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PHARMACOGNOSTIC STANDARDISATION OF PARTS OF THE WHOLE PLANT OF THE *Manihot Esculenta, Crantz (L)* AND ITS CHARACTERISTIC EVALUATION AND CONDUCT OF STUDY FOR ITS IDENTIFICATION

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ABSTRACT

Traditional plant information offers a generally recognised natural phytoconstituents for the treatment of a broad variety of ailments via different sources. Despite of its tremendous/ enormous medicinal value, there is nescience on the species' standardisation parameters. As a result, the current work was conducted in order to include a detailed report on the quality management and standardisation parameters of *Manihot Esculenta, Crantz (L).* The aim or rationale of present research study was to make establishment of parameters for authentication and standardisation of different plant parts (leaves, stem, and root/tubers.); for examination. Methods like microscopy and macroscopy, physicochemical parameters, were used to establish pharmacognostical standards. Pharmacognostic investigation including organoleptic, morphological and microscopic characters with anatomy of leaf and stem of plant were performed with the regular standard laboratory procedures, and the results were noted. **Results:** The macroscopic, microscopy, of different parts of *Manihot Esculenta, Crantz (L).* revealed various identification characteristics in the species, captured in the microphotographs. **Conclusion:** This is the first study providing complete pharmacognostic profile of *Manihot Esculenta, Crantz (L).,* and hence will be useful for correct identification and authentication of the species for future studies. The present study was not available in authentic literature, before the study of this research.

KEYWORDS: *Manihot Esculenta*, Phyto-study, Pharmacognostic standardisation and study, Pharmacognostic evaluation of crude drug.

1. INTRODUCTION

Medicinal plants have been used to treat a number of illnesses since ancient times. A stepwise pharmacognostic study is needed for the identification and authentication of plants used as a source of medicines. The GPhP's main aim is to identify methods and policies for creating pharmacopoeial norms, with the ultimate goal of harmonisation in mind. Herbs include crude plant materials/ unprocessed plant materials, such as: leaves, flowers, fruit, seed, stems, wood, bark, roots, rhizomes or other plant parts, which may be entire, fragmented or powdered. Guidance for herbal medicine monographs has been used to outline the structure and contents of a herbal medicine monograph. The identification section's purpose is to ensure that the herbal medicine under examination/consideration is the one stated on the label. Illustrations may be used to support macroscopic and microscopic descriptions. Identification tests should be specific for the herbal medicine under examination. Typically, several identification tests, using independent approaches, are required in order to confirm the identity. The tests given in the identification section are not designed to give a full confirmation of the chemical structure or composition of the herbal medicine. They are intended to give confirmation, with an acceptable degree of assurance, that it is the one stated on the label. Test methods should be able to detect substitutes or adulterants that are likely to be found.[1] This is because of increased global interest in traditional medicine/ herbal medicines. Therefore, pharmacognostic studies not only ensure plant identity and establish standardisation parameters, but they are also useful for detecting and preventing adulterations. Such research ensures reproducible quality of herbal products, which leads to natural product safety and efficacy. Traditionally, medicines or herbal medicines have been widely used as an alternative or as a main herbal constituent in many countries, including the: United States of America, Australia, China, England, Greece, India, and the Philippines. Also found in the European pharmacopoeias, British pharmacopoeias, and the American pharmacopoeias, as well as in the Indian pharmacopoeias. Ph. Eur., B.P., U.S.P., and the I.P. are all abbreviations for European pharmacopoeia, British pharmacopoeia, United States of America Pharmacopoeia, and Indian pharmacopoeia; respectively. Finer or improved analytical methods for chemical compounds may aid in reducing variation and improving plant drug resolution. Toxicological testing is becoming increasingly important as contamination can occur at any stage of the process, from collection, storage, analysis, or

processing to the extraction of active principles. These parameters should be recorded for years; a database should be generated, recorded, and statistically analysed to see if there is a difference in the quality and quantity of the chemical compounds. Traditional Chinese medicine is a medical system that has been used for thousands of years to prevent, diagnose, and treatment of disease. It is based on the belief that qi (the body's vital energy) flows along meridians (channels) in the body and keeps a person's spiritual, emotional, mental, and physical health in balance.[2] Kampo medicines are traditional Japanese herbal medicines used in Japan for more than 1500 years. They were introduced from China and are now fully integrated into the modern health care system. Around 148 Kampo formulas are officially approved as prescription drugs and are covered by the national health insurance. Kampo medicinal practice in Japan differs from traditional Chinese herbal medicine in some aspects. Kampo medicine is based on Chinese herbal remedies first introduced into Japan via Korea around the 5th century. It was subsequently modified to suit the environment and cultural practices of the 17th and 18th centuries (the Edo era) and adapted to Japanese culture.[3] Oriental medicine aims to restore the body's balance and harmony between the natural opposing forces of yin and yang, which can block qi and cause disease. Oriental medicine includes acupuncture, diet, herbal therapy, meditation, physical exercise, and massage. Also called TCM, Traditional Chinese medicine.[4] Of all the traditional medical systems being practiced today, Greek medicine has the most in common with Ayurveda. Both systems are constitutionally based, and deal with the relative balance of certain vital fluids or humors. Each humor or *dosha* has its own basic constitutional type, and mixed types exist. The word *ama* in Ayurveda; has its equivalent in the word "crudities," a common term for toxins in Greek medicine.[6] In the old Apothecary shops, herbal medicines, in various forms, were personally formulated and compounded, as directed by the patient's physician. Simple raw ingredients and/or preformulated standard preparations were mixed together in the right proportions, and in the right form, to produce the desired effect. Not only the herbs or medicines you take, but their dosage, form of preparation, and mode of administration are important to maximise the medicine's effectiveness.[8]

1.1 The Greco-Roman centres explains the human status of various physical aspects on human body and understanding for its improvements and as an alternative therapy to medicines.[7]

Table: 1.

1. The Crown Centre.	Greek: Koruphe Latin: Vertex.
2. The Brow Centre.	Greek: Enkephalos Latin: Cerebrum.
3. The Throat Centre.	Greek: Trachelos Latin: Collum.
4. The Heart Centre.	Greek: Phrenes Latin: Cor.
5. The Gastric Centre, or Solar Plexus.	Greek: Gaster Latin: Abdomen.
6. The Generative Centre.	Greek: Gonades Latin: Genitalia.
7. The Root Centre.	Greek: Hieron Osteon Latin: Os Sacrum.

1.2. The Apothecary's art: making and taking herbal preparations, medicines[9]

1.2. a. Herbal teas: Herbal teas are made in two ways: infusions are made by steeping herbs in hot or boiling water, and decoctions are made by boiling or simmering herbs in water for 10 to 15 minutes to half-hour or more.

1.2. b. Species, or powders

Powdered herbs are one of the most effective and versatile ways to prepare and take herbs. Powders are flexible and can be used as the basis for a number of other recipes. Pills, tablets, teas, tinctures, and electuaries are examples of these.

1.2. c. Pills and capsules

It's safer to take a powdered herb or herb mixture as a pill or tablet if it tastes bitter or foul. The simplest approach for the home herbalist is to put the powder into gelatin capsules, with the normal dosage being two to three capsules three times a day. Pills are a bit more complex. Pills are a more elaborate affair. To transform a powder into tablets, it must first be made into a dough, which necessitates the use of a binding agent. Acacia gum, Karaya gum, and Corn Starch are examples of binding agents. Licorice root powder is an example of a formula element that can also serve as a binding agent.

1.2. d. Medicinal wines and tinctures

For many factors, alcohol is an excellent medium or foundation for producing medicines: Alcohol works well as a preservative. Herbs extracted in 80 proof distilled spirits have an unlimited shelf life. There are two types of alcoholic extracts: medicinal wines and tinctures. A medicinal wine is made with wine or "soft" spirits, while a tincture is made with distilled or "strong" spirits, which are normally 80 proof or 40% alcohol. Two heaping tablespoons

per cup, or 2 ounces per pint, of herb or herb mixture to wine or liquor is double the amount used in herb teas.

1.2.e. Herb juices

One of the most potent and powerful ways of taking a herb, if you are able to get it fresh, is to juice it. "Juicing some fresh Ginger rhizome in a juicer." Or a simple leamon juice, mixed with honey.

1.2.f. Syrups

Syrups are thick, creamy, and have a molasses-like consistency. They're a tasty way to take herbal medicines that would be too astringent, harsh, sour, or bitter otherwise. They're also great for cough syrups because they have a calming, coating effect. Syrups are a therapeutic vehicle that takes advantage of the sweet taste's calming and emollient properties. Syrups are made up of two components: the medicinal component and the sweetener, or foundation. There are three types of syrups, according to Culpepper, depending on how the medicinal portion is produced.

