UNIVERSITY FOR DEVELOPMENT STUDIES, TAMALE

FLOW RATE AND QUALITY OF SHEA LATEX (*Vitellaria paradoxa*) AS INFLUENCED BY AN ETHYLENE GENERATOR; IN COMPARISON TO NATURAL RUBBER LATEX (*Hevea brasiliensis*)

BY

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DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in the university or elsewhere. Works that were consulted have been duly acknowledged by way of references.

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ABSTRACT

Hevea brasiliensis, a spurge, is known to be the most exploited latex producing tree species. Few decades ago, *Vitellaria paradoxa* known as the shea butter tree, a member of Sapotaceae was found to produce latex with beneficial properties although the rate of flow was minimal. Enhancing the flow rate, an ethylene stimulant used for stimulating the flow of latex in *Hevea brasiliensis* was applied. In this study, the quality of shea latex in comparison to natural rubber latex obtained from *Hevea brasiliensis* was investigated. Three samples were used; *Hevea brasiliensis* latex, shea latex (unstimulated) and shea latex (stimulated). Results showed that the stimulant positively influenced the flow rate of shea latex and consequently, influenced the latex quality biochemically in terms of proximate composition, ionic composition (metallic and non-metallic) and phytochemical constitution. Comparatively, the stimulated shea latex exhibited both physical and chemical properties which makes it suitable for confectionery purposes over natural rubber latex. These properties such as good smell, appearance (colour) and chewiness, as well as testing negative for flavonoids and saponins for phytochemical composition, similar to an already exploited gum base (*Manilkara zapota* L.) suggest a comparative advantage of shea latex over natural rubber latex when considered for gum making in the confectionery industry. Also, the relatively high stickiness and high adhesiveness of shea latex makes it a better option for bio-adhesive over natural rubber latex.

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DEDICATION

This thesis is dedicated to the Payne family.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The tropical and temperate regions have diverse plant resources and among these, approximately 2000 species of trees, shrubs, or vines produce latex; a sticky, creamy substance that is obtained from cuts in the tree's bark from which natural rubber or a closely related substance can be obtained(Subramaniam, 2000). Latex is composed mainly of the cytoplasm of special tube-like cells called laticifers, which are distinguished from vascular cambium. Latex exudes from the laticifers without mitochondria after the soft bark is cut or wounded (tapping) *(Zhang et al*., 2017).

Hevea brasiliensis, also known as the "Pará tree" belongs to the spurge family, *Euphorbiaceae*. A fast-growing tree, which seldom exceeds 25 m in height in plantations and is a commercially known source of natural rubber latex (Hobhouse, 2005). Although the Pará tree is native to South America (Vijayaram, 2009), history has it that the rubber treewas introduced by the British Empire to many of its more tropical former colonies, comprising Singapore,Indiaand Malaysia, which are presently classified as leading producers of rubber. Among the world's top ten countries of the largest rubber producing countries is Côte d'Ivoire which shares border with Ghana to the West. The country is not only recognized for producing cocoa, it is also a leading producer of rubber and the top-ranked in Africa. Ghana, a major rubber producer of Africa, in 2009, had nearly 11,255 hectares of land under cultivation, producingover 16,000 metric tonnes (Matthews, 2017).

The shea tree, or karité as called in French, belongs to the family*Sapotaceae* under the scientific name *Vitellaria paradoxa* (Gaertn. f.), previously called *Butyrospermum paradoxum*

(Gaertn. f.) (Henry *et al.*, 1983 in Hatskevich *et al*., 2011) is a deciduous tree indigenous to Tropical Africa. The tree is said to be native to the Guinea and Sudan Savanna zones, where the former is one region predominated by shea tree species found in the Guinea Savanna belt of West Africa. This spreads from Senegal in the West to Sudan in the East and the latter, the West Sudan Savanna running from the Atlantic Ocean to eastern Nigeria encompassing some geographical locations in Ghana (Dalziel, 1955;Nikiema and Umali, 2007; Kwasi *et al.*, 2012; Amissah *et al.*, 2013).Ghana as West Africa's second largest shea exporter (Hatskevich *et al.*, 2011) is marked by the abundance of shea trees growing in the wild. When quantified, almost half of the country's geographic land space, located practically in the whole area of northern Ghana with a land area of over 77,670 km^2 (Hatskevich*et al*., 2011) with an estimated shea tree population of roughly 9.4 million growing in Ghana (Dogbevi, 2009).The tree species is taxonomically separated into two subspecies, namely *paradoxa* and *nilotica* located in West-Central Africa and East Africa respectively (Gwali *et al.*, 2014). It supplies vital products and ecological services to the semi-arid region (Teklehaimanot, 2004). Matured shea trees grow to a typical height and girths of approximately 15 m and 1.75 m respectively with abundant branches and a thick waxy and deeply fissured fire-resistant bark (Fobil, 2007).

Among other economically important products, the shea tree has been found to produce latex – a research in progress (Fosu and Quainoo, 2013;Quainoo *et al*., 2015; Abdul-Azi*et al*., 2016; Quainoo and Dugbatey, 2016). However, *Hevea brasiliensis* is known to produce the most latex and considered as the only important commercial source of natural rubber (Subramaniam, 2000; Vijayaram, 2009). Upon damage of specialized canals in certain plant species a sticky emulsion referred to as latex exudes (Agrawal and Konno, 2009). In order to obtain rubber, incisions are made in the bark of the rubber tree, and a creamy sap from the latex vessels confined

in the inner bark oozes out and is collected. Latex is a mixture that contains water, proteins, phytochemicals such as tannins, resins and rubber in varying quantities (Cotter*et al.*, 2009). The flow rate of latex in diverse plants is known to be influenced by age (Data *et al.*, 1996), plant species (Agrawal and Konno, 2009)and other environmental factors such as time of tapping (Fosu and Quainoo, 2013).

Ethylene is used as a stimulating substance in latex production in *Hevea brasiliensis*. Bark treatment with an ethylene releaser (2-chloroethylphosphonic acid,) arouses latex regeneration (D'Auzac and Ribaillier, 1969) and this kind of treatment is now commonly used in rubber plantations to improve yield (Dusotoit-Coucaud *et al*., 2010). Significantly ethylene triggers the regenerating metabolism within the laticiferous cells (Pujade-Renaud *et al*., 1994). Insome clones of Hevea species with low latex metabolic rate, an ethylene releaser (ethephon) is applied to the bark which increases latex flow and latex regeneration from one tapping to the next (d'Auzac *et al*., 1997).

1.2 Problem Statement

Despite the progressive nature of research on shea latex, the flow rate of latex from the shea tree has not been encouraging and there is no single record of the use of stimulants as done in almost all Hevea plantations. Also, not much comparison has been done taking into consideration the most latex producing and highly considered important commercial source of natural rubber of about 44.3% (Agrawal and Konno, 2009), the latex from *Hevea brasiliensis* except in a study which compares the proximate composition of shea and rubber latex carried out by Fosu and Quainoo (2013).Considering the results obtained from the study, rubber latex revealed higher percentages of moisture, crude protein and carbohydrate than in shea latex. However, there was higher percentage of ash and crude fat in shea latex than in rubber latex (Fosu and Quainoo, 2013). According to Abdul-Aziz *et al.*, (2016), there is a potential for shea latex to be used to manufacture hypo-allergenic latex products owing to the lower protein content compared to latex from *Hevea brasiliensis.* This could be true but also debatable since no elucidation of the chemical structure of shea latex has been done to ascertain the presence of rubber particles and at what percentage it exists, although Agrawal and Konno (2009) report that both the stickiness and characteristically white colour of latex are often due to the presence of rubber particles distributed in the fluid. According to Lovett (2004),industry experts, use methods in the shea kernel extraction which may denature a percentage of the unwanted unsaponifiables including certain latexes that occur in unheated kernel. However, it should be noted that, though the latexes may not be necessary in the kernel extraction process, it may prove useful in other areas.

Further chemical analysis is thus required, which may also reveal certain other potentials of shea latex since other products of economic relevance are obtained from some latexes in other latex-producing plant species.

