SCANNING ELECTRON MICROSCOPE (SEM) IMAGE AND TOOTH HARDNESS EXPOSED TO JATROPHA CURCAS SAP

THESIS

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HASANUDDIN UNIVERSITY

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A RESEARCH

THESIS

Submitted to Hasanuddin University
to Complete One of The Requirements
to Achieve a Bachelor's Degree in Dentistry

By:
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APPROVAL SHEET

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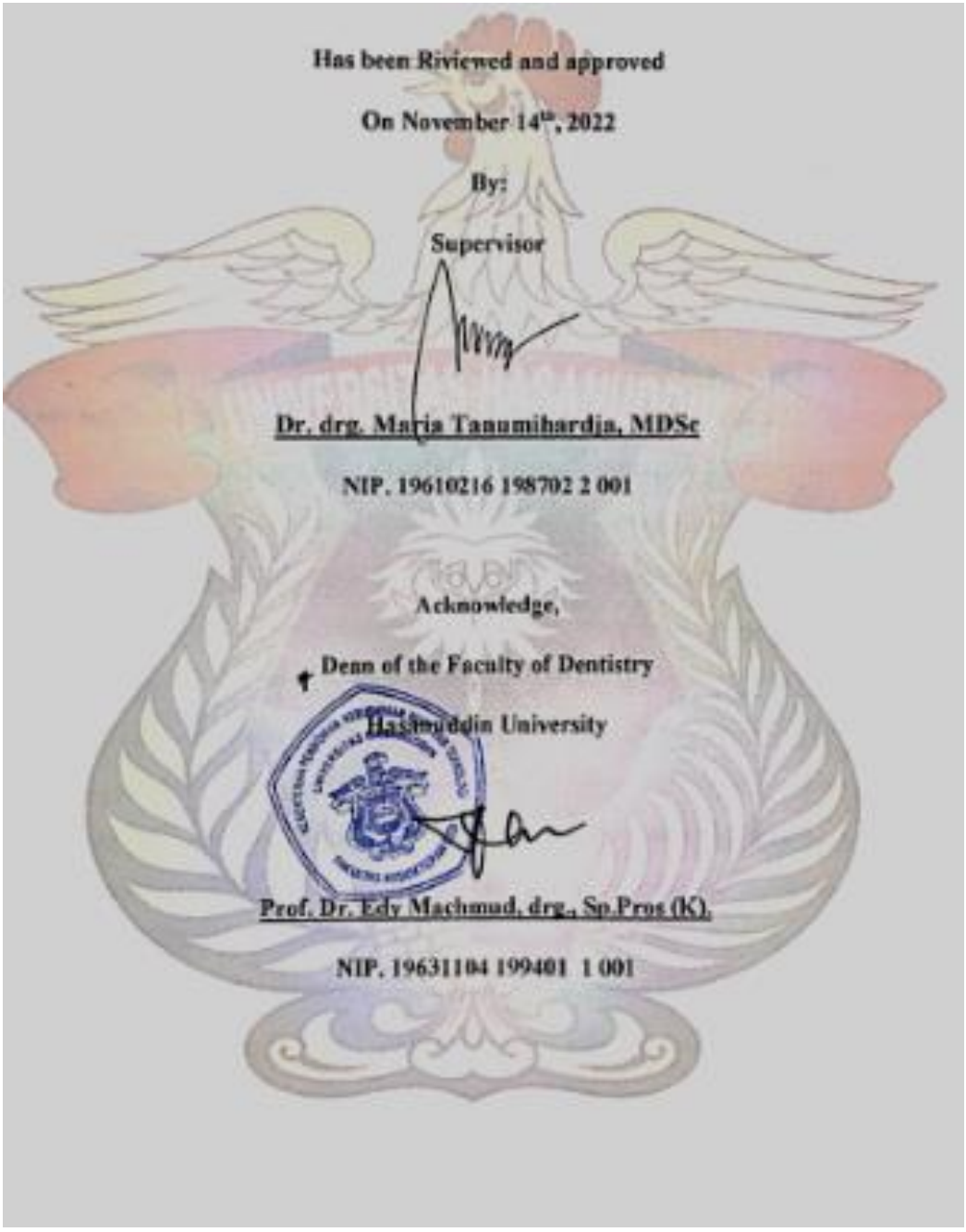