1) Syrups made from infusions,
2) Syrups made from decoctions,
3) Syrups made from juices.

Furthermore, these categories need not be mutually exclusive. A combination of any two, or all three, may be used for the medicinal extract portion.

1.2.g. Electuaries: Herbal pastes, or Jams.

An electuary, which is a herbal paste or jelly, is one of the most enjoyable ways to prepare and consume herbs. The electuary is the most complicated method of preparing herbs for internal use. An electuary is made up of two main ingredients: a powdered herb or herb mixture, and a sweetening and binding agent, typically clarified honey.

1.2.h. Simples versus formulas

A tea or other preparation made from just one herb is called a simple preparation. A medicinal preparation containing two or more ingredients is called a formula.

Adulterant is herbal material, a herbal constituent or other substance that is either deliberately or non-intentionally (through cross-contamination or contamination) added to a herbal material, herbal preparation or finished herbal product. Herbal dosage forms are the physical

form (liquid, solid, semi-solid) of herbal products produced from herbs, with or without excipients, in a particular formulation (such as: decoctions, tablets and ointments). They are produced either from herbal materials (such as: dried roots or fresh juices) or herbal preparations (such as: extracts).

1.3. Medicinal plants used by ancient Greek physicians

The climate in Greece is perfect/ideal for growing plants and herbs, which can be found in abundance on mountain sides where they naturally grow. The plants present in Greece today are the same plants that were collected thousands of years ago in ancient Greece, and their uses have remained unchanged, including not only flavoring delicious Greek cuisine but also for medicinal purposes. To mention few: Mint, Sideritis, Greek Mountain tea, Fennel, Olive, Parsley, Saffron, Licorice, Mandarke, Marshmallow, Balkan Penoy. Few of the other medicinal plants used and found in the U.K. are: Wild garlic (*Allium Ursinum*), Fennel (*Foeniculum Vulgare*), Stinging nettle (*Urtica Dioica*), Borage (*Borago Officinalis*), Corn mint (*Mentha Arvensis*), Dandelions (*Taraxacum Officinale*), Ground elder (*Aegopodium Podagraria*), Rosemary *(Salvia Rosmarinus) (Rosmarino, in Italian).*[13] Other plants include Ferula Assafoetida, also knowns as devil's dung known because of its strong odour. English names: Asafoetida, derived from Latin Fetid. Merde du Diable meaning devil's shit in French, Teufelsdreck in Deutsch, Duiwelsdrek in Afrikaans, and Asafedity in the U.S.; is a dried latex obtained as (gum oleoresin), which is an exudate of rhiziome or tap-root used as a condiment in food preparations. Oregano, (Origano, in Italian), Bay leaves (Alloro, in Italian), Basil (Basilico, in Italian), Parsley (Prezzemolo, in Italian).[26]

1.4. Other required regulatory information of herbal medicines

1.4.1. Snippet

Because of the high hepatotoxic, mutagenic, and cancerogenic effects of pyrrolizidine alkaloids (PAs) the German Federal Institute for Risk Assessment (BfR) recommends not to exceed a daily PA intake of 0.007 µg/kg body weight (0.42 µg/60 kg adult). In a recent study conducted by the BfR, up to 5647 µg PA/kg dried herbal material were detected in tea products marketed as food.[10]

1.4.2. Snippet

Risk assessment on the use of herbal medicinal products containing pyrrolizidine alkaloids.

Pyrrolizidine alkaloids (PAs) are amongst the most widely occurring natural toxins. Pyrrolizidine alkaloids (PAs) are toxins biosynthesised by plants and are known to be present

in approximately 6000 plant species, about 3% of all flowering plants. PAs are probably the most widely distributed natural toxins and represent a potential risk to human health, since poisoning caused by these toxins is associated with acute and chronic liver damage and may lead to death. One of the most common sources of PAs exposure in humans is honey consumption. There are developed methods which are quick and easy method to quantify nine different PAs (echimidine, heliotrine, intermedine, lycopsamine, lasiocarpine, retrorsine, seneciphylline, senecionine, senkirkine), in honey based on QuEChERS sample extraction and ultra-fast liquid chromatography coupled with mass spectrometry detection. A performed validation study of the method and it resulted in good precision and accuracy, high recoveries, and good linear calibrations were found. The limit of detection ranged from 0.021 to 1.39 μg Kg^{-1} and the limit of quantification from 0.081 to 4.35 μg Kg^{-1}. This new approach was applied to the quantification of PAs in retail honeys purchased in local supermarkets, classified by their country of origin: Italian honeys, blends of honey of European countries and blends of honey of European and non-European countries. The concentrations detected ranged from 1 to 169 μg PAs/kg^{-1} with higher concentrations in blends of European and non-European honeys. This study reveals that many samples tested would exceed the tolerable daily intake suggested for these substances and they could be a hazard to human health.[11]

1.4.3. Snippet

The cytotoxic capacity of the essential oils based on a prooxidant activity can make them excellent antiseptic and antimicrobial agents for personal use, i.e., for purifying air, personal hygiene, or even internal use via oral consumption, and for insecticidal use for the preservation of crops or food stocks.[14]

1.5. *Manihot Esculenta Crantz*

Manihot Esculenta Crantz is a dicotyledonous, perennial woody shrub that grows to 1-3 m in height and thrives in tropical climates. Cassava is a member of the *Euphorbiaceae* (Surge) family and has the botanical name *Manihot Esculanta*. Other common names for cassava include manioc, manihot, and yucca. *Manihot Esculenta Crantz* is a tropical-adapted dicotyledonous perennial woody shrub that grows to a height of 1-3 meters. *Manihot Esculanta* is the botanical name for cassava, which belongs to the *Euphorbiaceae* (Surge) family. Cassava is also known by the name's manioc, manihot, and yucca.[16] Cassava comes in varieties, ranging from low cyanide content (known as cassava) to higher cyanide content (known as bitter cassava). Bitter cassava requires more/extensive processing (sometimes

more than one day) than sweet cassava to remove the cyanogenic potential. (A Human Health Risk Assessment Technical Report Series no. 28). Cassava, also known as *Manihot Esculenta Crantz*, is a sweet, edible variety grown for its starchy tuberous roots, which feed over 500 million people. Since raw cassava contains cyanide, which is poisonous when consumed, it is vital to prepare it properly. Cassava comes in two varieties: sweet and bitter. Bitter cassava is tougher, but it contains much higher cyanide content. Most of the cassava used in the United States of America is sweet.[21] The crop is an important source of carbohydrate for humans and animals, with higher energy than other root crops, and is mostly grown on a small scale by farmers in developing countries because of rigidity and tolerance to the adverse environment. It is also grown in developed countries like U.S.A. in Brazil and Argentina. *Manihot Esculenta Crantz*. (*Euphorbiaceae*) also called Cassava, manioc, yuca, balinghoy, mogo, mandioca, kamoteng kahoy, tapioca is a perennial woody shrub in the *Euphorbiaceae* (spurge family) native to South America but now grown in tropical and subtropical areas worldwide for the edible starchy roots (tubers), which are a major food source in the developing world, in equatorial regions including Africa, South America, and Oceania. Also known as yuca, the dried root is the source of tapioca. The cassava shrub may grow to 2.75 m tall, with leaves deeply divided into 3–7 lobes. This food plant is also medicinally used to treat hypertension, headache, and other pains, irritable bowel syndrome and fever.[19]

Cassava (*Manihot Esculenta Crantz*) is a perennial shrub with a bulky storage root. The dry matter content of approximately 35% is mostly made up of starch; protein content being low. Both roots and leaves are used in food preparation.[20] Cassava (*Manihot Esculenta*) is a tuber vegetable used for both culinary purposes and in folk medicine.[21] All parts of the plant contain cyanogenic glycosides. Upon processing these are hydrolyzed to release cyanohydrins, which in turn release the toxic HCN. Anti-nutrients and other bioactive agents present in roots/leaves also include coumarins, nitrate, oxalate, phytic acid, saponins, tannins, and trypsin inhibitors. Roots with yellow parenchyma have elevated contents of carotenoids; some with vitamin A effect. Fresh peeled roots may be subjected to one of the following unit processes: drying, soaking (retting, steeping), cooking, frying, size reduction (grating), or fermentation. Leaves may be pounded and cooked. The fate of the cyanogenic glycosides, coumarin, scopoltin, and carotenoids during processing is reviewed.[20] It does, however, contain compounds believed to be anti-inflammatory and antioxidant, including phenolic acids, anthraquinones, saponins, and alkaloids.[21]

Manihot Esculenta tubers and leaves contain cyanides in two different forms: i) the glycosides; linamarin and lotaustraline which are considered "bound" and ii) the non-glycosides; hydrogen cyanide (HCN) and cyanohydride which are considered "free". Free cyanide comprises 8%-12% of the total tuber cyanide. This cyanide can, under some circumstances, lead to human toxicity problems and cassava for food used to be processed to remove cyanide-containing substances.[16] Free HCN has a lethal dose of 50-60 mg for an adult, but the toxicity of bound HCN is less well known. The endogenous enzyme linamarase, which is located in the human digestive tract, hydrolyses the glycosides to HCN. By releasing HCN from the glycosides, all conventional cassava processing methods minimise or eliminate toxicity. Since HCN is water soluble and has a high boiling point, by releasing HCN from the glycosides, all conventional cassava processing methods minimise or eliminate toxicity. HCN can be dissolved by soaking since it is soluble in water and has a boiling point of 25°C. Since the glycoside limamatine is heat resistant and the enzyme linamarase is inactivated at 75°C, boiling fresh cassava has no impact on its toxicity.

