International Journal of Education and Science Research Review
E-ISSN 2348-6457
Volume-10, Issue-1 Jan-Feb-2023
P-ISSN 2349-1817
www.ijesrr.org
Email- editor@ijesrr.org

Wound healing effect of leaves of *Centella asiatica*.

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Plants have been used for health and medical purposes for several thousands of years. The number of higher plant species on earth is about 2,50,000. It is estimated that 35,000 to 70,000 species have, at one time or another, been used in some cultures for medicinal purposes. A majority of the world's population in developing countries still relies on herbal medicines to meet its health needs. Herbal medicines are often used to provide first-line and basic health service, both to people living in remote areas where it is the only available health service, and to people living in poor areas where it offers the only affordable remedy. Even in areas where modern medicine is available, the interest on herbal medicines and their utilization have been increasing rapidly in recent years. According to World Health Organization (WHO), about 80% of the world's population relies on medicinal plants for their primary health care needs both in the developing countries and developed countries where modern medicines are predominantly used.[1-3]

Since time immemorial man has used various parts of plants in the treatment and prevention of many ailments.[4] Historically all medicinal preparations were derived from plants, whether in the simple form of plant parts or in more complex form of crude extracts, mixtures, etc. Today, a substantial number of drugs are developed from plants which are active against a number of diseases.[5] The majority of these involve isolation of active ingredient (chemical compound) found in a particular medicinal plant and its subsequent modification. In the developed countries, 25 percent of drugs are based on plants and their derivatives and the use of medicinal[10]

WOUNDS

Wound is defined as the disruption of cellular and anatomic continuity of a tissue. According to the Wound Healing Society (WHS), wounds are physical injuries that result in an opening or break of skin that causes disturbance in the normal skin anatomy and function. They result in the loss of continuity of epithelium with or without the loss of underlying connective tissue.[13,14] This includes injury of underlying tissues / organs caused by surgery, a blow, a cut, chemicals, heat/cold, friction / shear force, pressure or as a result of

disease. Wound may arise due to physical, chemical, microbial agents, thermal or immunological damage to the tissue.

CLASSIFICATION OF WOUNDS

There is no definite method of classifying wounds. There are many different types of wounds ranging from mild to severe to potentially fatal.

Based on anatomical site

Wounds can be referred by their anatomical site, e.g. abdominal or axillary wound.

Based on underlying cause of wound creation

Wounds are classified as open and closed wounds.[16]

Open wound

In this case, blood escapes the body and bleeding is clearly visible. It is further classified as incised wound, laceration or tear wound, abrasions or superficial wounds, puncture wounds, penetration wounds and gunshot wounds.

Incised Wound

It is an injury with no tissue loss and minimal tissue damage. It is caused by a sharp object such as knife. Bleeding in such cases can be profuse, so immediate action should be taken.

Abrasions or Superficial Wound

It is caused by sliding fall onto a rough surface. During abrasion, the topmost layer of the skin i.e. epidermis is scraped off that exposes nerve ending resulting in a painful injury. Blood loss similar to a burn can result from serious abrasions.

Laceration Wound or Tear Wound

This is the nonsurgical injury in conjunction with some type of trauma, resulting in tissue injury and damage.

Puncture Wound

They are caused by some object puncturing the skin, such as needle or nail. Chances of injection in them are common because dirt can enter into the depth of wound.

Gunshot Wound

They are caused by a bullet or similar driving into or through the body.

Penetration Wound

Penetration wounds are caused by an object such as a knife entering and coming out from the skin.

Closed Wound

In closed wounds blood escapes the circulating system but remains in the body. It includes contusion or bruises, hematomas or blood tumor, crush injury etc.

Contusion or bruises

Bruises are caused by a blunt force trauma that damage tissue under the skin.

Hematomas or blood tumor

They are caused by damage to a blood vessel that consequently causes blood to collect under the skin.

Crush injury

Crush injury is caused when great or extreme amount of force is applied on the skin over long period of time.

Based on the basis of physiology of wound healing

Wounds are popularly categorized by their level of chronicity as either an acute or a chronic wound.