1.3 Justification

According to Agrawal and Konno (2009), other plant species produce latex which several economically relevant products are obtained. Chicle is known to be obtained from *Manilkara* spp. belonging to the family *Sapotaceae* and is utilised in chewing gum, and lacquers produced from phenols in the latex of plants species in the *Anacardiaceae* (Cashew). Bioactive compounds which are essential to a couple of processes are obtained in latex from numerous plant species including alkaloids such as cardiac glycosides in *Asclepias* spp. (*Apocynaceae*); lactucin from lettuce (*Lactuca* spp. Asteraceae); terpenes such as the sesquiterpene lactone; morphine in *Papaver* spp. (Papaveraceae) and digestive cysteine proteases in *Carica papaya* (*Caricaceae*) and *Ficus* spp. (*Moraceae*). A study conducted by Aielo *et al.*, (2014), also demonstrated interesting properties such as the excellent biocompatibility and capacity to stimulate angiogenesis, adhesion to cells and the development of extracellular matrix, encouraging the replacement and rejuvenation of tissue, provided by natural rubber latex membranes combined with a measured release of drug, making it important for biomedical applications. In this work, a natural rubber latex (NRL) membrane is used to administer sodium diclofenac and notably the discharge time of diclofenac in a NRL membrane in vitro was increased from the characteristic 2-3 hours for oral tablets to about 74 hours. Murbach *et al.*, (2014) also reports that natural rubber latex membrane can discharge ciprofloxacin for up to 59.08% in 312 hours and the process is due to super case II (non-Fickian). It is therefore necessary to further study the quality of shea latex into detail in comparison to the natural rubber latex especially, and other known important latex producing plants to allow more research into finding out whether it merits being exploited in industrial processes including but not limited to the production of other latex products like gloves, balloons,

chewing gum and condoms and also in the confectionary industry, biomedical industry, pharmacological industry and pharmaceutical industry.

1.4 Objectives

The core objective of this study is to determine the quality of shea latex (*Vitellaria paradoxa)* in comparison to natural rubber latex obtained from *Hevea brasiliensis.*

1.4.1 Specific objective

1.0 To determine the flow rate of shea latex as influenced by ethylene generator.

2.0 To determine the biochemical and phytochemical constitution of shea latex as influenced by ethylene generator in comparison to natural rubber latex.

3.0 To predict the comparative advantage of shea latex over natural rubber latex and vice versa based on chemical analysis as well as other latex producing species with economic relevance.

1.5 Anticipation

It is expected that the ethylene generator will have significant effect on the flow rate of shea latex and its quality.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Profile of the Shea Tree

The economic importance of *Vitellaria paradoxa* has increased interest into its study, cultivation and management as well as ecological distribution (Lovett and Haq, 2000b, 2000a; Sanou *et al*., 2006; Bello-Bravo, Lovett, and Pittendrigh, 2015;Quainoo *et al*., 2015; Abdul-Aziz *et al*., 2016), little is still understood of the pattern of characterization within its natural range. Regarding the two known existing subspecies – *paradoxa* and *nilotica*, no clear distinction is made based on leaves, inflorescences and flowers, fruits or morphology. The difference is mainly based on the origin of the stand: in the Eastern part (subsp. nilotica) and Western part (subsp. paradoxa) of the natural range (Bouvet, Fontaine, and Sanou, 2004). More in-depth research needs to be carried out in order to understand the genetic diversity of *Vitellaria paradoxa* and distinctively characterize trees within its natural range. Lovett and Haq, (2000b) identified a high level of genetic variation within populations of *Vitellaria paradoxa* occurring in Ghana. As a result of traditional land management, Lovett and Haq, (2000a) reported a proposed and unconscious selection of semi-domesticated landraces. The report indicated that 294 trees were characterized at 24 different sites from 18 different locations in the Brong-Ahafo, Upper West, Upper East and Northern regions of Ghana with broad variations in several characters including tree height; diameter at breast height; number of stems; leaf and petiole size; fruit size, shape, weight and taste; seed size and colour; canopy size; bark colour and texture; leaf and petiole colour; and flower size. Considering these variations, a conclusion was drawn that unless tree age can be accurately determined, these variables are not to be recommended for *Vitellaria* diversity studies.

In 1977, the fourth session of the FAO Panel of experts on Forest Genetic Resources included *Vitellaria* as part of the African forest genetic resource priorities for in situ conservation as a result of the importance of its non-wood products. Tree improvement and conservation programmes are either underway or yet to be initiated in countries where this species is utilized. The Cocoa Research Institute of Ghana began research into the diversity, management and propagation of shea nut since the early 1980s. Shea trees bear fruits whose nuts are cherished both locally and internationally for the economic value they possess (Gwali *et al.*, 2014). Shea fruits are mostly amassed by hand pickers, sold or processed into butter for several purposes (Hatskevich *et al.*, 2011). Shea butter processing, a major source of livelihood in the Northern regions of Ghana with the potential to alleviate rural poverty in these areas (Irvine, 1961). The shea butter utilization as cocoa butter substitute and raw materials for both the food and cosmetic industries is rapidly growing on global market. This provide huge opportunities to boost income of the rural women in Ghana (Hatskevich *et al.*, 2011). The tree is considered a sacred tree by many communities and ethnic groups and plays important roles in religious and cultural ceremonies where is also believed to have some spiritual protective powers (Agbahungba and Depommier, 1989; Van der Watt and Pretorius, 2001). It has been claimed to possess potentials to improve nutrition, boost food supply in the annual hunger season (Israel, 2014; Masters *et al*., 2010), foster rural development, and support sustainable land care (Israel, 2014). In characterization of the trees, Nafan *et al.*, (2009), classified them into four categories according fruiting season and fruits fallen rate and called them early maturing trees – generally fruits fall starts in April to mid-May, mid-season trees – fruits fall of these trees begin in May and ends in June. Some trees which have maturity periods spread over the entire production time (that is,

early, intermediate and late fruiting seasons) and a late season trees - The fall of the fruits of these trees begins in mid-May and ends in late July.

2.2 Morphology of plants

The relevance of plant morphology, including morphogenesis to practically all fields of plant biology such as physiology, molecular genetics, evolutionary biology, ecology and systematics remains unparalleled. This significance comes from the fact that other fields refer to or infer to conceptual frameworks of morphology, morphological concepts, and morphological theories (Sattler and Rutishauser,1997).

The requirement for the existence of trees do not only comprise of the capability to build a functional multicellular organism, but also to produce the necessary construction, storage, defense and regulatory substances. These include the buildup of storage and other relevant substances into the cells such as starch, proteins, fats or substances of the defense system, namely, anthocyanins, tannins, dyes, aromatic and bitter substances, saponins, glycosides, alkaloids or metabolic waste products. Some accumulate into the cell walls such as cellulose, hemicellulose, pectin substances, cutin and other high molecular weight waxes, lignin, suberin, polysaccharide mucilaginous substances etc. or are localized in specific parts such as resins, essential oils, latex, gum, rubber and the rest.

Woody plants, Plantae lignosae are perennials with woody stems above-ground and root systems, exclusively adapted to the terrestrial environment and are able to live for at least two growing seasons, but usually longer, from many years, to approximately thousands of years with a few exceptions. Woody plants are studied by dendrology; however, dendrology does not cover the whole part of the system. They can be divided into Holoxyles and Hemixyles, whose stems

lignify in the same growing season as they elongate and whose stems fully lignify only as late as the following season respectively. A damage to an unlignified part in a rest season, does not present a severe loss to a plant. The opposite is true – if it survives, a hemixyle has a store in place when the photosynthesis starts at the beginning of the next growing season. According to a plant design, woody plants can be differentiated as trees - a distinctive main stem remaining unbranched in its lower part, and shrubs - higher than 0.8 m without a distinct main trunk, with the stem branching at its base above or below the soil surface. Trees have a large, extensive root system, which passes into a compact, space- minimized (intense) and clearly established trunk near the soil surface. Branches form on the trunk at certain height above the ground and carry a crown. The crown usually grows in its periphery, viz. in height and width and the stem can be found either around full length, or it may soon form strong, numerous skeletal branches (as solitary broadleaf). Shrubs, woody cushion plants and creeping woody plants also have extensive aboveground and belowground systems (Martinková *et al.*, 2014). However, they do not build a trunk, thus they develop new and stronger branches from the lower buds, viz. close to the soil surface. Shrubs with a root system covered in the soil are known as chtonophytes. Above-ground parts of woody cushion plants also have a nearly hemispherical shape with dense, short shoots around the surface. Above-ground parts of creeping woody plants are pressed against the surface of the soil and the ends of branches may be ascending. Living cells form a protoplast, consisting of the cytoplasm made up of cytosol constituting colloidal solution of water, protein, fat, saccharides, and complex membranes, organelles, nucleus, vacuoles and ergastic substances produced by cell activities such as crystals, rubber, oil, resin. Vacuoles are primary storage spaces of water, saccharides, amino acids, proteins and lipids; they might be part of disintegrative and excretory apparatus and might have protective, defense and alarming functions suited for toxins

and pigments. Ergastic substances include starch, lipids, proteins as storage substances, cellulose, and lignin as structural substances and substances with physiological and ecological functions (crystalline inclusions, aromatic substances etc.).

The trunk of a tree is the unbranched part connecting the root and the tree crown, stems found within the crown are called branches. Trunks usually have the radial symmetry, the most frequent shape is cylindrical, but may also be muscular, flattened etc. and it is extended by roots at the bottom (Martinková *et al.*, 2014).