While most processes depend on enzymatic hydrolysis to lower glycoside concentrations, the degree of glycoside breakdown is largely determined by fermentation time in operation.[18]

Wild *Manihot* and *Manihotoides* Species

Table 2: The wild relative of cassava is referred to as *M. Esculenta* subsp. *Flabellifolia*.[16]

Species	Approximate Geographical Region
M. acuminatissima,	eastern Brazil.
M. aesculifolia,	Mexico.
M. affinis,	southern Brazil.
M. alutacea,	central Brazil.
M. angustiloba,	southwest USA, Mexico.
M. anisophylla,	Argentina.
M. anomala,	central Brazil, Paraguay.
M. attenuate,	central Brazil.
M. auriculata,	Mexico.
M. brachyandra,	northeastern Brazil.
M. brachyloba,	central America, west Indies, northern & central south America.
M. caerulescens,	northern, northeastern, and central Brazil.
M. carthaginensis,	Colombia, Venezuela, west Indies.
M. catingae,	northeastern Brazil.
M. caudate,	Mexico.
M. cecropiaefolia,	central Brazil.
M. chlorosticta,	Mexico.
M. condensate,	Bolivia.

Species	Approximate Geographical Region
M. corymbiflora,	southeastern Brazil.
M. crassisepala,	Mexico.
M. crotalariaeformis,	central Brazil.
M. davisae Croizat,	southwest USA, Mexico.
M. dichotoma,	northeastern Brazil.
M. divergens,	central Brazil.
M. epruinosa,	northeastern Brazil.
M. falcate,	central Brazil.
M. filamentosa,	Venezuela.
M. flemingiana,	central Brazil.
M. foetida,	Mexico.
M. fruticulose,	central Brazil.
M. glaziovii,	northeastern Brazil; introduced throughout tropical America, Africa, India, Pacific Islands.
M. gracilis,	central Brazil, Paraguay.
M. graham,	southeastern Brazil, northern, Argentina, Paraguay, Uruguay.
M. guaranitica,	Bolivia.
M. handroana,	eastern Brazil.
M. hassleriana,	Paraguay.
M. heptaphylla,	northeastern Brazil.
M. hunzikeriana,	southern Brazil, Argentina.
M. inflate,	southern Brazil.
M. irwinii,	central Brazil.
M. jacobinensis,	central Brazil.
M. janiphoides,	eastern Brazil.
M. jolyana,	southeastern Brazil.
M. leptophylla,	Ecuador, Peru, western and central Brazil.
M. leptopoda,	southeastern Brazil.
M. longepetiolata,	central Brazil.
M. maguireiana,	Venezuela.
M. maracasensis,	northeastern Brazil.
M. marajoara,	northern Brazil.
M. michaelis,	Mexico.
M. mirabilis,	Paraguay.
M. mossamedensis,	central Brazil.
M. nana,	central Brazil.
M. oaxacana,	Mexico.
M. oligantha,	central Brazil.
M. orbicularis,	central Brazil.
M. paviaefolia,	central Brazil.
M. peltate,	central Brazil.
M. pentaphylla,	central Brazil, Paraguay.
M. Pilosa,	eastern Brazil.
M. pohlii,	eastern Brazil.
M. populifolia,	Paraguay.
M. pringlei,	Mexico.
M. procumbens,	southern Brazil, Paraguay.

Species	Approximate Geographical Region
M. pruinose,	central Brazil.
M. pseudoglaziovii,	northeastern Brazil.
M. purpureo-costata,	central Brazil.
M. pusilla,	central Brazil.
M. quinquefolia,	northeastern Brazil.
M. quinqueloba,	central Brazil.
M. quinquepartite,	northern and central Brazil.
M. reniformis,	northeastern Brazil.
M. reptans,	central Brazil.
M. rhomboidei,	Mexico.
M. rubricaulis,	Mexico.
M. sagittato-partita,	eastern Brazil.
M. salicifolia,	central Brazil.
M. sparsifolia,	central Brazil.
M. stipularis,	central Brazil.
M. stricta,.	Peru, western and central Brazil
M. subspicata,	Mexico.,
M. surinamensis	Venezuela, Guayana, Suriname.
M. tenella,	southern Brazil.
M. tomatophylla,	Mexico.
M. tomentosa,	central Brazil.
M. tripartite,	central and eastern Brazil.
M. triphylla,	central Brazil.
M. tristis,	Venezuela, northern Brazil.
M. variifolia,	Paraguay.
M. violacea,	central Brazil.
M. walkerae Croizat,	southwest USA, Mexico.
M. warmingii,	eastern Brazil.
M. websterae,	Mexico.
M. weddelliana,	central Brazil.
M. xavatinensis,	central Brazil.
M. zehntneri,	northeastern Brazil.
Manihotoides pauciflora,	Mexico.

1.5.a. *Manihot* is also used in

i. Diabetes

Cassava is a cellulose-rich insoluble fiber. This is a type of dietary fiber that aids in digestion and helps prevent constipation and diverticular diseases. It is also believed to be a prebiotic, a type of fiber that promotes the growth of probiotic bacteria as it ferments in the intestines.[22]

ii. Diarrhea

Despite cassava's ability to ease constipation, a 2015 study in the Journal of Ayurvedic and Integrative medicine suggests that an alcohol-based cassava leaf extract can also treat occasional diarrhea. For this study, lab mice with induced diarrhea were given either an oral

dose of the cassava leaf extract or one of two antidiarrheal drugs (loperamide or atropine sulfate). According to the researchers, mice given cassava achieved the same relief of symptoms as those prescribed such as: loperamide. At higher doses, the cassava extract was seen to be comparable to atropine sulfate in its slowing of intestinal motility.[22] MEC extracts decreased intestinal fluid volume in dose dependent manner no extract group was comparable with standard drug loperamide (5 mg/kg). MEC extracts also significantly inhibited gastrointestinal motility in dose dependent manner. MEC (100 mg/kg) and MEC (200 mg/kg) were comparable with standard drug atropine sulfate (5 mg/kg) in this aspect, <0.05 were considered to be significant. Ethanolic extract of MEC leaves exhibited significant antidiarrheal activity by decreasing intestinal fluid accumulation and the gastrointestinal motility in Wistar rats.[24] An outbred albino rat, genetically modified for testing purposes.

1.5.b. Manihot side effects

i. Allergies to latex: Cassava might cause an allergic reaction in people who are sensitive to latex.[23]

Anti allergic drugs such as NSAIDS can be prescribed in such cases where adverse event is noted.

1.5.c. Chemical constituents

(ppm unless otherwise indicated) Amentoflavone: LfME048 Ascorbic acid: RtME017 Caffeic acid: RtME017 Ent-kaurene: RtME006 Ent-primara-8(14)-15-diene: RtMEO06 Glucose: RtME017 HCN: Lf 0.018_0.180% ME030, ME035, StME007, Flou rME009, MEOlO, T u berME013, ME012.[25]

1.5.d. Pharmacological activities and clinical trials

Antibacterial activity. Ethyl acetate extract of dried aerial parts, at a concentration of 1.0 mg/ml on agar plate, was active on *Staphylococcus Aureus.* Water and acetic acid extracts of the dried aerial parts, at concentrations of 1.0 mg/disk, were inactive on *Escherichia Coli*, and the water extract was inactive on *Staphylococcus Aureus*.

