: 30% Hexane, 8(b) solvent system is 80% Ethyl acetate: 20% Hexane, 8(c) solvent system is 90% Ethyl acetate: 10% Hexane.8 (d) solvent system is 70% Ethyl acetate: 30% Hexane, 8(e) solvent system is 80% Ethyl acetate: 20% Hexane and 1(f) solvent system is 90% Ethyl acetate: 10% Hexane 3.5 Thin Layer Chromatography (TLC) Analysis for Both Mother Solutions (D3) of *Aloe capensis and Gentiana lutea* Mother Solutions (D3) & SLASS Prepared According to the Krauss Method

Thin Layer Chromatography (TLC) analysis for mother solutions of *Aloe capensis* and *Gentiana lutea* prepared according to the Krauss method. *Aloe capensis* (First spot (A) loaded) and *Gentiana lutea* (Second spot (B) loaded) on TLC plate was analyzed and images are captured and noted in Fig. 8.

The solvent system used as 70% Ethyl acetate: 30% Hexane (a), 80% Ethyl acetate: 20% Hexane (b), 90% Ethyl acetate: 10% Hexane(c). Images (a), (b), (c) are seen in UV 254 nm (Short wave), and (d), (e) (f) are seen in UV 365nm (Long wave).

3.5.1 Thin Layer Chromatography (TLC) analysis of SLASS prepared according to the Krauss method was analyzed and images are captured and noted in Fig. 9

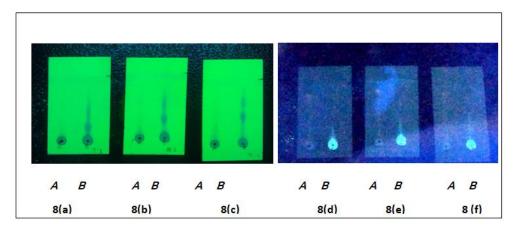


Fig. 9. Thin Layer Chromatography (TLC) analysis of SLASS prepared according to the Krauss method. The solvent system uses 70% Ethyl acetate: 30% Hexane 9 (a), 80% Ethyl acetate: 20% Hexane 9(b), 90% Ethyl acetate: 10% Hexane 9(c). Images (a), (b), (c) are seen in UV 254 nm (Short wave), and (d), (e) (f) are seen in UV 365nm (Long wave)

3.6 Fourier Transfer Infra-Red (FTIR) Spectroscopy of Extracts of *A. capensis* and *G. lutea* and SLASS Preparations According to the n Krauss Method of Spagyric Preparation as Mentioned in German Homoeopathy Pharmacopoeia (GHP) Method 30