The changes in the thickness of the bark which is reversible are well documented. In certain times of the season, these variations in bark thickness portray a distinct diurnal oscillation with shrinkage and swelling during the day and overnight respectively. The rhythm of shrinkage and swelling becomes more irregular on cloudy or rainy days and swelling increases. Lövdahl and Odin (1992) stimulated variations in bark thickness by regulating temperature and air relative humidity in chamber-grown Norway spruce seedlings (*Picea abies* (L.) Karst.). The study revealed, however, that the daily variations in trunk diameter remain unaffected, and established that changes in bark thickness signify the hygroscopic behaviour of tree bark and are not dependent of the rate of transpiration. Aside from the daytime changes in bark thickness during certain seasons, in summer particularly, there are also fluctuations in stem thickness which cannot be explained also occurring in winter, even at temperatures beyond the freezing point. Loris *et al.*, (1999) suggested that these forms of fluctuations in stem thickness can serve as agauge of water stress. This occurs mostly when soil water freezes and cuticular and peridermal transpiration rises in reaction to temperature ranges above freezing air temperatures. Loris *et al.*, (1999)established that water transfer along an osmotic gradient account for the changes in stem thickness, over the whole tree trunk and over a period of time. The time course of reversible bark thickness changes

coupled with the changes in relative humidity with a lag of 2 to 3 hours. However, the relation between changes in relative humidity and variations in bark thickness varied between periods of clear and sunny weather and periods followed by a rainy day. There was a correlation between relative humidity and vapor pressure deficit (VPD) due to the narrow temperature range within the experimental period. Therefore, the strong relationship between sap velocity and VPD shown to be above a threshold of about 300 Pa, suggests a strong relationship between sap velocity and relative air humidity. Nevertheless, this relationship was varied in some cases, as shown by results when there was a decrease in relative air humidity and a decrease in bark thickness, but no sap flow was detected. (Gall *et al.*, 2002).

Apart from classical conducting systems including the xylem and phloem, which are widely spread in vascular plants with well-defined basic functions in plant physiology, there are also laticifer structures, representing an independent secretory-like duct, whose intrinsic cellular metabolism and functions remain to be established in detail. Laticifers differentiate themselves not only in morphology but also in their functions from true conducting systems. Laticifers are true specialized cells, with the ability to differentiate and express distinct DNA information resulting in the synthesis of some specific proteins and metabolites. Natural rubber is the most common chemical product resulting from laticifer biochemistry.

2.3 Latex sources

Found in long branching tubes known as latex tubes laticifers is the milky juice known as plant latex. Depending on the plant species this juice may be white, yellow or pinkish in colour. It is a viscous fluid and colloidal in nature (Mahajan and Badgujar, 2008b).

In the 19th century, the latex system of plants was one that received attention and was subjected to various sorts of investigations. By the middle of that century, enough research on the features and structure of the latex system had been completed with a fairly accurate idea of the elements constituting those systems in a few of the more common families (Lommasson, 1962).

Esau (1965), reported that during the creation of articulated latex vessels – a type of latex vessel, cell fusion takes place and walls between adjacent differentiating cells break down. Sassen (1965) reported that the wall is dissolved enzymatically by means of electron microscopic investigation. Because latex is a term that covers dozens of types of secretions, hence there is an extremely wide diversity in their ultrastructure and it is at the level of electron microscopy that structural inconsistencies can be analyzed (Abd Razak, 2000).

Since cellulase takes part in the breakdown of wall material, the latex of various plants has been examined for the presence of this enzyme. The latex of several plants with non-articulated laticifers has also been investigated for cellulase; without cell fusion or breakdown of cell walls, laticifers of this type develop by intrusive growth (Esau, 1965). Evidently it was realized that cellulase is present in latex from articulated laticifers, and was absent in latex from non-articulated laticifers, strongly indicating that the enzyme cellulase is concerned with the removal of wall material during differentiation. The activity of cellulose is varied among different species, some are high, compared to other reports of cellulase activity in certain plant tissues; for example, Sheldrake, (1969) reported a cellulase activity of *Hevea brasiliensis* latex which is 50-150 times that in expressed sap from the stems and leaves of tobacco plants Tracey (1950).

The latex system, apart from the articulated may be of branched non-articulated laticiferous cells. These laticifers are spread out regularly or ostensibly at random through the cortex of root and stem depending upon the species. There are still lactiferous cells apparent the root and stem even

when the outer cortex is destroyed by periderm in the root or the inner cortex by crushing in the stem. However, in the leaf is found the most extensive branching network of laticifers. The commonest latex system in the leaf was found to be the sub-epidermal system (Lommasson, 1962). The latex systems of the root and stem remain active after periderm formation in the root and crushing of the cortex in the stem. The intrusive growth and subsequent expansion of surrounding cells accounted for the irregularities of the pathways of the laticiferous cells. Straight laticifers may probably be as a result of symplastic growth. Branching occurred most frequently near the point of origin of lateral structures (Lommasson, 1962).

In *Hevea brasiliensis*, the absence of cytoplasmic connections or plasmodesmata between anastomosed cells and their neighboring cells is a unique feature of the laticifers (Hebant, 1981). Therefore, contents of the cytoplasm should be the only component of the exuded latex from laticifers, uncontaminated by those of other cells (Kush *et al*., 1990). In addition to that, the colour of the latex may differ between species. Metcalfe (1967) added, for example, that it might be yellow (Cannabis), orange (Papaver), red, or even greenish in different taxa.

2.4 Laticiferous cells

These cells are regularly distinguished as single cells from the cambium and arranged in concentric layers (mantels). Upon maturation, the laticifers joined in a tube-like manner within each mantel, forming laticifer networks, referred to as a para-circulatory system(de Faÿ and Jacob, 1989.; Hébant and Faÿ, 1980).Morphologically, laticifers are classified into two. They may be either articulated laticiferous cells or non-articulated laticiferous cells. These laticifers are similar to xylem vessels to such an extent that laticiferous vessels originate from rows of meristematic cells in which the transverse septa become absorbed at an early stage of

development (Lommasson, 1962). The simple laticifers can be said to be created from single cells while the compound laticifers are derived from series of cells. In specialized states, the series of cells in a compound laticifer are unified by dissolution of intervening walls. Due to presence of this junction of cells the compound laticifers are commonly known articulated laticifers, whereas the simple laticifers are referred to as non-articulated. These two types of laticifers may be branched or unbranched and are mostly referred to as 'vessels', probably due to their resemblance in origin to that of the conducting elements (Abd Razak, 2000). They are however living and coenocytic in nature. In mature plants, they may exist as non-anastomosing systems, or they may form several branches. The vessels form the anastomosing system by the joining of more or less parallel ducts through connecting living cells. Members of the families *Papaveraceae*, *Caricaceae*, *Musaceae* and the genera; Hevea and Manihot in the *Euphorbiaceae* are known to have laticiferous vessels (Lommasson, 1962). Laticiferous cells are structurally coenocytes created from a single cell. Initial cells may be distinguished in the young embryo at the inner margin of the primary cortex in the cotyledonary node. As the plant mature the laticiferous cells grow into a branching system ramifying throughout the entire plant body and it is referred to as intrusive growth. Also a considerable amount of growth is svmplastic growth (Lommasson, 1962). In study conducted to determine the distribution and location of laticifer vessels in the plant, Transmission Electron Microscopy (TEM) was used to characterize the ultra-structural features of the cells secreting and storing the latex. The TEM showed that the laticifer cells have well-preserved cytoplasm and organelles from which a conclusion can be drawn that metabolic activity is still intact. The mature laticifer cell has a large central vacuole full of latex accumulated in a large osmiophilic droplet. Smaller droplets of the latex are also adhered to the tonoplast. Surrounding the vacuole was a thin layer of well-preserved parietal cytoplasm. Plastids,

mitochondria and rough endoplasmic reticulum profiles well-structured were present and showed no signs of degradation. In particular, the laticifer cell plastids held numerous osmiophilic droplets, while the plastids in the surrounding parenchymal cells contained only starch (Sacchetti*et al*., 1999). The findings derived from microscopy on all plant laticifers showed no significant structural differences between the laticifer cells in the different organs (Sacchetti *et al.,* 1999). Emphatically, the plastic response to biotic environmental factors, including induced production of plant defense chemicals in response to herbivory and pathogens, and the effects of endophytic fungi and other symbionts on host plant development and life history (Cheplick, 1997). Using optical and electron microscopy, the distribution, cytological organization and development of laticifers in some latex producing plants have been investigated. The pattern of distribution of laticifers in all species studied exhibited similar characters. They were found in the stem cambium, petioles, leaves and roots, or closely located within the vascular bundle. Chalk (1983) gives a detailed report on the structure and distribution of laticifers. In the advent of technological advancement in microscopy, including preparation techniques such as cryo-preparation and low temperature electron microscopy, it has been possible to retain the exactness of the cells without using destructive chemicals. These methods have offered opportunity to further unravel laticifer architecture and cellular organization. Generally, laticifers were known to function as a special type of storage or excretion system in plants. Again, it is noted that in almost all of the laticiferous plants, latex has been observed to have some repellent properties against insects, providing the plants with a defense mechanism against pest attack.