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PREFACE

بِسْمِ اللهِ الرَّحْمٰنِ الرَّحِيْمِ

All praise and gratitude to Allah SWT for all His blessings and guidance so that the author can complete the writing and preparation of the thesis titled “Scanning Electron Microscope (SEM) Image and Tooth Hardness Exposed to Jatropha Curcas Sap.” This thesis was written as a requirement for the completion of studies in achieving a bachelor's degree in dentistry at the Faculty of Dentistry, Hasanuddin University. Salawat and greetings are also extended to the great Prophet Muhammad SAW as an example who leads people from a dark path to a path of knowledge.

The author acknowledges that the writing of this thesis would not be possible without the support of many parties. In this opportunity, the author would like to express her gratitude and respects to the author’s parents, **Sudirman, S,Pd.,M.pd** and **Liati, S,Pd.,M.pd** , for it is their prayers and their blessings, as well as their love and patience in providing both material and moral support so that this thesis can be completed.

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The author is fully aware that this thesis is still far from perfection, because perfection belongs to Allah alone. Therefore, the author apologizes if there are errors in the writing of this thesis. The author appreciates criticism and suggestions for the improvement of similar writing in the future. The author hopes that this thesis can be useful and can be of positive value for all parties who need it.

Makassar, October 2022

Author

SCANNING ELECTRON MICROSCOPE (SEM) IMAGE AND TOOTH HARDNESS EXPOSED TO JATROPHA CURCAS SAP

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ABSTARCT

Background: Root canal treatment is one way to maintain teeth that are severely inflamed which can be treated using local anesthetics or devitalizing Medicine. Natural ingredients from plants have been studied as alternative agents. Jatropha is a plant Euphorbiaceous family that has been studied for this purpose. However, information is scarce. **Purpose:** To find out the Scanning Electron Microscope (SEM) image of teeth exposed to jatropha curcas sap,to determine the hardness of the teeth after the application of jatropha curcas sap extract. **Methods:** Laboratory experimental research with post test design with control group. Sixteen premolars were divided into 2 groups. All teeth were prepared for Class I preparation. One group as a control group and the other 1 as treated group were stored in artificial saliva for 7 days after placement of jatropha sap extract into the prepared cavity and filled with GIC. The jatropha sap is cleaned in the area around the cavity, grinded and polished with 1200 grit to 3000 grit abrasive paper. The samples were tested for hardness using a Vickers hardness tester. **Results:** The hardness of the samples carried out decreased with the slight category. Control group = 6.112 HV, Treated group = 5,909 HV. Results of SEM photos with 6.000-10.000 times magnification on the surface of the sample showed white lines showing exposed dentinal tubules with more intensity than the control group and looked consistent in all samples. **Conclusion:** Jatropha Curcas Sap reduces the hardness with slight category and dissolves several components in the dentinal tubules.

Keywords: *Jatropha curcas sap, Vickers Hardness test Scanning Electron Microscope*

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CHAPTER I
INTRODUCTION

1.1 Background

Dental caries is one of the most common diseases, the main cause of tooth pain that can cause tooth loss[1]. Dental caries destroys the structure of the hard tooth structure of the teeth, namely enamel and dentin. Dental caries is caused by bacteria that produce acid and demineralize enamel to form cavities. Dental caries usually occurs slowly and when left untreated, it can cause extensive tooth decay, pain and eventually be extracted [2].