Acute Wounds

Acute wound is a tissue injury that normally precedes through an orderly and timely reparative process that result in sustained restoration of anatomic and functional integrity. Acute wounds are usually caused by cuts or surgical incisions and complete the wound healing process within the expected time frame.[12]

Chronic Wounds

Chronic wounds are wounds that have failed to progress through the normal stages of healing and therefore enter a state of pathologic inflammation. Chronic wounds either require a prolonged time to heal or recur frequently. Local infection, hypoxia, trauma, foreign bodies and systemic problems such as diabetes mellitus, malnutrition, immune deficiency or medications are the most frequent causes of chronic wounds.[13, 14]

Evaluation of formulation

The physical parameter such as color and appearance were observed for all the formulations. The color of prepared formulation was pale yellow and opaque and also it was smooth on application. The pH value of all formulations were studied using digital pH meter and it was found to be in the range of 6.5 – 6.8. All the formulations (F1-F6) showed a desirable pH quite similar to that of the skin pH.

Table 1 Evaluation of topical formulation

Batches	Color	Appearance	pH	Viscosity (cps)	Spreadability (g.cm/s)	Extrudability (%)
F_1	Pale yellow	Homogeneous	6.6 ± 0.01	23600 ± 88	28 ± 1.8	61.25 ± 5.6
F_2	Pale yellow	Homogeneous	6.7 ± 0.03	23340 ± 56	28 ± 2.4	67.88 ± 6.1
F_3	Pale yellow	Homogeneous	6.5 ± 0.12	25323 ± 71	28 ± 1.6	53.27 ± 7.3
F_4	Pale yellow	Homogeneous	6.5 ± 0.16	24730 ± 85	27± 2.6	60.11 ± 4.8
F_5	Pale yellow	Homogeneous	6.8 ± 0.18	27560 ± 58	31± 1.8	77.56 ± 5.8
F_6	Pale yellow	Homogeneous	6.8 ± 0.21	27646 ± 73	32 ± 1.8	81.21 ± 6.7

Data represented as mean ± SD, n=3

The viscosity of formulations was recorded. The viscosity of F5 and F6 was found to be 27560 and 27646 centipoises respectively which indicated that the formulations are easily spreadable by small amounts of shear. F5 and F6 showed a better spreadable property and extrudability than other formulations.

***In vitro* drug release studies**

Diffusion studies were carried out using Franz diffusion cell for F1–F6 formulations in pH 7.4 phosphate buffer saline solution. Percentage drug diffused for 8 h was reported in Table. 8.3. Drug release from F5 and F6 were found to be satisfactory. Hence F5 and F6 can be considered as the optimized formulation.

Table 2 *In vitro* drug diffusion study of formulation

Formulation	Cumulative % release				
	Time (h)				
	1	2	4	6	8
F_1	14.56 ± 1.3	21.78 ± 1.8	36.18 ± 3.8	41.25 ± 1.1	58.86 ± 4.5
F_2	16.87 ± 1.8	28.65 ± 0.8	31.58 ± 3.6	48.66 ± 0.8	58.14 ± 3.1
F_3	13.23 ± 1.6	20.01 ± 1.7	26.41 ± 2.5	45.80 ± 3.5	57.66 ± 5.1
F_4	15.18 ± 1.7	26.57 ± 1.8	33.28 ± 1.8	48.21 ± 2.6	67.25 ± 1.8
F_5	18.45 ± 1.8	21.45 ± 0.8	57.25 ± 4.3	67.54 ± 2.1	78.78 ± 2.1
F_6	22.12 ± 2.1	37.54 ± 2.8	58.14 ± 5.2	71.32 ± 1.5	82.18 ± 4.2

Data represented as mean ± SD, n=3

Table 3: Evaluation of Drug content of formulation

Formulation	Drug content (% w/w)
F1	61.53 ± 5.3
F2	58.77 ± 6.1
F3	68.12 ± 1.8

F4	70.04 ± 6.3
F5	81.55 ± 6.8
F6	86.17 ± 5.1

Data represented as mean ± SD, n=3

Table 8.5: Stability testing of selected formulation

Formulations	Properties	Storage conditions					
		25° ± 2°C / 65 ± 5%RH			40° ± 2°C/ 75 ± 5%RH		
		0 month	3 months	6 months	0 month	3 months	6 months
F5 (DVFO 2.5%w/w)	Colour	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow
	Appearance	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
	pH	6.8 ± 0.01	6.8 ± 0.03	6.8 ± 0.11	6.8 ± 0.06	6.8 ± 0.01	6.8 ± 0.31
F6 (DVFO 5%w/w)	Colour	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow
	Appearance	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
	pH	6.8 ± 0.03	6.8 ± 0.12	6.7 ± 0.06	6.8 ± 0.17	6.8 ± 0.23	6.8 ±0.07