2. MATERIALS AND METHODS

2.1. The plant material was collected from various locations in the Nashik, and Bombay regions, which have the same habitat requirements as *Manihot Esculenta, Crantz (L)*, i.e., Bombay region; also known as (Navi-Mumbai), Victoria Terminus-CSMT. Botanical specimens and prepared herbariums were collected and stored at the Botanical Survey of

India in Pune. A certificate of authentication with a specimen identification number was obtained in 2015. Fresh sections of plants, such as: leaves, roots, and stems, were collected from fully grown and stable plants. These obtained parts were used for macroscopical and microscopical research, as well as for identification studies. To remove impurities, whole plants were washed and dried in the shade, and dried parts such as: leaves and stems were stored in airtight bags or containers for later use. The plants were also grown in-house so that they could be thoroughly studied.

2.1.2. Plant authentication

The plant of *Manihot Esculenta Crantz* were grafted and grown with asexual propagation, apomixis vegetative reproduction in Maharashtra, Nashik, Nashik, India. The plant specimen: steam, leaves, and tubers; along with a plant herbarium, and photographs were taken to the 'Botanical survey of India,' Pune. Where the herbarium and sample specimens of plants were submitted to the 'Botanical survey of India,' Pune, western region centre, Koregaon road, Pune-411001, for the further plant authentication purpose. Later, the plant authentication certificate was collected from the 'Botanical survey of India,' Pune.

2.2. Pharmacognostic characteristics of plant

2.2.1. Macroscopic Evaluation

The colour and shape of various plant parts, size, fracture of stem, inflorescence, and leaf characteristics such as: margin, apex, colour, odour, base, surface, venation, leaf arrangement, and so on were evaluated for *Manihot Esculenta, Crantz (L).* Leaf size and petiole length, for example, were both measured and recorded. All macroscopic and microscopic evaluations were completed.

2.2.2. Morphological characterisation of *Manihot Esculenta Crantz,* (Macroscopy)

2.2.3. Morphological characterisation of leaf

2.2.3.A. Colour of apical leaves

i. The colour of the apical leaves were visually inspected, identified, and noted.

2.2.3.B. Colour of petiole and orientation of petiole

ii. Colour of petioles were visually inspected, identified and the middle part/region of the stem was observed for the orientation of petiole and were noted.

2.2.3.C. Colour of leaf and number of leaf lobes

iii. Colour of leaf was visually inspected, were identified and the number of leaf lobes were observed and, were noted.

2.2.3.D. Shape of central leaf

iv. Shape of central leaf was inspected visually, observed and, noted.

2.2.4. Morphological characterisation of stem

2.2.4.A. Prominence of foliar scars and colours of stem exterior

i. Observed from the middle third of the plant stem and, noted down.

2.2.4.B. Colour of stem epidermis

ii. The epidermis layer was peeled and scraped off with the aid of a sharp operation blade: scalpel, or bistoury, inspected visually and the colour was noted.

2.2.4.C. Branching habit

Observation was made at the lowest and, the first branching site.

2.2.5. Morphological characterisation of tubers

2.2.5. A. Tuber shape, external colour and texture of the tuber surface

i. The occurrence of root shape; the colour of the tuber was observed inspected visually and noted. The texture of tuber surface was observed and noted with the sense of touch.

2.2.5. B. Colour of tuber cortex

ii. Colour of root cortex was observed by peeling off the epidermal layer of the tuber.

2.3. Microscopic evaluation

Thin transverse sections of fresh root, leaf and stem were taken. To obtain a clear image, the sections were treated with chloral hydrate solution. Thin transversal sections were stained to detect and localise chemicals in the tissues such as: starch, proteins, lignin, calcium oxalate, and mucilage.

Chemicals such as: calcium oxalate, lignin, mucilage, proteins, and starch were detected and localised in tissue sections using staining.

2.3.1. Microscopy of

2.3.1.a. Leaf

i. Using a sharp operation blade: scalpel, or bistoury, a thin transverse section of the leaf was obtained. Thin transverse parts were cut and placed in the watch glass to be stained with the staining medium, which was prepared and standardised in the laboratory.

2.3.1.b. Stem

i. A sharp operation blade: scalpel, or bistoury, was used to cut a thin transverse portion of the stem. Thin transverse parts were cut and placed in the watch glass to be stained with the staining medium, which was prepared and standardised in the laboratory.

2.3.1.c. Tuber

Using a sharp operation blade: scalpel, or bistoury, a thin transverse section of tuber was collected. Thin transverse parts were cut and placed in the watch glass to be stained with various staining mediums that were prepared and standardised in the laboratory.

3. RESULT AND DISCUSSIONS

3.1. Plant authentication

The plant was identified to be *Manihot Esculenta Crantz* by the 'Botanical survey of India,' Poona with certificate number as BSI/WRC/Tech./ 2015/SDB-1; which is attach in the appendix section. As shown in figure 2 below, the herbarium was submitted to the 'Botanical Survey of India,' Pune.

1. Kingdom: Plantae,
2. Subkingdom: Angiosperms,
3. Division: Angiosperms,
4. Class: Eudicots,
5. Order: Malpighiales,
6. Family: Euphorbiaceae,
7. Subfamily: Cortonoideae,
8. Tribe: Manihoteae,
9. Genus: Manihot,
10. Species: *M. Esculenta.*
11. Binomial name: *Manihot Esculenta.*
12. Saurabh D. Bhandare, Nashik, India, collected, identified, and authenticated the specimens before obtaining the official identification certificate.

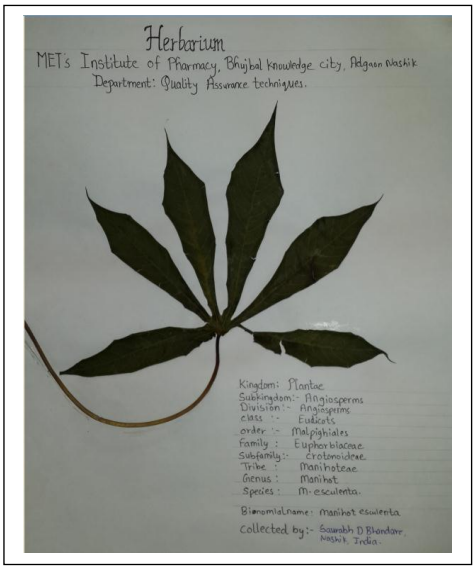


Figure 2: Photograph of the herbarium submitted to the, 'botanical survey of India,' Pune.

3.2. Herbarium: A collection of dried plant specimens mounted on sheets of paper. The mounted plants are labeled with their scientific names, the collector's name, and, in most cases, information about where they were collected, how they grew, and general observations. A herbarium is a valuable conservatory of flora plant material. Collected from various parts of the world. As a result, they provide basic material for studying the flora and vegetation of various places or regions in one location.

3.2.1. Herbarium preparation

Table: 3.

1. **Step 1:** Collecting-where to collect.	2. **Step 1:** Collecting-what to collect.
3. **Step 2:** Preparation-protecting the specimens.	4. **Step 2:** Preparation-arranging the specimens.
5. **Step 3:** Pressing-Pressing the specimens.	6. **Step 4:** Mounting-Mounting the specimens.

Figure 3: Photographs that are used to authenticate the plants at identification site or botanical centre.

3.3. Pharmacognostic characterisation of plant

3.3.1. Morphological characterisation of *Manihot Esculenta Crantz,* (Macroscopy)

3.3.1.1. Morphological characterisation of leaf

The morphological characteristics of the leaf of *Manihot Esculanta Crantz* were studied, as shown in figure 4 below, and the findings/observations are summarized in table 4 below.

Table 4: Morphological characterisation of *Manihot Esculenta Crantz,* leaf.

Parameter	Observation
a) Colour of apical leave,	Light green.
b) Colour of petiole and orientation of petiole,	Reddish green, inclined downwards.
c) Colour of leaf and number of leaf lobes,	Dark green, 7 lobes.
d) Shape of central leaf,	Lanceolate.
e) Size of the leaf,	10-12 cm.

Figure 4: Morphological features of *Manihot Esculenta Crantz,* leaf.

3.3.1.2. Morphological characterisation of stem

The morphological characteristics of the stem of *Manihot Esculenta Crantz* were investigated, and the results are shown in figure 5 and are summarised in Table 5.

Table 5: Morphological characterisation of *Manihot Esculenta Crantz*, stem.

Parameter	Result
a) Prominence of foliar scars and colour of stem exterior,	Prominent, silver.
b) Colour of stem epidermis,	Green.
c) Branching habit,	Trichotomous.

Figure 5: Morphological features of *Manihot Esculenta Crantz*, stem.