This process initially occurs in enamel as a result of acids that can dissolve hydroxyapatite characterized by demineralization, which will make teeth more brittle. Demineralization of enamel is followed by dissolution of organic substances that causes further bacterial invasion deeper into the tooth, reach the pulp and cause tooth pain that may reduce masticatory function.[3,4]

Caries that have reached the pulp can become into reversible pulpitis and if it late to be handling it will be irreversible pulpitis which need to be root canal treated to clean and shape the infected root canal, either by anesthesia and/or applying devitalizing agent[6]. Devitalizing agent usually used in dental practice is arsenic trioxide which is toxic, that cause pain and periapical inflammation after application due to its unlimited effect.[5]

Another devitalizing agent that often used is formaldehyde, which shows mild pain after application with less adverse effects than arsenic trioxide. Formaldehyde has disadvantage that devitalizes nerves more slowly. Various studies have been carried out to find alternative materials, one of them is utilizing natural sources.Jatropha curcas is a type of shrub that has been used by people as a traditional medicine to improve blood circulation and relieve swelling,[10] as well as to relieve pain. From previous studies, local application of Jatropha Curcas to mice teeth showed an analgesic effect

with optimal results in the first 3 hours, but decreased in the next 6 hours and ended at 24 hours,[9] odontoblast cell lysis is observed which indicates that the blood vessels pulp ruptures and causes pulp necrosis.. [12.13]

Research that has been conducted to evaluate the effect of combination of sidaguri root extract and Jatropha on the microhardness and morphology of the teeth tested. The results showed their potency to be used as a herbal-based devitalizing agent. The combination of sidaguri root extract and jatropha is reported to cause porosity and decrease the hardness of the cavity in contact with the extract, but there is lack of data related to the characteristics of each extract.[7]

Porosity of tooth following application of jatropha extract is evaluated by using *Scanning Electron Microscope* (SEM) because it has a large depth of field, which can focus a larger number of samples at a time and produce a good image of the three-dimensional sample.[11] SEM observation needs to be followed by tooth hardness evaluation because morphological changes in tooth structure is closely related to the components of the teeth in the form of inorganic and organic substances.

Based on the above reasons, this study is purposed to examine the porosity of teeth using Scanning Electron Microscope (SEM) and the hardness of teeth after exposed to Jatropha Curcas sap.

1.2 Problem Formulation

How is the image of teeth and tooth hardness following exposure to jatropha curcas sap?

1.3 Purposes of Research

1. To find out the Scanning Electron Microscope (SEM) image of teeth exposed to jatropha curcas sap
2. To determine the hardness of the teeth after the application of jatropha curcas sap extract.

1.4 Benefits of research

Adding insight and knowledge about the effect of Jatropha application on tooth hardness and Scanning Electron Microscope (SEM) images.

CHAPTER II

LITERATURE STUDIES

2.1 Tooth structure

Teeth consist of several components, namely enamel, pulp-dentin complex, and cementum

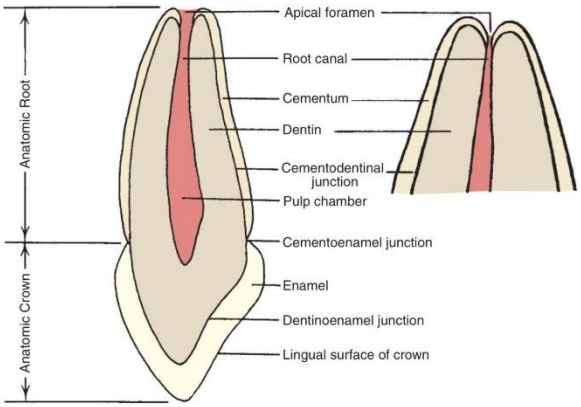


Figure 2.1. tooth structure [15]

2.2 Enamel

Enamel is a white layer located on outermost surface of the teeth that protects the crown of the teeth, the hardest and strongest tissue among other tissues in human body. Enamel has several components including hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) mineral. The stability of this mineral is influenced by oral conditions, such as mouth's pH.[15,16]