Data represented as mean ± SD, n=3

Stability testing

Stability studies were performed for formulation F5 and F6 with 25 ± 2° C/ 60 ± 5% RH and 40 ± 2° C / 75 ± 5% RH conditions for 6 months. The results of stability studies are shown in Table 8.5. The samples were analyzed for 0 month, 3 months and 6 months interval for colour, physical appearance and pH. It was found that they were homogenous in appearance and the pH values were identical to the initial formulation. At both experimental conditions, the formulations F5 and F6 were found to be stable.

Compatibility studies

Fourier Transform Infrared Spectroscopy (FTIR)

FT-IR spectroscopy was employed to ascertain the compatibility of fraction with the base. The spectra of fraction and the final formulation containing excipients were compared for confirmation of common peaks. The ethyl acetate fraction, base and formulation were analyzed in a FT-IR instrument using KBr pelletization method and the spectrum is obtained in the wave number of 4000 cm^{-1} - 400 cm^{-1}.

The functional groups present in the fraction, base and formulation are shown in the Figure 8.1. FTIR spectrum of fraction includes 3367 cm^{-1} (polymeric OH stretch), 2927 $cm^{-1,}$ 2852 cm^{-1}(C-H group in aromatic ring), 2317 cm^{-1} (C=H stretching vibrations), 1645 cm^{-1}(C=O stretch), 1377cm^{-1}(phenol (or) tertiary alcohol, OH bend), 1137 cm^{-1} (C-O stretch) and 1048 cm^{-1} (C-C stretch).

The FTIR spectrum of formulation showed the peaks at 3369 cm^{-1} (polymeric OH stretch), 2924 $cm^{-1,}$ 2852 cm^{-1}(C-H group in aromatic ring), 2322 cm^{-1} (C=H stretching vibrations), 1658 cm^{-1}(C=O stretch), 1377cm^{-1}(phenol (or) tertiary alcohol, OH bend), 1137 cm^{-1} (C-O stretch) and 1048 cm^{-1} (C-C stretch).