3.3.1.3. Morphological characterisation of *Manihot Esculenta Crantz*, tuber

The morphological characteristics of *Manihot Esculenta Crantz* tubers were studied, and the results are shown in figure 6 and are summarised in Table 6.

Table 6: Morphological characterisation of *Manihot Esculenta Crantz*, tuber.

Parameter	Result
a) Tuber shape, external colour and texture of the tuber surface,	Conical, conical cylindrical shaped, dark brown to faint brown coloured and rough, surfaced.
b) Colour of tuber cortex,	Pink, muddy rusty brown.

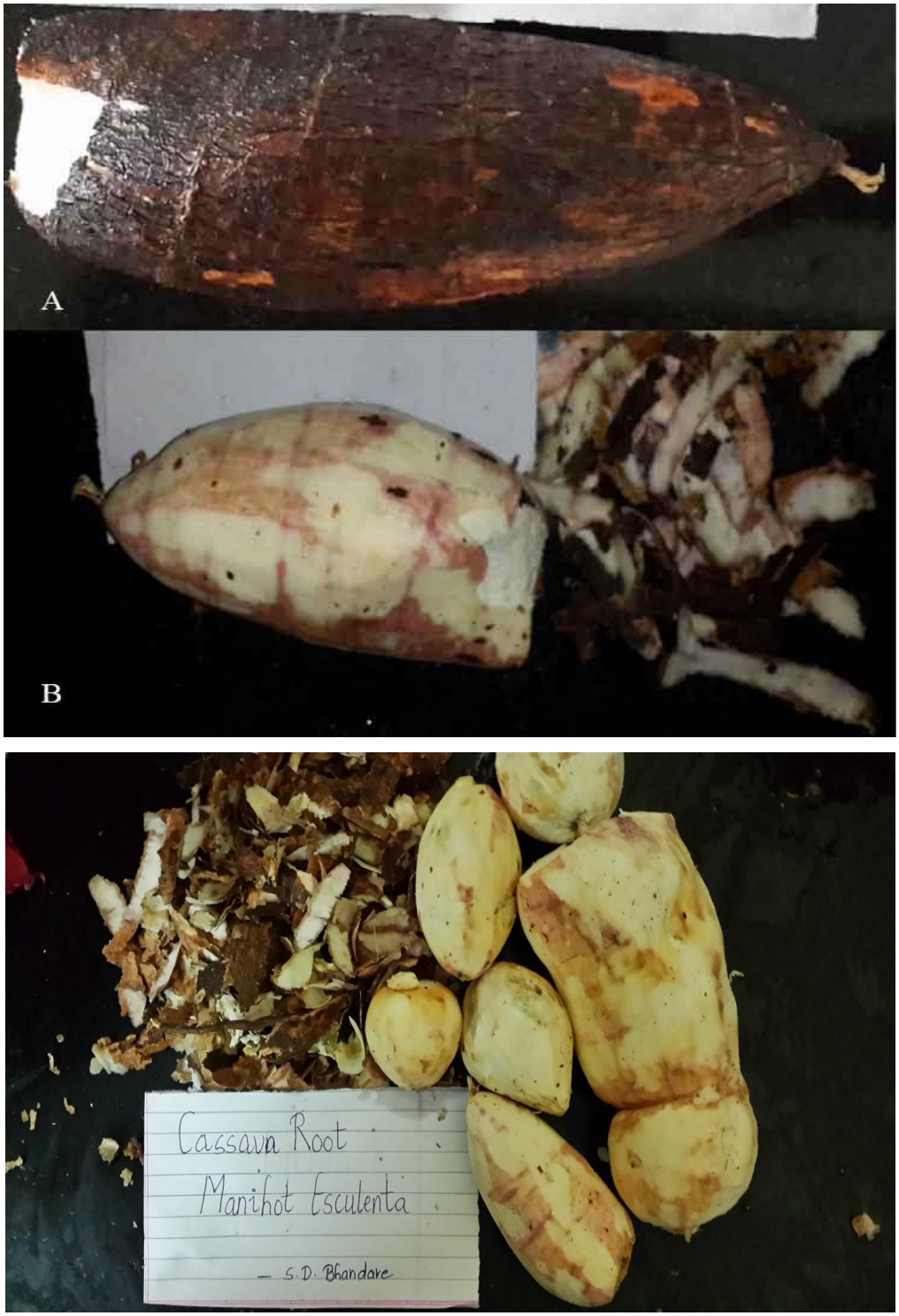


Figure 6: Morphological features of *Manihot Esculenta Crantz*, tubers.

3.4. Microscopy

a) Leaf

Transverse section of leaf of *Manihot Esculenta Crantz* stained with staining medium. Figure 7.

A transverse section of a typical leaf shows that it is covered by epidermis on both surfaces the upper epidermis on the upper surface and lower epidermis on the lower surface.

b) Stem

Manihot Esculenta Crantz stem transverse section stained with staining medium. Figure 8 shows an example of a formalised formal. A plant's axis is represented by a cross section of a stem. The epidermis is the stem's outer layer. Phloem vessels are sap-carrying tubes. The term "cambium" refers to new stem parts. Xylem vessels are the woody portions of the stem.

c) Tuber: Transverse section of tuber of *Manihot Esculenta Crantz* stained with staining medium. **Figure 9.** The transverse structure of tubers varies but as a common feature they possess a high number of parenchyma cells for storage.

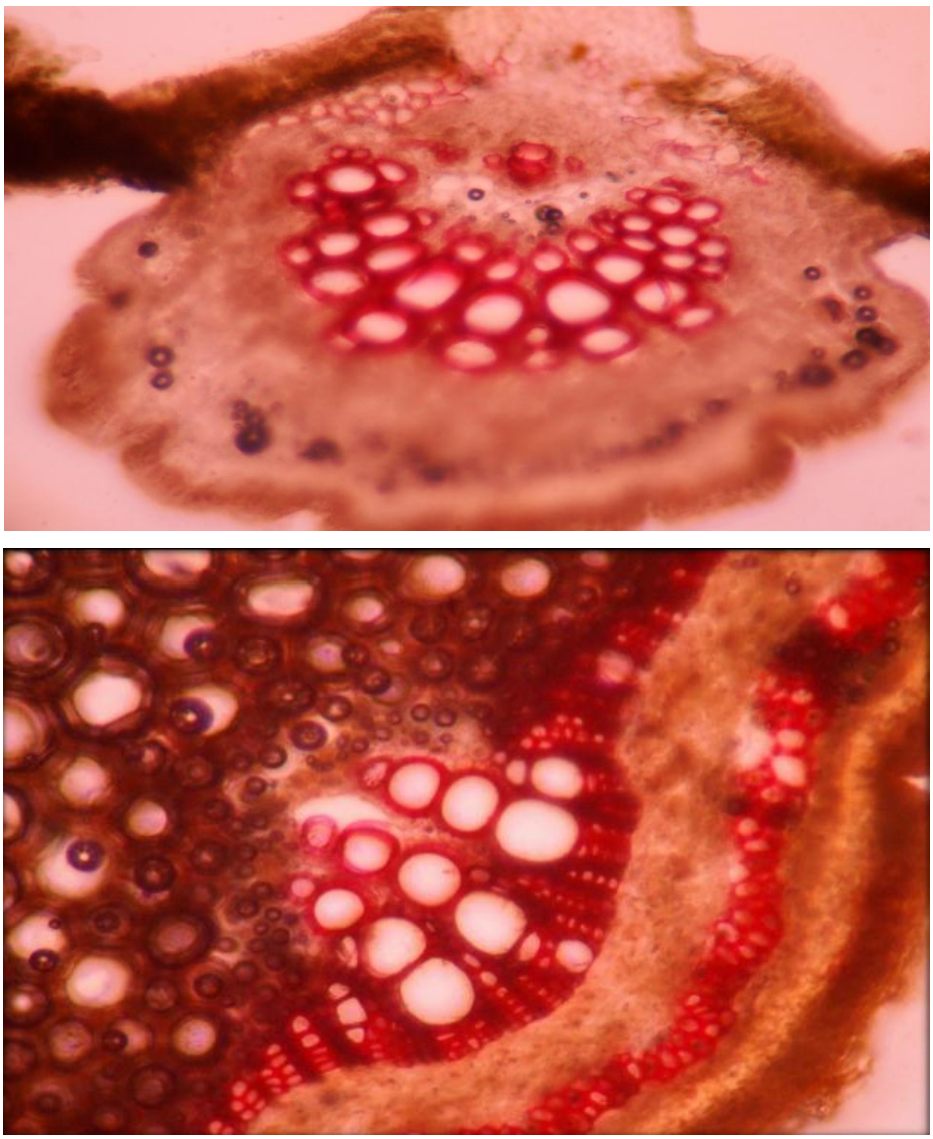


Figure 7: Photomicrography of microscopy of T.S. of *Manihot Esculenta Crantz* leaf.