2.4.1 Articulated Laticifers

Basically, there exist difference between articulated laticifers and non-articulated laticifers in terms of development and structure. The articulated laticiferous vessels are concentrically

arranged in rings in the phloem (Zhang *et al*., 2017).Huang and Sterling, (1970) observed in Allium that plasmodesmata connects two adjacent laticiferous cells, however in other tissue there is usually some form of perforation of the common wall. These structural variations of the articulated laticifers allows a further division into two subgroups. Several of the articulated laticifers comprise of long cell chains or compound tubes not laterally connected with each other. These are known as articulated non-anastomosing laticifers whereas others form lateral anastomoses with similar cell chains or tubes, coming together to form a net-like structure or reticulum and are also known as articulated anastomosing laticifers (Esau, 1965). The articulated laticifers can be present in all parts or tissue of the plant body, independent of the age, vegetative or floral. However, in *Hevea brasiliensis*, the most important laticifers are found in the bark from where the latex or rubber exudes when it is tapped (Gomez, 1982). Laticifers are found in all organs of pawpaw, and its laticifer are classified as articulated, anastomosing, multinucleate at maturity (easily seen in mature fruit), and especially common in the periphery of green fruit from which latex is collected commercially (Fisher, 1980).

2.4.2 Development of Articulated Laticifers

According to Esau (1965), the articulated laticifers develop into extensive tube-like structures by the consecutive addition of new primordial cells to the existing ones and not necessarily the growth of individual cells. This form of growth takes place by the continuous initiation of the nearby parenchyma cells, which are later converted to laticifer cells. As the newly added cells adjacent to older existing laticifers cells differentiate, the common wall becomes perforated, and the new cells are added to the laticifer, which is similar to the synthesis of the xylem vessel where new vessel elements are added (Mauseth, 1988).

Depending on how the developing laticifers interrelate with the neighboring cells during the differentiation, there are two types of developments in articulated laticifers, namely the non-anastomosing articulate laticifers and anastomosing articulate laticifers. Non-anastomosing articulated laticifers occur in some species which mostly bears single laticifer cell, which although are in row with neighboring cells, does not merge with another laticifer during its differentiation. Plant families such as *Achras* (*Sapotaceae*), *Allium* (*Liliaceae*), *Ipomoea* (*Convolvulaceae*) and *Musa* (*Musaceae*) are mostly characterized by this type of laticifers. On the other hand, *Meconopsis, Papaver* (*Papaveraceae*); *Carica* (*Caricaceae*); *Cichorium, Lactuca, Taraxacum, Tragopogon* (*Compositae*); and *Hevea, Manihot* (*Euphorbiaceae*) are characterized by articulated anastomosing laticifers, one in which laticifer can fuse with others, forming an extensive three-dimensional network that runs through the entire plant. Branching of laticifer system makes this formation possible. However, the laticifer itself does not grow out to form the branch; regular parenchyma cells that lie between two existing laticifers are induced as an alternative, differentiating into latex-bearing cells (Blaser, 1945; Vertrees and Mahlberg, 1978). In a study conducted by Sheldrake and Moir (1970) using electron microscopy on seven species with articulated laticifers and 4 species of non-articulated laticifers, strongly reported that the dissolution of walls in articulated laticifers is enzymatic, and it is by cellulase present in the laticifers itself. Also, suggesting that high level of auxin could also help increase cellulase activity by weakening or loosening the structure of the cell wall. The presence of enzymes in the laticifers is another criterion used to divide laticifers into two groups of - articulate and non-articulate. An assay study conducted on a species with articulated laticifers showed a very high content of cellulase, whereas there is hardly any evidence of cellulase in the non-articulated species. This also explains why laticifers are abundant and concentrated in certain species, for example *Hevea*

brasiliensis, where the cellulase content is estimated to about 50-150 times higher than the other species (Sassen, 1965; Sheldrake and Moir, 1970). Tracey (1950), also reported that the amount of cellulose is higher in the young tree as compare to the mature tree, probably due to the active differentiation of cells taking place.

2.4.3 Non-Articulated Laticifers

Single cells are the origin of non-articulated laticifers, which through continuous growth develop into tube-like structures, mostly branched, but naturally do not have fusions with other similar cells. The degree of complexity in non-articulated laticifers vary in their structure. Some undergo extra development into long, relatively straight tubes; others branch frequently, each cell ending up in the formation of an immense system of tubes. Esau (1965) suggested that these two types of structures can be called non-articulated unbranched laticifers and non-articulated branched laticifers, respectively.

Naturally the non-articulated laticifers are extraordinarily long cells, often stretch from the root up into the stem and leaves. Still, in certain species, such as Cryptotesgia, Jatropa (Dehgan and Craig, 1978) and *Parthenium argentums* (Metcalfe, 1967) the non-articulated laticifers are small, rather isodiametric idioblasts, resembling myosin cells to some extent. In some species they are unbranched as for instance *Cannabis* (*Moraceae*); *Urtica* (*Urticaceae*) and *Vinca* (*Apocynaceae*). In others, they branch repeatedly, forming an even more extensive network as in *Asclepias*, *Cerepegia*, *Crypstostegia* (*Asclepiadaceae*); *Broussoetia*, *Ficus*, *Madura* (*Moraceae*); *Nerium* (*Apocynaceae*) and *Euphorbia*, *Jatropha* (*Euphorbiaceae*).

2.4.4 Development of Non-articulated Laticifers

Non-articulated laticifers can be found in any part of the plant, usually in the softest regions such as the pith and cortex, still they can enter leaves and leaf gaps as well as wood and phloem (Mauseth, 1988). Blaser (1945) describe how the tip of the non-articulated laticifer may invade the margins of the shoot and root meristems, but as the meristem grows further, the laticifer continuously invades stem and root tissues which are newly formed. In some species, older plant will have more laticifers than a younger plant as in the new tissue new initials are formed. On record, there are varied forms of non-articulated laticifers and at maturity some plants species portray laticiferous cells that may develop into very large systems which extend throughout the different shoot and root tissues.

A research carried out by Roy and De (1992) on differentiation of non-articulated laticifers of *Calotropis gigantea* (Linn.) highlight the anatomy, distribution, structure and ultra-structural organization using both light and electron microscopy. They reported that prior to a sequential breakdown of the components of cells with preservation of a thin layer of peripheral cytoplasm and the creation of central vacuole is the broadening and fusion of the small vacuoles together with the degeneration of cell components. From the observation, Calotropis is comparable *Ficus carica* and tobacco in formation of vesicles from peripheral cytoplasm and the release of electron dense osmiophilic globules into the vacuole of the cells (Rachmilevitz and Fahn, 1982). However, there are still possibilities for non-articulated laticifers to growth at their tips portraying an intrusive manner of growth. According to Wilson *et al.*, (1976) the intrusive growth and extensive elongation of non-articulated laticifers discovered in milkweed, *Asclepias syriaca* L, proposes that there should an activity of a pectolytic enzyme system. The pectinase may play the role of loosening or dissolving the middle lamellae between cells and allowing intrusive growth of the

growing tip of the laticifer. Supposing that the enzyme is synthesized in the laticifer, even though the specific site of synthesis within the cell is not yet known, they however concluded that the dissolution of pectic substance of the middle lamella is as a result of laticifer secreting pectinase ahead of the growing cell tip and hence facilitates penetration of the laticifer among other cells during its growth throughout the plant. Still, the pectolytic enzyme may be responsible for loosening cell wall material proximal to the tip of the laticifer to allow cell elongation. Abd Razak (2000), referred to this criterion of enzyme releasing mechanism as a significant grouping criterion between articulated laticifers and non-articulated laticifers; that is toward the tips of the laticifers instead of in the laticifers and dissolving cell walls at any points along the laticifers.

2.4.5 Comparison between articulated and non-articulated laticifers

Table 2. 1: Comparison between articulated and non- articulated laticifers

	Articulated	Non-articulated
Mode of development	Cell wall degradation	Intrusive growth
Initial development	Compound cells - involves Parenchyma cells	Simple single cell
Enzyme activity present	Cellulase within the laticifers. Assisted by auxin a plant hormone	Pectinase in cell wall toward the laticifers.
Laticifers distribution	All part of the plants	All part of the plants

Source: (Abd Razak, 2000)

2.5 Location of Lactiferous Cells

Esau (1965), established that laticifers might occur in any plant organ and are not designated to only certain parts of the plants. This theory was further buttressed by other researchers such as of Gomez (1982) in Hevea, Roy and De (1992) in *Calotropis gigantean* and Fineran (1983) in *Euphorbia pulcherrima*. Nonetheless, this does not mean that all plants have laticifers in their tissues or some of their tissues.