2.2.1 Email Demineralization

The enamel consists of solid mineral crystals of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) and can be demineralized by acid exposure. Demineralization is a state of mineral ions loss from tooth enamel, refers to the breakdown of enamel and usually the first step in dental caries formation. Composition, pH, antibacterial activity, quantity and viscosity of salivary flow greatly affect the rate of demineralization.[17]

Lower pH will increase the concentration of H+ ions, which will harm the hydroxyapatite of enamel. When teeth come into contact with acidic drinks or foods, hydrogen ions (H+ ions) will diffuse on the enamel surface, resulting in demineralization that dissolves hydroxyapatite in tooth enamel, eventually releasing Ca^{2+}, PO_4^{3-}, OH^- ions. These ions will bind with hydrogen ions (H+) from the acid and form complex compounds that will dissolve and cause loss of minerals from enamel surface.[18]

Demineralization of enamel can also cause alterations in surface morphology and reduce microhardness of the enamel surface due to the release of calcium ions.

2.3 Dentin

Dentin is a dental hard tissue, has slightly yellowish color, forming most of the tooth that supports the enamel at crown. In root, dentin is covered by cementum which surrounded with periodontal ligament fibers that provide support for various tooth movements during mastication. Dentin extends in the center of the tooth from the pulp cavity outward to enamel of the crown or cementum at the root. Dentin consists of 70% calcium hydroxyapatite, 18% organic matter or collagen fibers, and 12% H_2O. [15]

2.4 Pulp

The pulp is an unmineralized soft tissue surrounded by dentin except for the apical foramen and has formative, sensory, nutritive and defensive function.[15]

Dental pulp is a unique and complex tissue, provides the main source that constructing the tooth. The pulp anatomy is divided into two parts, the coronal pulp and the root canal pulp. The main role of pulp is to produce dentin, secreted from odontoblast cells, which are in direct contact with the dentinal matrix peripherally.[19]

2.5 Cementum

Cementum consists of 65% calcium hydroxyapatite, 35% organic matter (collagen fibers), and 12% water. Cementum is hard like bone, but softer than enamel.

2.6 Devitalizing Ingredients

Pulp devitalization is an operative technique used in the treatment of pulpitis teeth. Devitalizing agents are able to deaden nerve cells so that pain can be permanently eliminated (painless). Arsenic trioxide is the most widely used pulp devitalizing agent by paralyzing nerve fibers, destruction and decomposition of axons in the medullary sheath, causing vasodilation, hyperemia and hemorrhage which results in disruption of blood circulation, impairs mitochondrial respiration activity so that vital cell functions are lost, lead to cells death. [13]

2.7 Jatropha Curcas

Plants are source of natural biological compounds that play an important role, some of which can be used as herbal medicines. Indonesia is a tropical country that has abundant and diverse biological natural resources, thrive in almost all regions. Some plants produce secondary metabolites that can be used as medicine for certain diseases.

Jarak pagar or in Latin Jatropha curcas is one of the plants thats often used as an alternative source of environmentally friendly diesel fuel.[20] Jatropha has many benefits and quite easy to obtain, so it is not only used as a substitute for fuel by the community but also as traditional medicine. Parts of Jatropha plant provide many benefits including seeds, leaves, roots, bark and sap, those part functions are treating vaginal discharge, ear inflammation, toothache, skin disorders, wounds and bleeding, rheumatism, intestinal worms in children and cure cough disorders.

2.7.1 Morphology of jatropha curcas

Jatropha L. is a family group of Euphorbiaceous

Classification of plants as follows:

- Kingdom : Plantae
- Subkingdom : Spermatophyta
- Division: Magnoliophyta

- Subclass : Rosidae
- Order: Euphorbiaceae
- Genus: Jatropha
- Species: Jatropha curcas L.