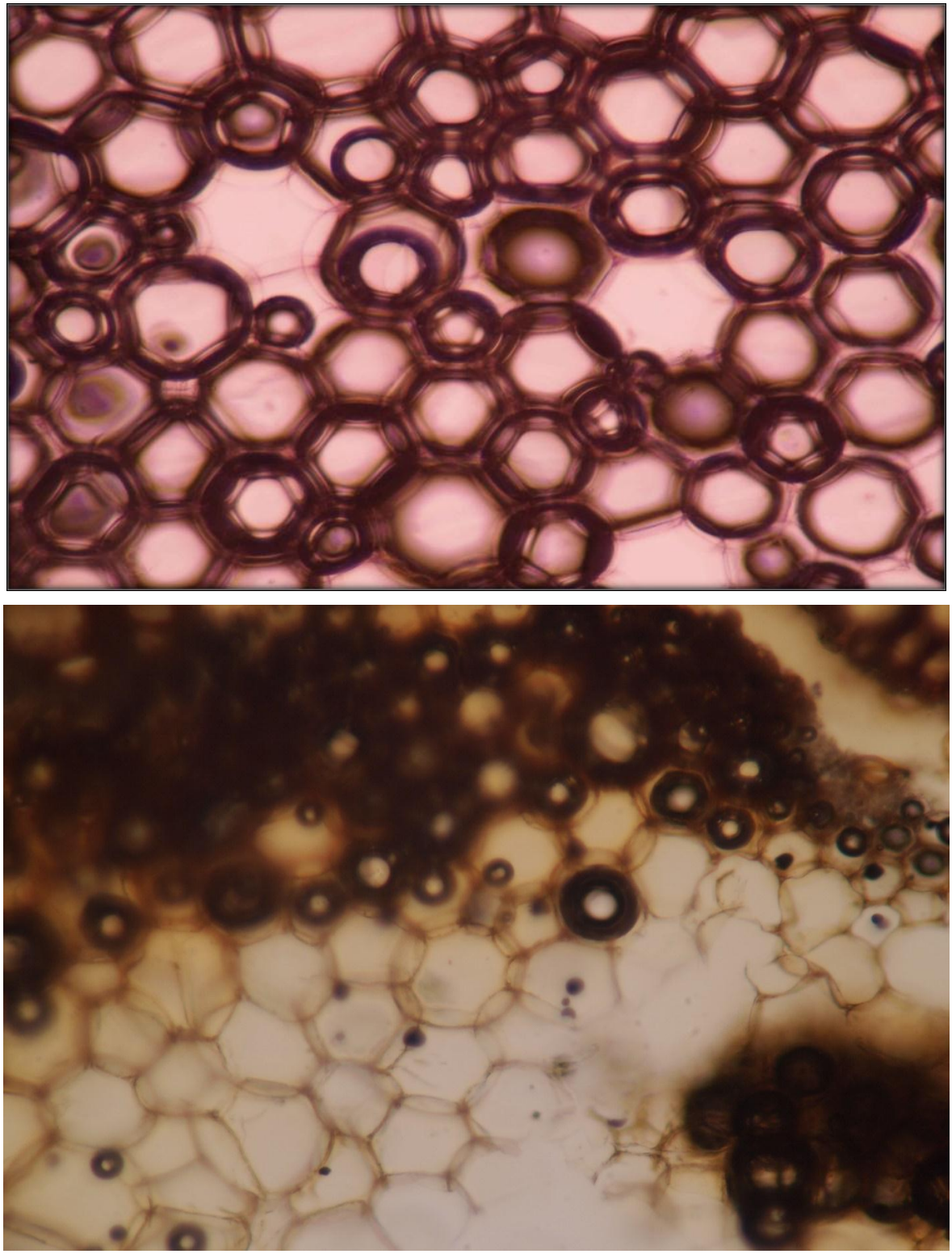


Figure 8: Photomicrography of microscopy of T.S. of microscopy of the stem of *Manihot Esculenta Crantz*.

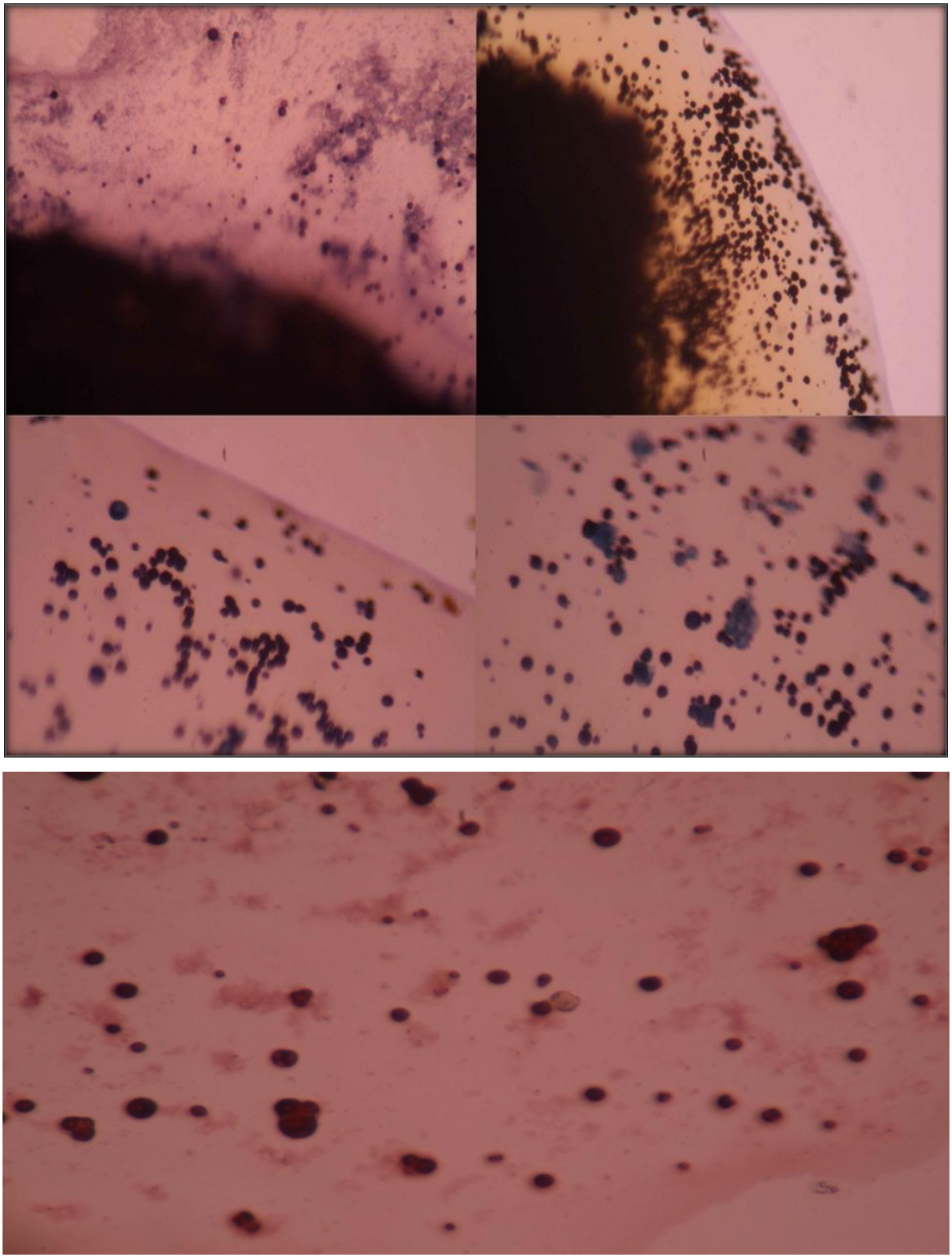


Figure 9: Photomicrography of microscopy of T.S. of *Manihot Esculenta Crantz*, tuber.

Thus, based on the microscopy, it can be concluded that the leaf contains xylem: lignified cells and phloem non-lignified cells, as well as a lower amount of polysaccharide stained in blue, the stem contains spongy parenchyma and a scarce amount of polysaccharide stained in blue, and the tuber contains an abundant amount of polysaccharide stained in blue. Xylem is

a complex plant tissue that transports water and other nutrients to the plants. Phloem is living tissue that transports food and other organic materials. Xylem is made up of dead cells (parenchyma is the only living cells present in the xylem).