The distribution of laticifers within the plant body varies, and it mainly depends on the species in question. Most often they go together with the vascular bundle, and occur prominently in the phloem, in which case is difficult to distinguish them from sieve-tubes. At certain times, laticifers are more widely spread out in the parenchymatous tissue, e.g., *Nerium oleander* L. Laticifers are generally restricted to the rays in the xylem tissue. Countless number of plants have laticifers spreading out into the leaves and may have ramifications extending into the mesophyll where they sometimes reach the hypodermis, if present, or the epidermis. Conversely, some species still have laticiferous cells restricted to certain organs of the plant body (Metcalfe, 1967).

In some plants the laticifers are scarcely present or they do not exist at all. With regards to known latex bearing plants, the presence of laticiferous cells may either be localized or exclusively found only in certain or specialized tissues of plants (Abd Razak, 2000) as found in the interesting *Decaisnea insignis* Hook. f., a shrub which belongs to the family *Lardizabalaceae* which is related to the more familiar Berberis family, the *Berberidaceae*. The latex is restricted to the fruits where it is to be found in a system of canals.

Moreover, Metcalfe (1967) stress that laticiferous tissues are not restricted to plants with any one particular type of habit nor are they confined to plants from any particular type of habitat. They

can find in a broad range of members in the kingdom plantae including herbs, including both xerophytic succulents and water plants, as well as in trees, shrubs, and lianas.

2.5.1 Plant roots

The laticiferous cells in the roots of certain species occur most frequently in the cortex and since the development of cork usually damages the cortex of the older roots, most of the evidence of laticifer cells are destroyed alongside. However, in cases of this sort, certain cells of the phloem seem to be differentiated as laticifers. In *Euphorbia macalata*, the root laticiferous cells in the cortex are large and oval and can be easily distinguished from other cells of the cortex. In other roots, such as those of *Euphorbia supina*, only a small number of the cells bearing latex are conspicuous. The path of the laticiferous cells through the cortex of the root may be a tortuous pathway and at other places it may be straight. The difference in the nature of the pathway may probably be associated with the two types of growth. The tortuous appearance and straight pathways is most likely to be a result of intrusive growth of the laticiferous cells through the tissues and also the result from symplastic growth respectively (Lommasson, 1962).

According to Sacchetti *et al.*, (1999), when conventional optical microscopy was used to observe sections of roots of *Vinca sardoa* belonging to the family *Apocynaceae*, there were numerous laticifer cells which appeared dark and were distributed throughout the cortical region of the roots. Histochemical reaction with chloroauric acid solution on longitudinal sections, latex stained deep purple-red showing several unbranched, articulated laticifers distributed throughout the entire cortical region.

2.5.2 Plant stem

Development of laticiferous cells in the stem is similar to that found in the root. Lommasson, (1962), investigated the latex system of some spurges (*Euphorbaceae*). Discoveries were reported as follows; in *Euphorbia fendleri* an intriguing development is observed, as the occurrence of latex cells may be observed in both perennial and first year stems. The development of the periderm in the perennial stems damages all traces of the cortical cells and sclerenchyma fibers which are located in first year stems. The laticiferous cells found in the perennial stem and the first-year stem are all in the phloem and the in the cortex respectively. Some laticiferous cells are found between the sclerenchyma groups and neighbouring to the outermost part of the phloem. In the stem of *Euphorbia stictospora* two systems of branching exit. One is made up of larger laticiferous cells within the inner cortex and the other are much smaller and more numerous in the outer portion of the cortex. The stem of *Euphorbia supina* shows an abundance of latex cells in the cortex. The cortex of the stem of *Euphorbia missurica* var. *intermedia* however has a morphological feature that allows for laticifers only to exist in the innermost part of the cortex. In transverse sections of the stems of *Euphorbia rnaculata*, *Euphorbia hurnistrata*, *Euphorbia serpens* and *Euphorbia. glyptosperma* generally have the same type of distribution of the laticifers and similar orientation in the stem - the laticiferous cells appear as a single layer of large oval cells. The differences amongst these may relate to number, spacing and wall thickness of laticifers. In *Euphorbia missurica* var *typica* the laticifers have irregular arrangement but are located within the inner cortex where they are clearly distinct by their larger size.

In the stem, the laticifers of *Vinca sardoa* were located in the cortical region, both close to the vascular bundles and below the epidermis. Unbranched and articulated laticifers with varying

alkaloid reactivity were found below the epidermis and close to the vascular bundles (Sacchetti *et al.,* 1999).

2.5.3 Plant leaf

Cross-sections of fresh and resin-embedded leaf specimens of *Vinca sardoa* showed laticifer cells, particularly close to the abaxial surface just below the epidermis. No particular interaction between laticifer vessels and vascular bundles were found here. Cross-sections of resin-embedded petiole specimens indicated a large number of laticifer cells both below the epidermis and in the phloem parenchyma (Sacchetti *et al.,* 1999).

At the nodes of stems, branching of the laticiferous cells occur most regularly. These branching can be traced in the petiole. In the petiole, the laticiferous cells and vascular tissue are in close proximity to each other laterally and abaxially (Lommasson, 1962).

The leaf blade contains profusely branching network of laticiferous cells due to proliferation that takes place with the laticifers. The laticifers are approximately the same size and rather close to the midrib in the base of the leaf blade, and as opposing the structural arrangement in the petiole, they are not intimately associated with the vascular tissue. In any tissue of the leaf blade, the large laticifers present are oriented multi-directionally. No anastomosing structure has been observed in any species. A noticeable network may be located in almost all layers of the leaf blade. Among certain individuals in the family *Euphorbiaceae*, *Euphorbia serpens* has been found to possess the most easily observable subepidermal system where the laticiferous cells are in connection with the upper epidermal cells and also the upper ends of adjacent palisade cells. In *Euphorbia supine* a prominent network of laticiferous cells occurs in the spongy parenchyma just above the hypodermis even though it possesses a rather poorly developed hypodermis above the lower

epidermis. Taking all the Euphorbia species studied by Lommasson (1962) into consideration, the most usual latex system in the leaf lies just underneath the epidermal cells. No laticifers were found in a sub-cuticular position. The next to those in beneath the epidermal cell in regularity is the system found in the spongy parenchyma as noted in *Euphorbia supina*. The least common system was those located adjacent to the bundle sheath cells. Laticiferous cells either ends in the mesophyll or in contact with either epidermis.

2.6 Defensive property of latex

The functionality of the latex system in plants in times past has resulted in concepts which are in conflict, not knowing which exact role it plays in plants (Groom, 1889; De Barry, 1884). Seemingly, James (1887) proposed the first defensive hypothesis for latex. It was said that it had properties that made it become a better protection to the plant from predators than all the thorns, prickles, or hairs that could be provided. Lommasson, (1962) reported that not much progress has been achieved in the understanding of the function of latex has been made even during the sixty's and that textbooks in plant physiology typically exclude any discussion of latex functions. According to Kniep (1905), slugs readily ate damaged leaves of a plant in the *Euphorbiaceae*, which no longer produced latex, but did not eat the leaves of intact plants that were not drained of their latex. In the ninety's however, Data *et al.*, (1996) investigated the potential of latex produced by sweet potato as a defense mechanism against the sweet potato weevil. The application of latex to the surface of root cores reduced feeding and oviposition. Also, there was significant reduction in the number of feeding punctures when latex was added to a semi-artificial media. Young vine materials of sweet potato used in the study produced more latex and recorded less weevil feeding damage than older more mature portions of the vine. Latex in some

laticiferous plants acts as a natural defense system against certain herbivores.Latex-containing tissues or cells, known as laticifers mostly constitute chitinase or lysozymes because they are particularly critical for plant survival and also because they are rarely susceptible to invasion by a pathogen. Thus, it might be particularly difficult for an invading microorganism to be contained in a wounded laticifer without constitutively expressing defense proteins (Martin, 1991). No strong alternative hypotheses have stood the test of time (Agrawal, 2005).

Evidences, ranging from observational to the experimental and even comparative, have been accrued to buttress that theory that latex acts as a plant defense against plant feeders (Agrawal and Konno, 2009), although before, Agrawal (2005), reported that not much findings have been tied to this assertion. The defensive advantage of latex production for plants have not been well quantified (Agrawal, 2005). The high level of chitinase activity found in laticifers, combined with the occurrence of highly toxic secondary metabolites which are already described in literature may explain how *E. tirucalli* plants are protected against herbivory and infectious organisms (Souza *et al.*, 2010).

2.7 Phytochemicals in latex

Phytochemicals are biologically active compounds, found in plants in small quantities which are not well-known nutrients. However, they seem to contribute meaningfully to plant defense against degenerative disease (Dreosti, 2000). This terminologyis not applicable to compounds used in relation to treating an established acute disease, rather to substances that are defensive at low levels against the growth of degenerative diseases over a period (Dreosti, 1998). They are natural bioactive compounds that interact with nutrients and dietary fibre to defend against diseases. Rao (2003), classifies phytochemicals with disease-preventing functions into major

groups including, dietary fibre, antioxidants, detoxifying agents, immunity-potentiating agents and neuropharmacological agents and stating that each class of these functional agents consists of a wide diversity of chemicals with differing potency.