Jatropha plant parts used in the health sector:

A. Jatropha Curcas leaves

The leaves of Jatropha curcas are curved, striped to the edge, a single leaf has an angle of 3-5 cm, spread throughout the stem, on the top and bottom surfaces are green, but on the underside it is slightly paler. Broad leaves resembling a heart or oval with a length of 5-15 cm, connected by a stalk that is about 4-15 cm long. The leaf bones have 5-7 main veins.

Jatropha leaves have various benefits believed by the community, including medicine for flatulence, overcome constipation, stabilize blood sugar and treat canker sores. In toddlers, it used as an alternative medicine to deal with irritation of the skin.

Figure 2.7 a) Leaves and twigs of Jatropha sap

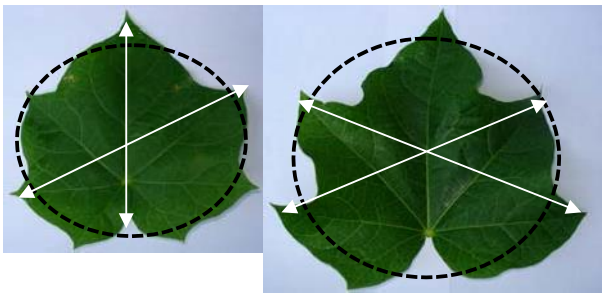


Figure 2.7 b) Leaf shape during seedling phase (left) and mature leaves (right).

B. Jatropha stems and roots

Figure 2.7 c) Stems and roots of Jatropha curcas.

Jatropha stems are long, round, and gray-green. In older stem, it is found peeled-off dead skin on its surface. If the plant has few primary branches, then the growth is upright, but if the number of primary branches is large, the growth looks like a bush [22]

2.7.2. Ingredients of Jatropha Sap

Jatropha is considered a versatile plant, has been used in traditional human medicine and veterinary medicine for a long time, can reduce soil degradation, desertification, and deforestation. Jatropha has great adaptability to dry and semi-arid environments.[20]

Jatropha curcas has a white sap, slightly cloudy and has a bitter taste. It is commonly used as a mouthwash for bleeding gum. Besides that , the sap is also used for blood circulation, eliminating swelling, as well as anti-inflammatory and antioxidant[13]

Jatropha sap is obtained by slashing or injuring the jatropha stem using a knife, then the sap that comes out is collected using a sterile container. Jatropha tree sap contains flavonoids, alkaloids, saponins, tannins, can stop bleeding in wounds, and have antimicrobial properties. It can eliminate bacteria such as E. coli or Streptococcus bacteria in the wound healing process.

Flavonoids in Jatropha sap function as antifungal, antiseptic, and anti-inflammatory. Flavonoids are very effective as antioxidants, besides that they can also prevent cardiovascular disease by reducing Low Density Protein (LDL) oxidation. Flavonoids contained in Jatropha bark extract have biological activities such as antimicrobial, anti-allergic, and antioxidant. Saponins can stimulate the growth of collagen in the healing process, relieving pain and stimulating the formation of new cells.

2.8 Scanning Electron Microscope

Scanning Electron Microscope (SEM) is an important microscope for the characterization of materials at the nano scale. SEM can reach sub-nanometer spatial resolution.[26] Scanning Electron Microscope (SEM) depicts specimens by scanning them with a high-energy electron beam in a scanned raster pattern [26]

Jatropha curcas shows potential as a devitalization material but the mechanism is not yet understood. Morphological changes in tooth structure after application Jatropha sap extract can be identified using SEM, to observe and determine the depth of microporosity occurs in the tooth structure.