3.5. Labelled transverse section of the leaf, steam, tubers

Labelled transverse sections of the leaf, stem, and tubers were studied and photographed under a light-illuminated electronic microscope. (Photomicrograph of transverse sections were obtained by use of an Olympus microscope.). Labelling is done in accordance with laboratory standards for the analysis or identification of crude drugs obtained from plant sources.

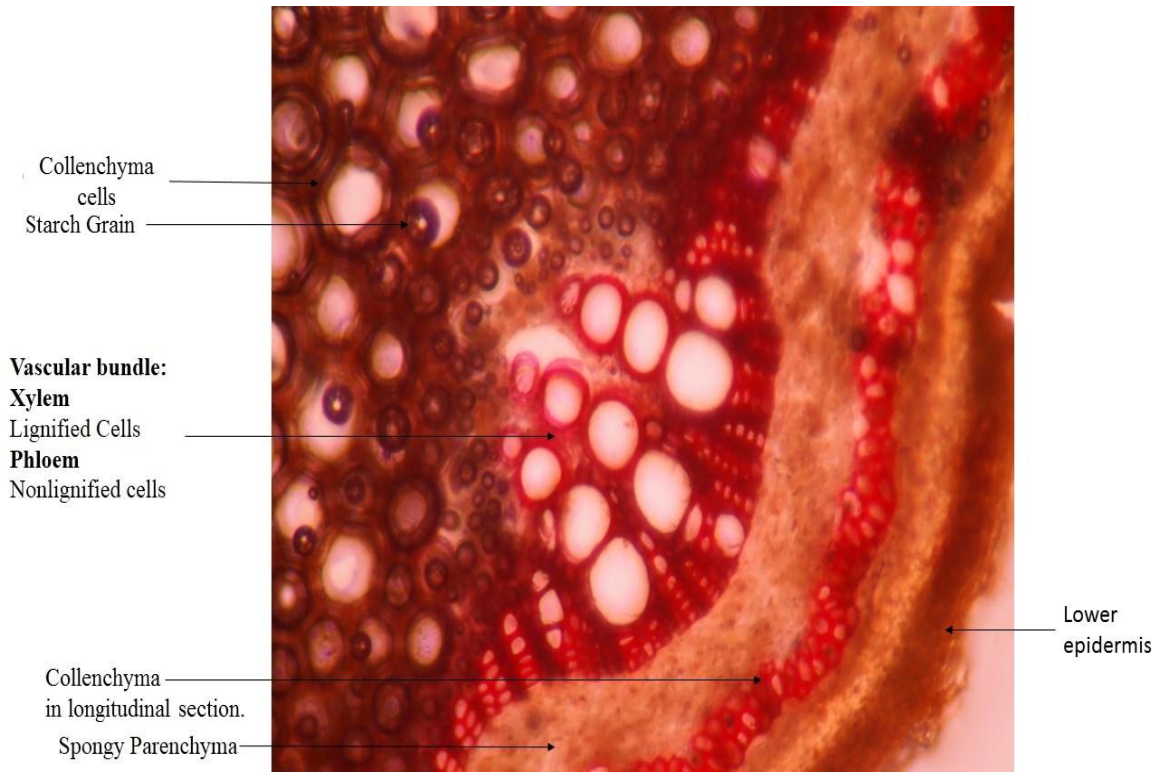


Figure 10: Labelled microscopy of *Manihot Esculenta Crantz*, leaf. (T.S. Photomicrography.).

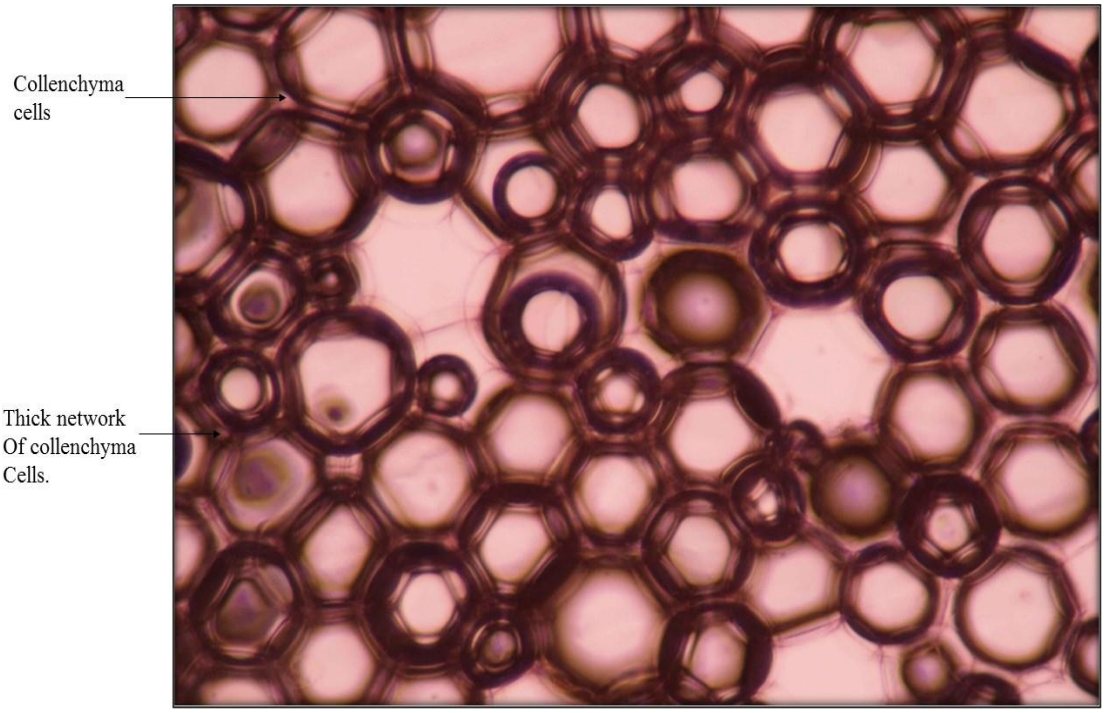


Figure 11: Labelled microscopy of *Manihot Esculenta Crantz*, stem. (T.S. Photomicrography.).

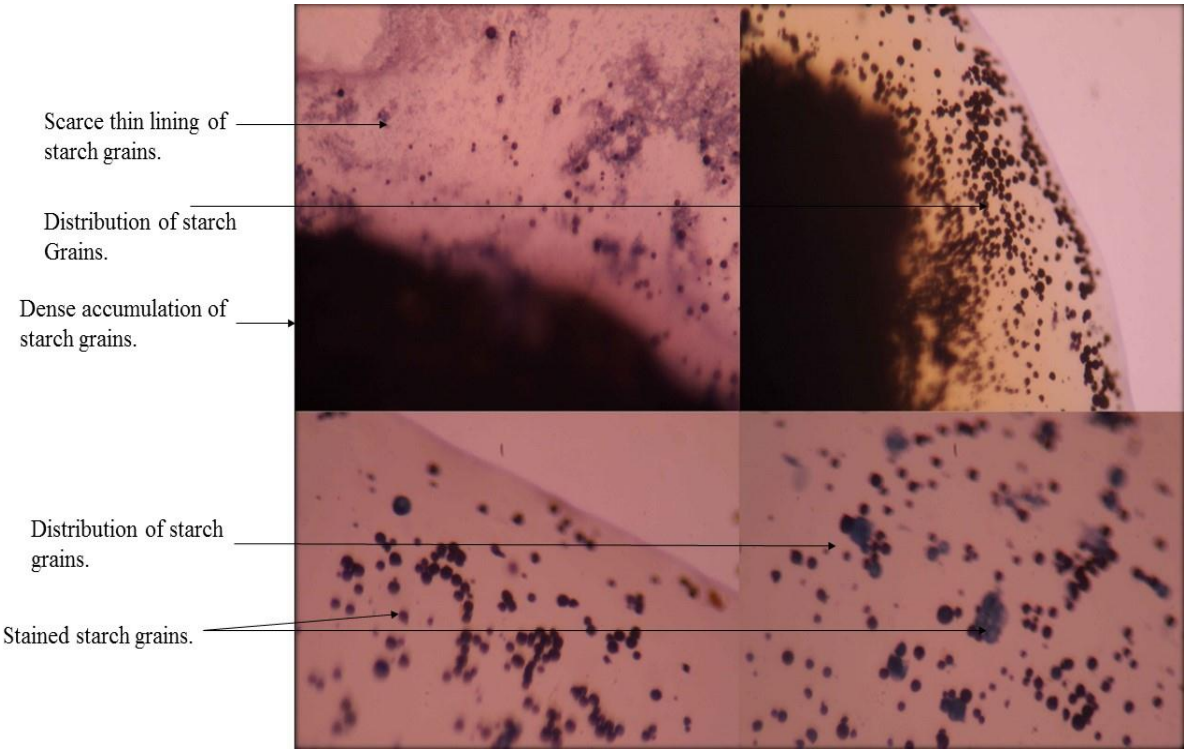


Figure 12: Labelled microscopy of *Manihot Esculenta Crantz*, tuber. (T.S. Photomicrography.).