It is also well known that plants produce these compounds as part of their defensive system, but recent research proves that they are able to protect humans against diseases along with assisting in risk reduction for a variety of chronic and inflammatory conditions (Adesuyi *et al*., 2012).

Phytoconstituents isolated from the latex of *Euphorbia nivulia* belonging to Euphorbia genus are reported for cytotoxic activity (Veluri *et al.,* 2003). According to Manoorkar and Gachande (2015), the constituent of latex are proteins, alkaloids, tannins, terpens, starch, sugars, oils, resins, gums and enzymes. Histochemical analysis of alkaloids present in the latex of *Vinca sardoa* (Stearn) Pign. (*Apocynaceae*) proved positive, indicating a close relationship between laticifer cells and the presence of alkaloids in the plant (Sacchetti *et al.,* 1999). The biosynthesis of terpenes in general, has already been demonstrated (Grumbach and Forn, 1980).

According to Saratha and Subramanian (2010), partially purified *Calotropis gigantea* latex extract is an important source of potentially useful compounds for the development of novel chemotherapeutic agents as a result of its antifungal effect on some human pathogenic fungi in-vitro compared to Amphotericin B. Qualitative phytochemical screening of the extract revealed certain bioactive compounds such as flavonoids, alkaloids, triterpenoids, steroids, saponins, phenols and glycosides.

Adesuyi *et al*., (2012) reported on the presence of Phenols, Saponins, Alkaloids and Flavonoids which are indications of Cosmetic and medicinal Value of *Aloe barbadensis*. Tannin, Phytate and Oxalate contents were also present and were described to affect the availability of Minerals in

Aloe barbadensis. Also, the rich source of minerals such as sodium and potassium indicate the tendency of *Aloe barbadensis* to be able to regulate or control the osmotic balance of the body fluid as well as body pH. *Aloe barbadensis* is also found to be rich in phosphorus and magnesium which are essential for bone formation and lowering the blood pressure respectively.

Table 2. 2: Qualitative Phytochemical analysis of latex of some laticiferous plants.

Sr. No	Botanical Name	Family name	Common name	Alkaloids	Cyanogenic Glycosides	Phenolics	Flavonoids	Terpenoids	Tannins	Saponins
1	*Alstonia scholaris*	Apocynaceae	Satwin	+	-	+	+	-	-	-
2	*Calotropis gigantea* (L.)	Asclepiadaceae	Rui	+	+	+	-	-	+	-
3	*Calotropis procera*	Asclepiadaceae	Rui	+	-	+	-	-	+	+
4	*Carica papaya* L.	Caricaceae	Papaya	+	+	+	+	+	+	-
5	*Euphorbia hirta* L.	Euphorbiaceae	Christ Plant	+	-	+	+	-	+	-
6	*Euphorbia milii* Desmoul.	Euphorbiaceae	Dudhi	-	-	+	-	-	-	-
7	*Euphorbia nivulia* Buch-Ham.	Euphorbiaceae	Sabar	+	+	+	-	-	+	+
8	*Euphorbia prunifolia* Jacq.	Euphorbiaceae	Dudhi	+	-	+	-	-	-	-
9	*Ficus carica* L.	Moraceae	Anjir	+	-	+	-	-	+	+
10	*Ficus hispida* L.	Moraceae	Bhui-umber	+	+	+	-	-	+	-
11	*Ficus racemosa* L.	Moraceae	Umber	-	-	+	-	-	+	-
12	Ficus religiosa L.	Moraceae	Pimpal	+	+	+	+	-	-	+
13	*Ipomoea carnea* Jacq.	Convolvulaceae	Besharam	-	-	-	+	-	+	-
14	*Manilkara zapota* (L.)	Sapotaceae	Chiku	-	+	+	-	+	-	-

15	*Pedilanthus tithymaloides* (L.)	Euphorbiaceae	Vilayati sher	+	+	+	-	-	-	+		
16	Plumeria rubra L.	Apocynaceae	Lal chafa	+	-	+	-	-	-	+		
17	*Plumeria rubra* L. forma acuminata (Ait.) Santapau and Irani ex Shah	Apocynaceae	Pandhara chafa	+	+	+	+	-	+	+		
18	*Synadenium grantii* Hook. F.	Euphorbiaceae	Irhonda	+	+	+	-	-	-	+		
19	*Tabernaemontana citrifolia* L.	Apocynaceae	Chandani	+	+	+	+	-	-	-		
20	*Tabernaemontana divaricata* (L.) R. Br.	Apocynaceae	Chandani	-	-	+	+	-	-	-		
21	*Thevetia peruviana* (Pers.) K.Shum.	Apocynaceae	Piwali Khaner	+	+	+	-	-	-	-		

Positive sign "+"and Negative sign "-" denote the presence and absence of a specific phytochemical in the latex samples respectively (Mahajan and Badgujar, 2008a,b).

2.7.1 Alkaloids

Phytochemically, latex tends to be more diverse and often contains complex combinations of terpenoids, phenolics, proteins, and alkaloids (Langenheim, 2003). Alkaloids are basic rather than acidic natural products containing nitrogen, many of which are toxic and typically do not have a

primary function in plants. Alkaloids are produced by a variety of animals, microorganisms, and plants and have been noted to be a constituent of the latex of many species, occasionally spread among angiosperm families, including *Papaveraceae* and *Moraceae* (Agrawal and Konno, 2009). It has been reported that synthetic enzymes involved in the early stage and late stages of alkaloid synthesis are contained in parenchymal cells surrounding laticifer cells and inside the laticifer respectively (Samanani *et al*., 2006; Weid *et al*., 2004). Rao (2003)reported that, some alkaloids function as neuropharmacological agents, antioxidants, and in cancer chemoprevention. Kaufman and his associates (1999) had the view that alkaloid compounds are abundant among plants and are best known for their often-potent pharmacological properties. Consequently, many of the common drugs are alkaloid based. Relatively mild examples include caffeine, quinine, and nicotine. More potent examples include cocaine, morphine, and strychnine. Biosynthetically, they may be derived from amino acids, terpenes, or aromatics depending on the specific alkaloid structure.

2.7.2 Terpenoids

Indeed, the latex of most species contains a diversity of biologically active compounds. Terpenoidsare carbon-based compounds which are diverse and are derived from five-carbon isoprene units. Terpenoids probably have many roles in plants including pollinator attraction, defense, and roles in primary metabolism such as carotenoids that provide additional pigments for harvesting light energy, and can be produced abundantly in latex(Agrawal and Konno, 2009). The latex of *Lactuca sativa* containssesquiterpene lactones (SL) whichhave some antifungal activity against the pathogenic, *Cladosporium herbarum*; and lettucenin A, which is induced in latex by microorganisms, strongly inhibited the growth of *Cladosporiun herbarum*(Sessa *et al*., 2000).

The latex of some *Euphorbiaceae*, such as *Euphorbia biglandulosa*, contains diterpenes such asphorbol and its derivatives (Noack *et al*., 1980). These compounds have toxicity against insects and mammals, have tumor-promoting activity, and cause skin inflammation(Gershenzon and Croteau, 1991). Further, triterpenoids are reported as the major components ofthe latex ofsome *Euphorbia* spp.(Mazoir *et al*., 2008). It is reported by Rao (2003) that, some terpenoids have properties that allows them play vital roles as neuro-pharmacological agents, antioxidants, and cancer chemoprevention. Terpenes have, however, been shown to act as anticancer agents (Elson and Yu, 1994).

2.7.2.1 Triterpenes

Triterpenes are C_{30} compounds, based on six isoprene units and are derived from squalene. They are often colorless solids with high-melting points, and are broadly distributed among plant resin, cork, and cutin. Triterpeneshave several important groups, including common triterpenes, steroids, saponins, sterolins, and cardiac glycosides. Only a small number of the common triterpenes are widely distributed among plants. These include the amyrins and ursolic and oleanic acid which form on the waxy coatings on leaves and also as protective layer on some fruits. Other triterpenes include the limonins and the cucurbitacins. Nearly all plant steroids are hydroxylated at the third carbon and are in fact sterols. In the mammals, the steroids have weighty importance serving as hormones, coenzymes, and provitamins. However, the role of the phytosterols is less well understood. Saponins are high-molecular-weight triterpene glycosides. They contain a sugar group joined to either a sterol or other triterpene. They are widely distributed in the plants and are made up of two parts: the sugar part called glycone and the triterpene part called aglycone or genin. Characteristically, they have detergent properties, foams

in water, tastes bitter, and are piscicidal. Many of the plants that contain saponins have been used in history as soaps including soaproot (*Chlorogalum pomeridianum*), soapbark (*Quillaja saponaria*), soapberry (*Sapindus saponaria*) and soapnut (*Sapindus mukurossi*). Saponins are constituents of many plant drugs and folk medicines, especially among Asian peoples (Kaufman*et al*., 1999).