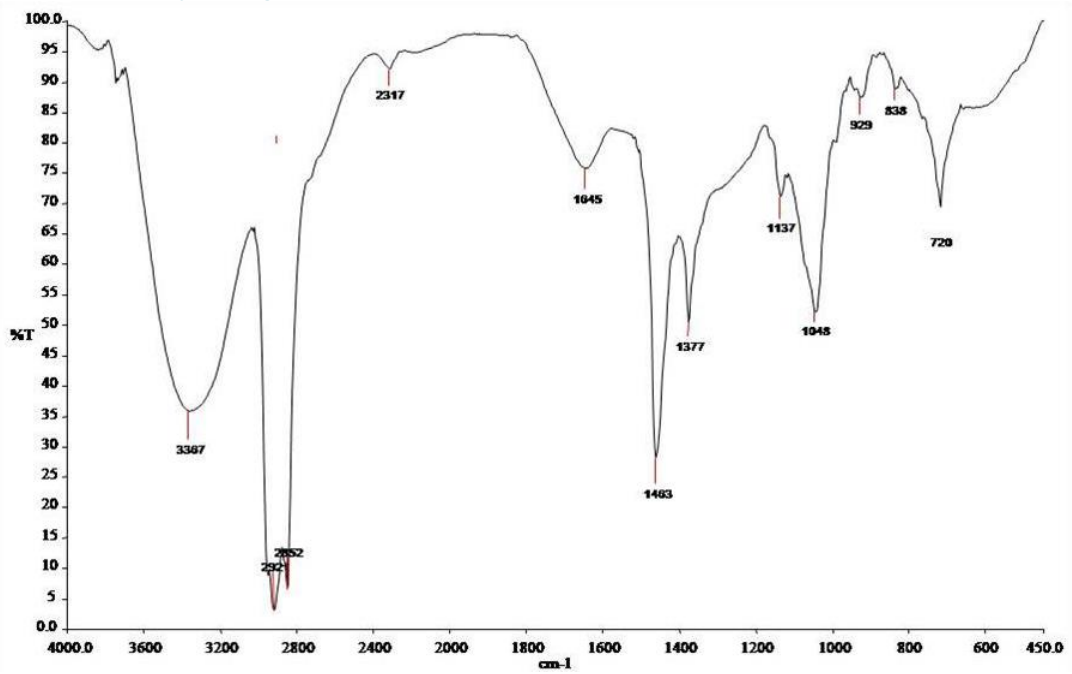

Fig (a): FTIR spectrum of fraction (EFDV)

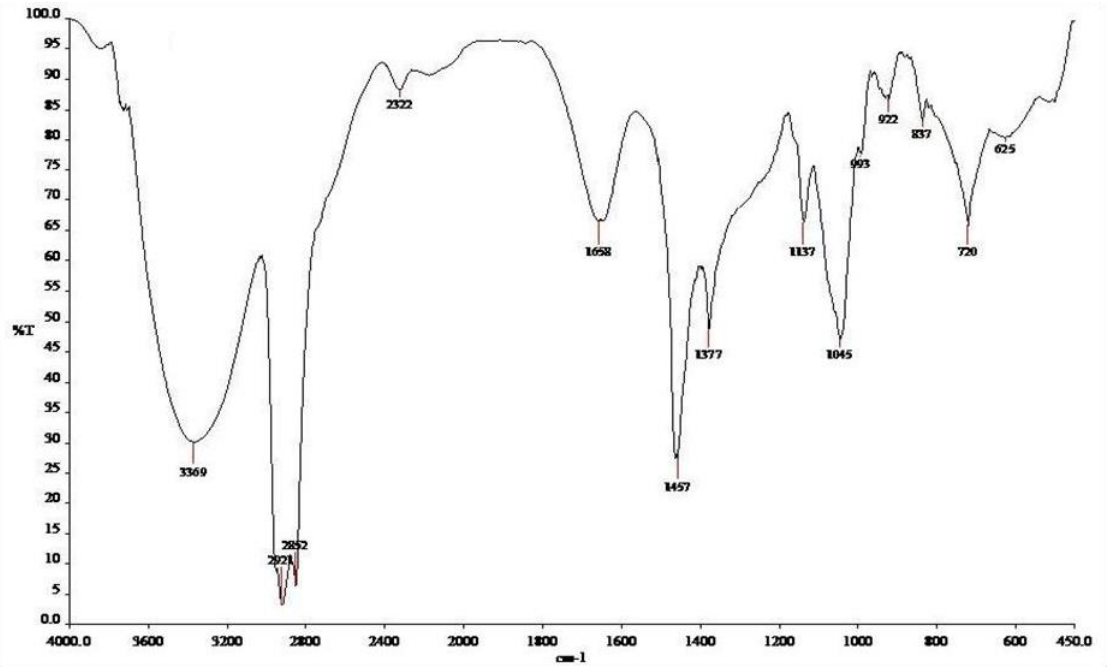

Fig (c): FTIR spectrum of formulation (F6)

Drug excipient compatibility study was carried out by FT-IR studies for fraction, base and formulation. IR spectra showed that the developed formulation of topical preparation showed similar peaks compared with the fraction. This indicated no interaction between the drug and the excipients.

DSC studies

DSC is a very important tool in carrying out drug excipient compatibility studies. The DSC thermograms for the ethyl acetate fraction of *Centella asiatica* and formulation with hydrophilic ointment base (F6) are shown in the figure 8.2. Melting endothermic peak was obtained at 132.2°C for fraction and in the formulation it was seen at 130.5°C. The images obtained from DSC studies suggested that there is no major shift in the thermogram of the formulation compared to the fraction which confirmed that there was no interaction involved between base and the fraction EFDV.