Figure 2.8a).SEM Device [27]

SEM works at high magnifications of up to 300,000 × and even 1,000,000 × to produce extremely precise images of wide variety of materials. Energy Dispersive X-ray Spectroscopy (EDS) works in conjunction with SEM to provide qualitative and semi-quantitative results. The two techniques, together, have the potential to introduce fundamental information about the material composition of the scanned specimen, which general laboratory tests cannot provide.[27]

The main equipment contained in the Electron microscope or SEM include:

1. Electron guns, generally consist of filaments made of elements that are easy to release electrons, such as tungsten.
2. Lenses for electrons, i.e. magnetic lenses because negatively charged electrons can be deflected by a magnetic field.
3. Vacuum system, because electrons are very small and light, if there are other air molecules, electrons traveling towards the target will be scattered by collisions before hitting the target, so removing air molecules is very important.

The working principle of SEM is as follows: [26]

1. An electron gun produces electrons beam and accelerated by the anode.

2. Magnetic lenses focus electrons towards the sample.
3. The focused electron beam scans the entire sample directed by the scanning coil.
4. When electrons hit the sample, the sample will release new electrons which will be accepted by the detector and sent to the monitor

There are several signals that are important by SEM. From the inelastic reflection, a secondary electron signal and X-ray characteristics are obtained, while from the elastic reflection, a backscattered electron signal is obtained. These signals are described in the following figure.

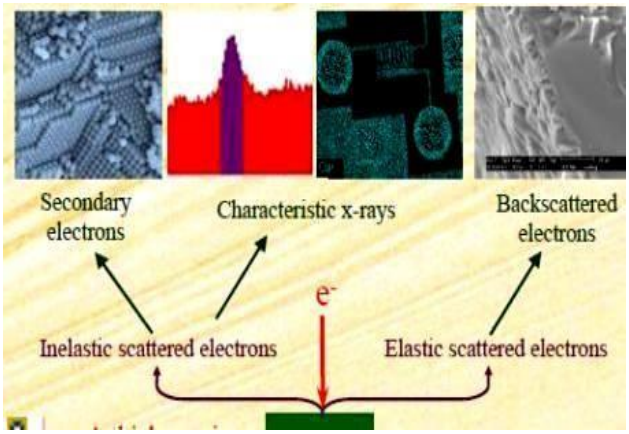


Figure 2.8 b). Detection of the signal generated by SEM result of electron reflection.

SEM is a major mechanical assembly, particularly suitable for viewing point by point visual images of high caliber molecules. SEM can provide some data such as subjective data of the sample including morphology, arrangement and crystallographic data. In addition, it provides data about the surface, shape, size and on the sample being tested. SEM is a multipurpose instrument that can view and investigate material at high magnification.[28] with a lot of advantages of using SEM, this is the reason it is very appropriate to be used in this research.

2.9 Hardness Test

Hardness test is the most suitable method to determine the mechanical properties of a material. In the devitalization material used, Jatropha shows the potential as a devitalizing material. So far, there has been no study on teeth hardness after application of Jatropha sap, hence it is necessary to test the hardness.

The hardness test is included in the American Dental Association (ADA) specifications for dental materials. There are several types of surface hardness tests which are partly based on the most commonly used tests in determining the hardness of dental materials known as Vickers, Knoop, Brinell and Rockwell. [23]

2.9.1 Vickers Hardness Tester

The Vickers Hardness taster hardness test is a common method used to characterize the hardness of materials. Hardness testing with the Vickers method aims to determine the hardness of a material as material durability. [24]

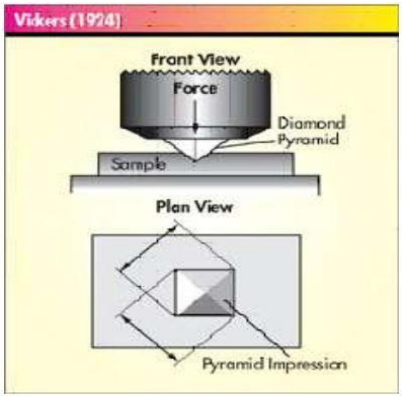


Figure 2.9.1 The working principle of Vickers hardness measurement [29]