2.7.3 Phenolics

Phenolics, which include that include tannins, lignins, and flavonoids are a vast group of multifunctional carbon-based secondary metabolites produced by the shikimate pathway (Agrawal and Konno, 2009). Rao (2003)stated that there are antinutritional and toxic factors present in various foods including phytates and tannins, which hinder with iron absorption. Tannins are said to consist of various phenolic compounds that react with proteins, forming water-insoluble co-polymers. Industrially, this reaction with proteins has been exploited for the conversion of animal skins into leather. Plant tissues with high tannin content have a highly bitter taste and are avoided by most feeders (Kaufman *et al*., 1999). However, further report suggests that, some phenols are detoxifying agents which play key roles in inhibiting procarcinogen activation, inducers of drug metabolizing enzymes, binding of carcinogens, and inhibitors of tumorigenesis (Rao, 2003).

2.7.4 Sugars or Carbohydrates

Sugars are the primary products of photosynthesis and are essential as a source of energy to plants. They may be stored as starch or fructans, used as sucrose, and polymerized to form

cellulose, the main cell wall structural material of plants. Sugars are optically active aliphatic polyhydroxlyated compounds which are soluble in water.

2.8 Economic benefits of latex

2.8.1 Rubber

Bonner and Galston (1947), pointed out that rubber formation is a feature scattered through numerous families of the plant kingdom and does not have any clear evidence of regular pattern. The families *Moraceae*, *Euphorbiaceae*, *Apocynaceae*, *Asclepiadaceae* and *Compositae* are particularly well represented. Not every genus within any one family are ordinarily rubber forming, and within a genus, the species may vary greatly in rubber forming capacity, as seen in *Ficus* and *Euphorbia*. However, it is reported that there are certain rules regarding distribution of rubber in the plants. Rubber for instance is a property confined to the dicotyledons branch of the angiosperm. Recently, accounts show that latex-producing plants belong to rather more than 22 dicotyledons families, although a few monocotyledonous families are also recognized, together with one genus of *Pteridophyte*, *Regnellidium*, of the *Marsileaceae* (Labouriau, 1952).Euphorbia as a single genus, include both the tropical and temperate zone representatives. Those in the tropics may include numerous species which form and accumulate substantial amounts of rubber, whereas those in the temperate regions may form, in general, little or no rubber (Bonner and Galston, 1947).

Metcalfe (1967), emphasizes that the quantity of rubber present in latex varies considerably across species and due to the difficulties involved in tapping the latex from many species in quantities that are large enough to be of economic importance, a small number of the latex-bearing plants have ever been exploited commercially.

2.8.2 Medicinal properties

Currently, secondary plant metabolites called phytochemicals have been extensively investigated as a source of medicinal agents (Balandrin *et al.,* 1985). Enough evidences prove that the latex producing plants are used in the management of various diseases namely diabetes, asthma, dysentery, diarrhea, malaria and skin problems (Nadkarni, 1976; Wealth of India, 1948). *Ipomoea carnea* Jacq. and *Euphorbia hirta* L. are known for wound healing activity and flavonoids (Ambiga *et al.,* 2007; Jaiprakash *et al.,*2006). Ethnobotanical values of latex-bearing plants as used by tribal people of Khandesh region has been reported by (Mahajan and Badgujar, 2008a) and they described various traditional medicinal uses of some indigenous plants. Latex of *Calotropis procera* (Ait.) R.Br. was suggested for wormicidal activity (Shivkar and Kumar, 2003) and larvicidal activity (Mahajan and Badgujar, 2008a). Pandey (2001), reported that plant latex has broader ethno-pharmacological relevance as it is used by tribal communities. *Euphorbia hirta* latex has been exploited traditionally as ear drops and in the treatment of boils, sore and wound healing (Igoli *et al.,* 2005). Jatropha latex is claimed to have some ethno-medicinal use like wound healing, coagulant activities of blood.

Nath and Dutta (1992), reports on the presence of Curcain, a proteolytic enzyme isolated from latex of *Jathropha curcas* Linn. Curcain has been reported to play a role in wound healing activity. *Alstonia scholaris* R. Br. is popularly recognized for various activities such as antimicrobial, antiplasmodial, antiamoebic, hepatoprotective, antidiarrhoeal, immunomodulatory, antiasthmatic, anticancer, free radical scavenging, antioxidant, analgesic, anti-inflammatory, antiulcer, antifertility and wound healing activities (Arulrmozhi *et al.,* 2007). Mahajan and Badgujar, (2008b) suggested that result obtained from phytochemical investigations of some

laticiferous plants may lead to development of potent bio-products in the treatment and management of various disease ailments.

However, the potency of these bio-compounds preventing a disease may be the sum of the bioactivities of all phytochemicals present with a common property. Whether these chemicals work in synergy with each other, or are additive or only one or two components account for the functions in question that remains unanswered (Rao, 2003).

2.9 Protein component of latex

According to Pandey (2001), latex constitute compounds. Among these are proteins in concert with several others. In a study conducted by Souza and associates (2010), proteins were extracted from laticifer cells of three plants namely *Cryptostegia grandiflora* , *Plumeria rubra* and *Euphorbia tirucalli* and examined by electrophoresis, mass spectrometry and characterized in respect of proteolytic, chitinolytic and anti-oxidative activities by means of zymography and colorimetric assays. Acidic proteins predominated in laticifers of *Plumeria rubra* but was not found in laticifers of *Cryptostegia grandiflora* and *Euphorbia tirucalli*. The later was poor in respect of proteins. Catalase (E.C. 1.11.1.6) was detected only in laticifer cells of *Cryptostegia grandiflora*. Chitinase (E.C. 3.2.1.14) was the sole activity found in laticifer cells of *Euphorbia tirucalli*, but was also detected in the other latices. This study reports new protein data of laticifers from plants that have been poorly investigated in this respect and contributes to the understanding of biochemical and functional aspects of laticifers in plants. Although only three latices were investigated here, it is clear that the protein content in laticifers of different species differs intensely. Using mass spectrometry and two-dimensional polyacrylamide gel electrophoresis (2-DE) analysis, soluble proteins were found in these fluids when observed. This suggests that very

specific inherent activities take place in different latices. Possibly these differences in enzyme activity involved in secondary metabolite biosynthesis pathways directly account to differences observed in latex from different species. However, latex from *Euphorbia tirucalli* indicated the absence of soluble proteins (Souza *et al.,* 2010). The latex of Manilka zapota is another where proteins are scarce (Selvaraj and Pal, 1984). However, the laticifers of *Cryptostegia grandiflora* and *Plumeria rubra* exhibited an important number of protein spots. Complex protein profiles were also observed in the latices of *Calotropis procera*, *Hevea brasiliensis* and *Papaver somniferum.* In the latex of *Hevea brasiliensis*, approximately 200 distinct protein spots were found in 2-DE and more than 300 in the latex of *P. somniferum* (Decker *et al.,* 2000; Freitas *et al.,* 2007; Posch *et al.,* 1997). Considering these results, laticifer cells can be classified as sources of unknown proteins certainly of biological relevance. Results obtained suggest that there is a low rate of endogenous proteolysis during latex processing (Souza *et al.,* 2010).

The endogenous oxidative process involving secondary metabolites in plants and within these specialized cells is proof that anti-oxidative activities are occur in laticifers. Pathogenesis-related proteins as called by Van Loon *et al.,* (2006), anti-oxidative enzymes such as peroxidases, catalase and superoxide dismutase activities are up or down regulated upon infection.

Svalheim and Robertsen (2006), also reported that anti-oxidative enzymes are synthesized and occur after initiation of an infection. Souza *et al.,* (2010) reported abundant anti-oxidative activity in the laticifers of *Cryptostegia grandiflora* and *Plumeria rubra*, but minimal activity in *Euphorbia tirucalli*. Caruso *et al.,* (2001) also proposed the involvement of anti-oxidative metabolism in defensive mechanisms against fungi. In conclusion, the results of Souza *et al.,* (2010) show that latices exhibit proteins or enzymatic means to protect themselves against

reactive oxygen species in vivo and this network might play an important defensive role against infection.