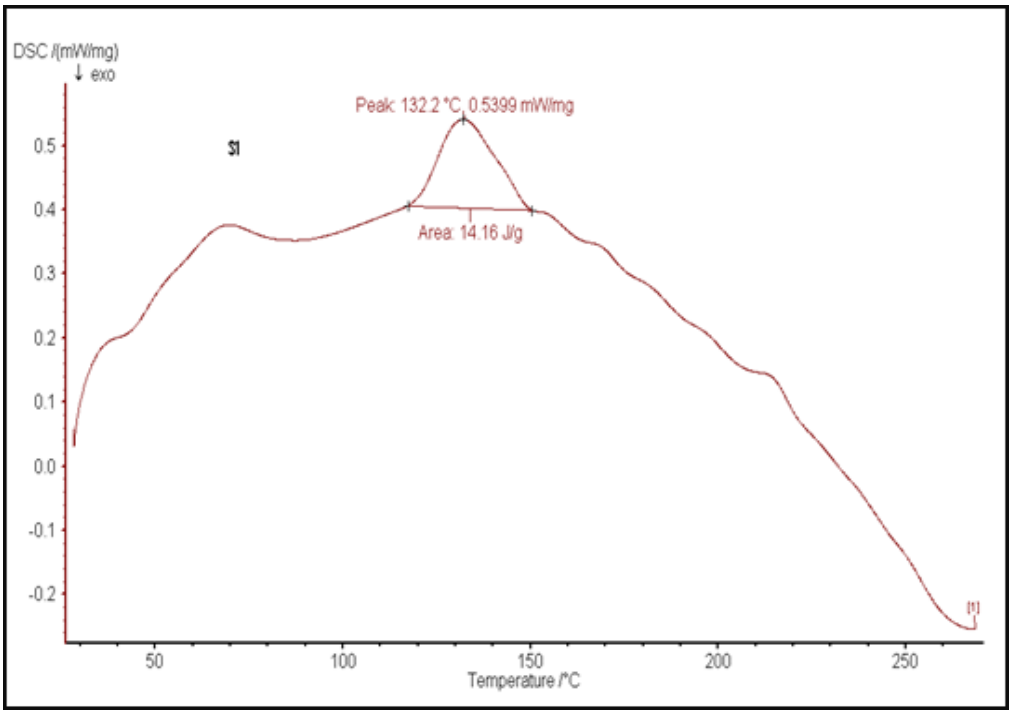

Fig. (a): DSC Thermogram of fraction (EFDV)