Tests with the Vickers method were carried out by emphasizing a straight diamond pyramid with a square base and a peak angle of 136° to the surface of the test object for a certain time. This test is important to determine the hardness of a material, i.e. the resistance of the material to a fairly small diamond indenter[25]

The Vickers Hardness Number (VHN) is a number related to the force and surface area.

$$VHN = \frac{1{,}8544\ x\ P}{d2}$$

Description =

VHN = Vickers hardness value (HV)

P = amount of load (kgf)

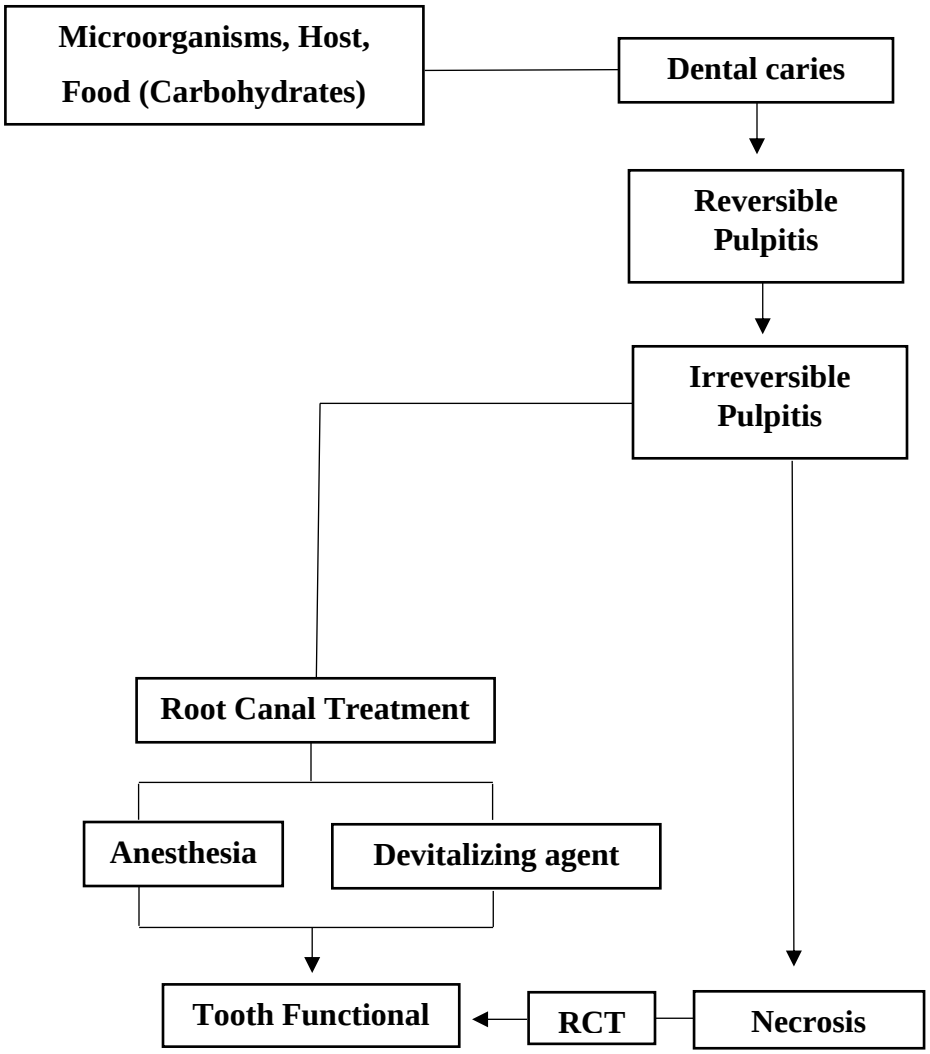
D = diagonal width (mm)

The advantage of this Vickers test is that it does not damage the material, because the indentation is very small then test material can be reused. In addition, the load range in test can be selected from light to heavy loads.[25]