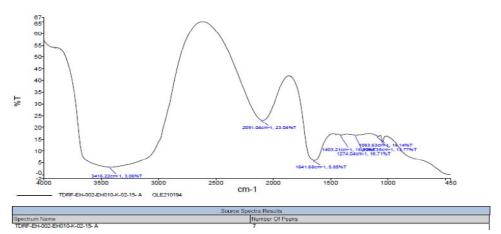


Fig. 10 (a). Infra-Red spectra of Aloe Capensis (Part A); Total 7 peaks obtained

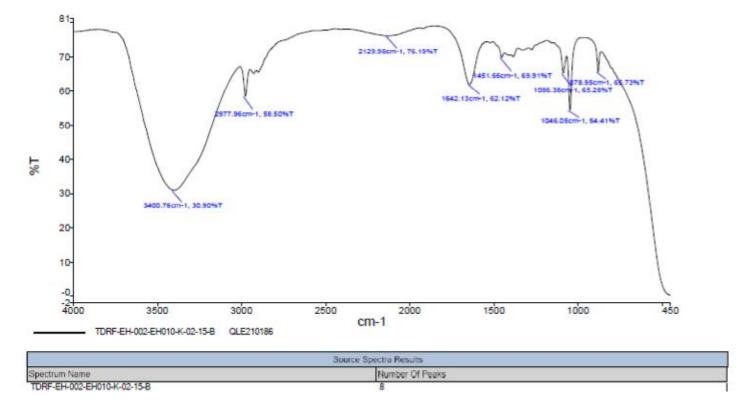


Fig. 10 (b). Infra-Red Spectra of Aloe Capensis (Part B). Total 8 peaks obtained

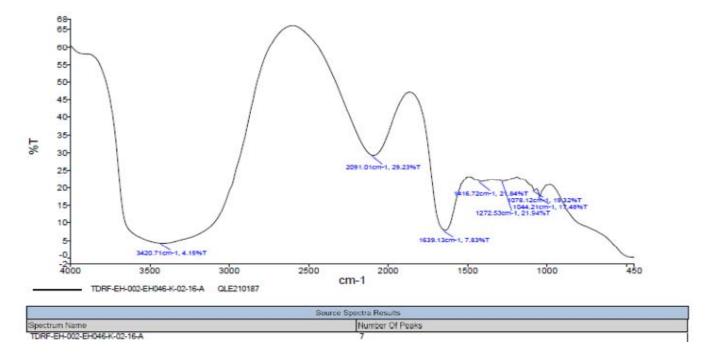


Fig. 11(a). Infra-Red Spectra of Gentiana Lutea (Part A). Total 7 peaks obtained

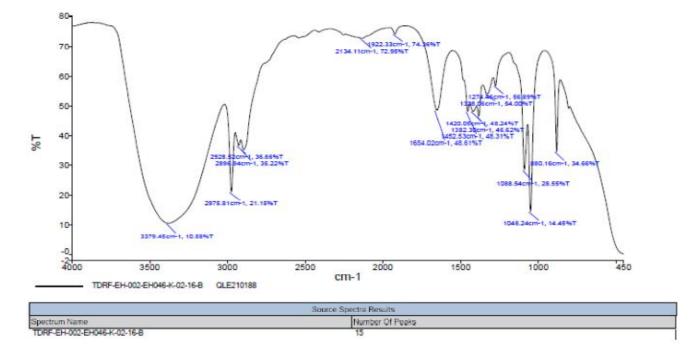


Fig. 11(b). Infra-Red Spectra of Gentiana Lutea (Part b); Total 15 peaks obtained

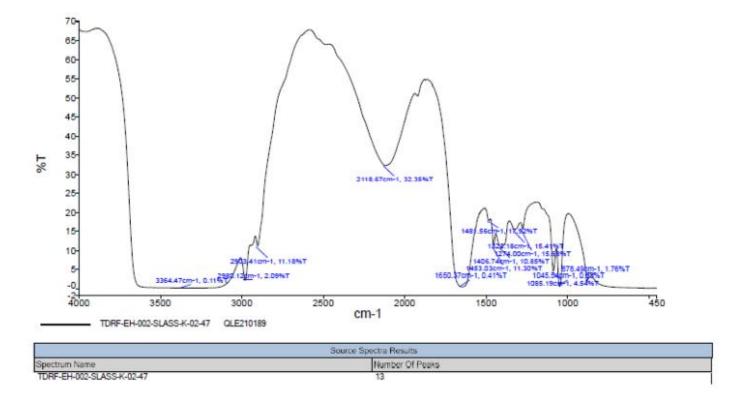


Fig. 12. Infra-red spectroscopy of SLASS remedy according to krauss method total 13 peaks obtained

4. CONCLUSION

The Electrohomeopathy or Electropathy is a purely herbal system of medicine has a history of nearly 160 to 170 years of existence with lots of differences among other existed medical systems in its unique principles, diagnosis, preparation and selection of remedies. SLASS is one of the common remedy using by electrohomeopathy medical practitioners. There is no documents available regarding the qualitative and phytochemical studies of the SLASS. Preparation of Slass according Krauss method and is compared with our modified method showing significant differences in qualitative analysis, TLC and FTIR studies. SLASS remedy according to our method Total 24 peaks found where as in Krauss method 13 Peaks observed Glicosides are present and yield is also less in Krauss method of preparation. Further detailed studies required for qualitative characterization of phytochemicals. Hence the present research investigations outcome with reproducibility will become standard markers or signatures to assess the guality, safety, and efficacy of the electro homeopathy medicine as well as reduce the risk of adulterations helps to develop specifications of the Electrohomeopathy remedies. Future work is essential to explore its therapeutic applications

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist

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