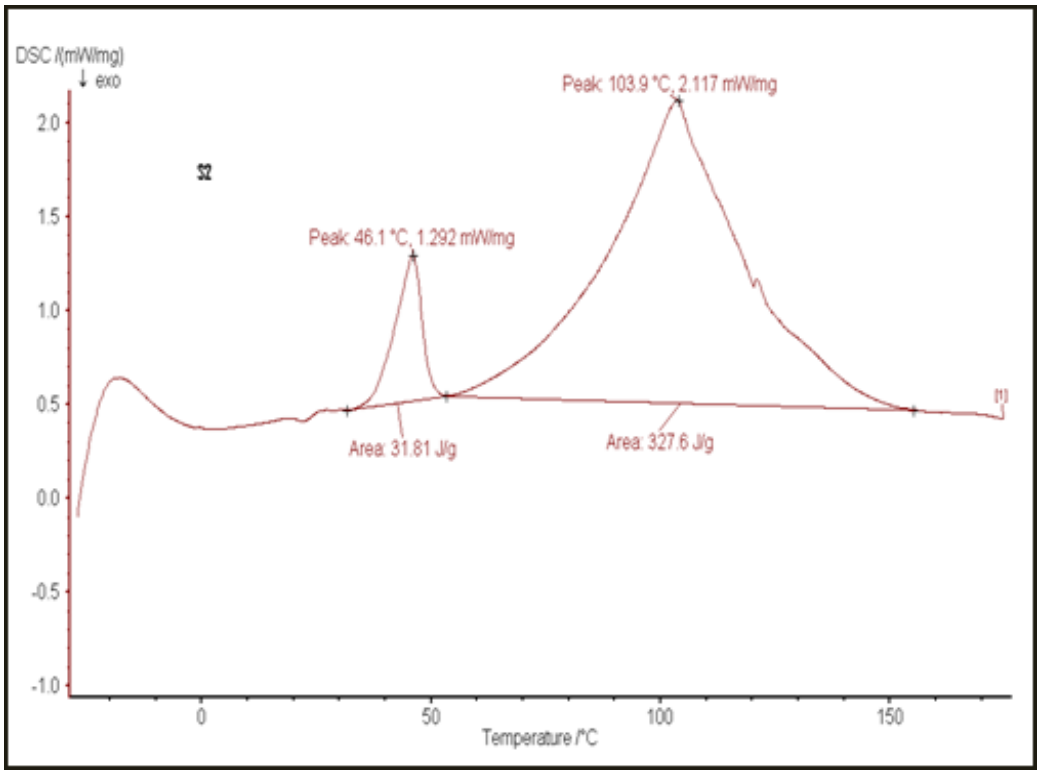

Fig. (b): DSC Thermogram of ointment base

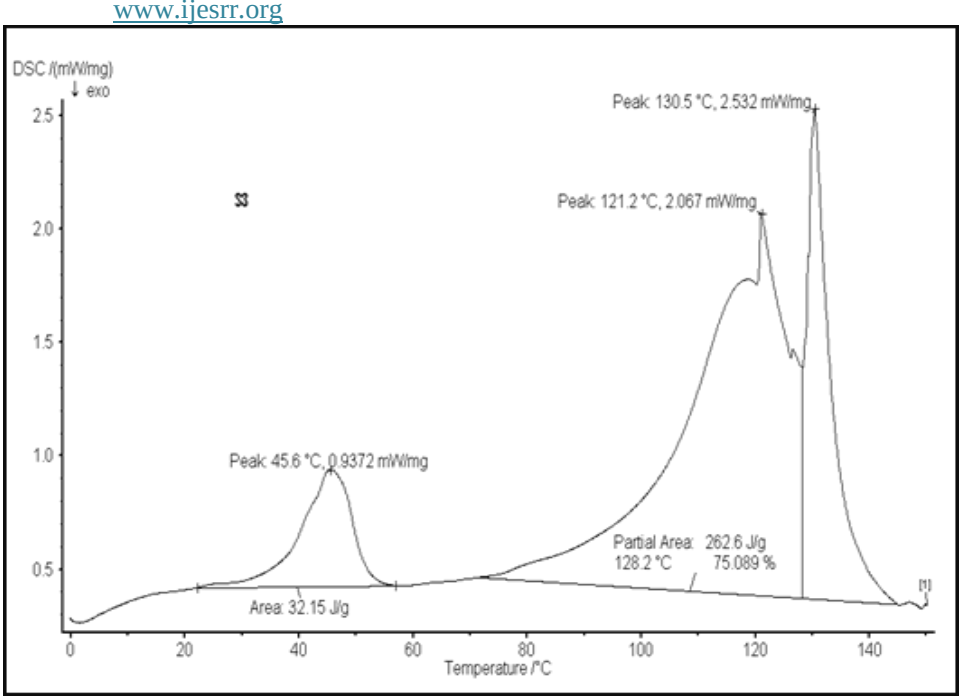

Fig. (c): DSC Thermogram of formulation (F6)

SUMMARY

Centella asiatica Linn. (Sapindaceae) is an evergreen woody perennial shrub distributed throughout India. This plant is commonly known as Hop bush plant in English and Sinatha in Hindi. The stem, leaves, seeds, roots, bark and aerial parts are used in traditional medicine. Traditionally, leaves are used in the treatment of fever, malaria, ulcers, diarrhea, dysmenorrhoea, rheumatism, sprains, bruises, burns and wounds. It is proved to have antibacterial, antiviral, analgesic, anti-inflammatory, antiulcer and antioxidant activity. Literatures showed the presence of flavonoids, diterpenoid acids, saponins, P-coumarin acid ester, sterols, essential oils and tannins. Based on the folklore claim and the phytoconstituents present, the plant *Centella asiatica* was selected for the study to explore the wound healing potential of this plant.

- Heavy metals such as lead, cadmium, mercury and arsenic were estimated and it was found to be within the limits of WHO standard. The elemental analysis of powdered drug was carried out to determine the levels of copper, zinc, sodium, potassium, selenium and iron.
- Physico-chemical and elemental analysis of powdered drug was carried out which will act as a standardization tool for future identification of the plant.

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