CHAPTER III

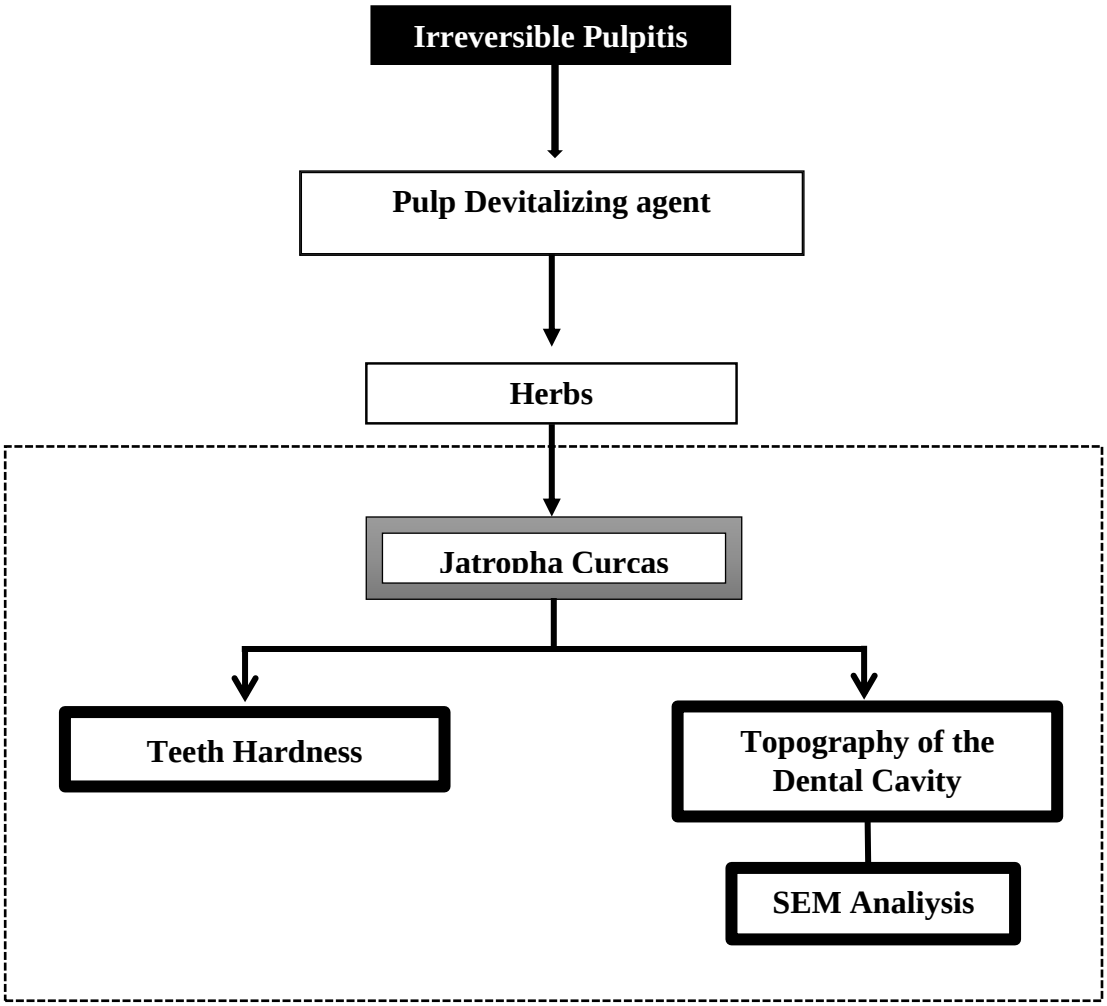
THEORY FRAMEWORK AND CONCEPT FRAMEWORK

3.1 Theoretical Framework



Source: Torabinejad M, Walton RE. Endodontic principles and practice. Saunders Elsevier; 2009. PP: 53-56, 84-85

3.2 Conceptual Framework



Description:

: Dependent variable

: Independent variable

: Control Variable