3.5.1. General morphology and composition of the tuber of *Manihot Esculenta Crantz*

The periderm: This comprises the roots outermost layer. It is mostly made up of dead cork cells that seal the roots surface. The periderm is only a few layers of cells thick, and as the root grows in diameter, the outermost portions of it slough off and are replaced by new cork formations from the periderm's inner layers.

The starchy flesh is the central portion of the root that is made up primarily of parenchyma cells packed with starch grains. On a dry weight basis, it contains 30-35 percent amyloses and amylopectins; it is thus a predominantly starchy food that can be identified from the tuber's transverse section.

4. Abbreviations

1. AAPNA- Association of Ayurvedic Professionals of North America,[28]
2. ACEAM- The first east Asian medical school in Greece,
3. ANZFA- The Australia New Zealand Food Authority,
4. APA- Ayurvedic Practitioners Association,[29]
5. BfR- Federal Institute for Risk Assessment,
6. CCAOM- Council of Colleges of Acupuncture and Oriental Medicine,
7. COC- Committee on Carcinogenicity of Chemicals in Food,
8. COT- Committee on Toxicity of Chemicals in Food,
9. EFSA- European Food Safety Authority,
10. ETCMA- European Traditional Chinese Medicine Association,
11. EUAA- The European Ayurveda Association,[27]
12. FDA- U.S.- Food and Drug Administration,
13. GPhP- Guidance on good pharmacopoeial practices,
14. IARC- International Agency for Research on Cancer,
15. JSOM- Japan Society for Oriental Medicine,
16. JSP- Japanese society of pharmacognosy,
17. MAAA- Maharishi Ayurveda Association of America,[29]
18. MOE- Margin of Exposure,
19. NOAEL- No-observed-adverse-effect-level,
20. OTC- Over the counter drugs,
21. PANOs- Pyrrolizidine Alkaloid N-Oxides,
22. PAs- Pyrrolizidine Alkaloids,

23. SOS- Sinusoidal obstruction syndrome,
24. T. Med-Traditional Medicine,
25. T.S.-Transvers section,
26. TCM- Traditional Chinese medicine,
27. TDI- Tolerable daily Intake,
28. UFLC- Ultra fast liquid Chromatography,
29. VOD- Veno-occlusive disease,
30. WHO- World Health Organisation.

5. CONCLUSION

The optical light assisted microscope was used to obtain more accurate and illuminated microphotographs of the transverse sections of the plant parts that were examined during the research of the *Manihot Esculenta* plant for identification. Leaf, stem, and tubers which are the three sections of the plant used to identify the whole plant source for the crude pharmacognostic drug. According to the microscopy, the leaf contains xylem: lignified cells and phloem: non-lignified cells, as well as a lower amount of polysaccharide stained in blue, the stem contains spongy parenchyma and a small amount of polysaccharide stained in blue, and the tuber contains a large amount of polysaccharide section that can be seen stained in blue. The drug's phytochemical assessment would be useful for isolating different constituents and subjecting it to extensive pharmacological testing. The quantitative estimation given may be useful in compiling a plant monograph and gaining global acceptance of the investigated drug or crude drug.

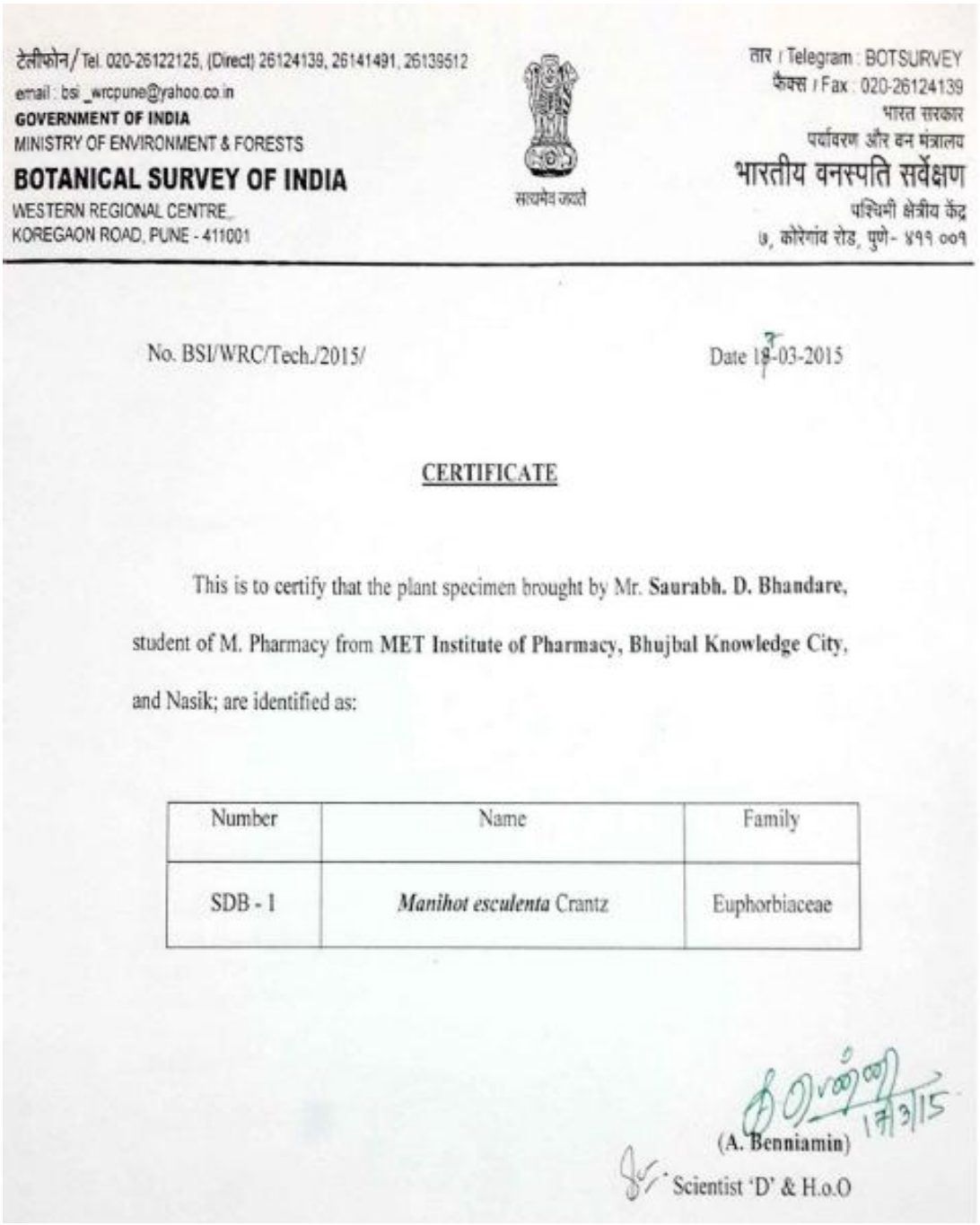
6. ACKNOWLEDGEMENTS

The author would like to express his gratitude to the "World Journal of Pharmaceutical Research," and Dr. Valentina Petkova, Vice Dean of International Integration Bulgaria for recognising and publishing his work in the journal.

7. CONFLICT OF INTEREST

The author declares that there is no conflict of interest in the publication of this article. Individual permission was granted to publish this academic research individually.

8. APPENDIX



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पश्चिमी क्षेत्रीय केंद्र
७, कोरेगांव रोड, पुणे- ४११ ००१

No. BSI/WRC/Tech./2015/ Date 18-03-2015

CERTIFICATE

This is to certify that the plant specimen brought by Mr. **Saurabh. D. Bhandare,** student of M. Pharmacy from **MET Institute of Pharmacy, Bhujbal Knowledge City,** and Nasik; are identified as:

Number	Name	Family
SDB - 1	*Manihot esculenta* Crantz	Euphorbiaceae

17/3/15
(A. Benniamin)
Scientist 'D' & H.o.O

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10. Other related image