Endogenous proteolytic activity is the most common and frequent activity investigated of the metabolism of laticifers, with the synthesis of natural rubber being an exception. Proteolytic enzymes are known to play a vital role in a number of relevant events in plant life-cycle. This includes leaf senescence, breakdown of stored proteins during germination of seeds, development and ripening of fruits, regulatory mechanisms and programmed cell death (Chowdhury *et al.,* 2008; Domsalla and Melzing, 2008). Synthesis and accumulation of proteinases in highly specialized cells such as laticifers are intriguing. Endoproteinases are now recognized as pathogenesis-related proteins. The defensive role of proteolytic enzymes in plant defense was revised by Van der Hoorn and Jones (2004). Konno and associates (2004) described the protective activity of papain, a proteinase from Carica papaya latex, against herbivorous by direct experimental evidence. Currently, Souza *et al.,* (2010) are trying to prove that the proteolytic activities of latices play a role in the defensive action against insects and phytopathogens. A conclusion was drawn that the latices of *Cryptostegia grandiflora* and *Plumeriarubra* possess relevant enzymatic activities including pathogenic related proteins. This buttresses the theory that laticifer cells of both plants are directly involved in plant protection. The latex of *Euphorbia tirucalli* according to Souza *et al.,* (2010) is poor in protein activity and does it imply that it is less protected. This hypothesis still requires experimental investigation. The Ninhydrin-Schiff's protein reaction indicated a greater accumulation of proteins in the laticifer cell cytoplasm than in the surrounding cells (Sacchetti *et al.,* 1999).

Chitinases/lysozymes have also been identified in the laticifers of numerous other latex-producing species, including monocotyledons, dicotyledons, and a gymnosperm (Glazer *et al.,*1969;

Howard and Glazer, 1967; Lynn, 1989; Martin and Gaynor, 1988). In many of these species chitinases/lysozymes also appear to represent from 2 to 30% of the total soluble protein in the latex (Martin and Gaynor, 1988).

2.10 Ultrastructure of laticifers in *Hevea Brasiliensis*

In the examination and comparison of the development of laticifers in a range of species of latex bearing plants from five different families, Abd Razak (2000) emphasizes the application of a range of microscopical techniques, and in particular a comparative effects of preparation techniques such as the use of different fixatives (such as immunofix, osmium tetroxide, and osmium tetroxide plus zinc iodide), and embedding media (wax, resins and low temperature embedding) on tissue preservation for both screening purposes. Different staining reagents were also used to compare for these purposes, including the use of histological and biochemistry stains. Optical microscopy and electron microscopy (using both scanning electron microscope and transmission electron microscopes) were used in the observation of the cell biology of developing laticifers. With such technical protocols for identifying laticifers Abd Razak (2000) reported that laticifers in hevea show a large quantity of rubber particles suspended in a matrix and when observed appeared in a dark colour, seemingly soaked by the osmium tetroxide and other heavy metals from the staining. An emphasis was made that all rubber particles were spherical but of different sizes. The process of cell wall disintegration was described to be the starting point where two or more neighbouring laticifers are joined to form a more complex articulated laticifer system. Laticifers were found in almost all plant parts. Results from histochemical studies of laticifers indicated that latex was observed in the root, leaf cotyledon, petiole, leaf and the stem. In addition, they were located in the cambial region of the tissues.

2.11 *Hevea brasiliensis* Latex

According Gomez (1982), the sizes of latex particles in *Hevea brasiliensis*, vary from 0.01 nm to 50nm and over and may vary in diameter in various species. Furthermore, the shape of latex particles may be different, ranging from spherical to pear- or rod-shaped. In *Hevea*, the properties of size and shape of the latex particles is dependent on the age or growth stages of the plant. Mature and older plants tend to have larger and spherical shapes whereas young plants have smaller and spherical shapes (Gomez, 1982). Some investigators reported the colour may vary in different parts of a single plant, and was observed in *Hevea brasiliensis* that the colour may change after the latex has exuded from the plant (Gomez, 1982). According to Homans and Van Dalfsen (1948), centrifuged latex separates into different fractions that is the white and the yellow fractions. Though the original latex coagulates spontaneously after about twelve hours, the white and the yellow fractions may stay fluid for days and coagulate within few hours respectively. This was attributed to the probability that all enzymatic reactions in the yellow fraction are much more noticeable than in the white fraction, allowing for an enhanced splitting of large-molecule complexes and the formation of free fatty acid which, in the presence of some cations effect spontaneous coagulation. Homans and Van Dalfsen (1948) further reports that the viscous matter responsible for viscosity in latex is robust, and the amount and the properties are dependent on several biological including age and strain of the tree, place and frequency of tapping; possibly the season and weather and even the soil conditions.

As reported by Abd Razak (2000), there is evidence of rubber particle coagulation and free microhelices amongst rubber particles from the broken lutoids - an organelle exclusively found in this genus. Lutoids are reported to be very sensitive to osmotic pressure and when under turgor pressure during sectioning, the structure of the lutoids is hard to maintain. Lutoids as in *Hevea*

brasiliensis (*Euphorbiaceae*), are named to be the second most numerous organelle after rubber particles(Dickenson, 1969; Southorn, 1969; Gomez and Yip, 1975). The size of lutoids which is 0.5-3 µm, has been described as similar as rubber particles (Southorn, 1964, 1969). Fresh section from all parts of *Hevea* produced white milky latex. Over time which turned pale to yellowish and will coagulate if left at room temperature just as reported by (Gomez, 1982). Lutoids are directly linked in the coagulation process of latex which enables the ceasing of the flow (Southourn, 1968).

In a paper by Martin (1991), results showed that chitinases/lysozymes makes up about 20% of the soluble protein in the latex of *Hevea brasiliensis*. The chitinase/lysozyme activity in the latex of *Hevea brasiliensis* was further resolved into seven proteins and characterizing the physical and catalytic properties of each. The activity of a marker enzyme for soluble vacuolar proteins - acid phosphatase, was measured in both the cleared cytosolic and pellet fractions. Almost all the activity was localized in the pellet, confirming the presence of intact vacuoles in that fraction. Greater than 98% of both the chitinase and lysozyme activities was also found in the pellet, showing that in latex these activities also are located exclusively in the vacuoles or lutoids. Protein determinations indicated that 70% to 80% of the soluble protein in latex was in the pellet or bottom fraction with chitinases and chitinases/lysozymes representing more than 20% of the total soluble protein (Martin, 1991).

However, the isolation of chitinases/ lysozyme from the latex of *H. brasiliensis* resulted in three groups, namely; “those having chitinase activity but lacking lysozyme activity, those behaving as "typical" plant chitinases with low lysozyme activity and those having both high chitinase activity and very high lysozyme activity”. Hevein is a known chitin-binding protein that has been found in laticifers of the rubber tree (Broekaert *et al.*, 1990). It is said that hevein may be a proteolytic

fragment of a chitinase/lysozyme (Martin, 1991). Archer *et al.,* (1969) reported hevein to be one of the major proteins in the lutoid bodies of rubber tree latex. Hevein has been shown to bind chitin and to inhibit the growth of several chitin-containing fungi (Parijs *et al.,* 1990) and thus suggesting that hevein plays a role in the protection of wound sites from fungal attack. In the latex of *Hevea brasiliensis*, approximately 200 distinct protein spots were found in using 2 dimensional electrophoresis (Posch *et al.,* 1997). The fraction of latex expelled from the severed laticiferous mantles during tapping is estimated to contain about 30-50% (wt/wt) of cis-1, 4-polyisoprene (Kekwick, 1989). The specialized activity of laticifers in the biosynthesis of rubber (isoprenoid) makes it rational to expect the major proteins in latex to be enzymes involved in the biosynthesis of rubber (Martin, 1991).

2.12 Allergenicity

A total of 57 Hevea latex polypeptides out of the approximately 240 different Hevea latex polypeptides identified are reportedly allergenic (Slater and Chhabra, 1992; Czuppon *et al.,* 1993; Lu *et al.,* 1995). Presently, the use of cornstarch donning powder in natural latex products is the only mode of treatment of allergic reactions as it prevents direct contact and exposure to airborne latex allergens. However, according to Tomazic *et al.,* (1995) there is a different group of soluble Hevea latex proteins, which readily attach to cornstarch and can be readily aerosolized, and is able to sensitize individuals who are occupationally exposed. It has become almost impossible to replace Hevea rubber-containing medical products such as catheters for individuals sensitized to Hevea latex, such as children with spina bifida who require frequent surgical procedures, because synthetic materials have not always possessed the physical properties required. Currently, no perfect synthetic substitute for some Hevea latex-containing medical devices are available.

Hence, the search for a rubber source that provides a non-allergenic alternative to Hevea latex is necessary for devices that must be highly elastic (Siler *et al*., 1996). Siler *et al.,* (1996) reported that the IgE antibodies in the sera of children and occupationally exposed adults with Hevea allergy, and those with spina bifida may react to different proteins at different degrees, depending on their mode of initial sensitization. Individuals who undergo multiple operations and a minority of adults who have dermatitis tend to develop IgE antibodies to these proteins.

2.13 *Vitellaria paradoxa* latex

Despite the progressive nature of research on shea latex little is still known about it. Reports from researches so far on shea latex show that it has low protein content compared to rubber latex (Fosu and Quainoo, 2013) when proximate analysis results were carried out on samples of both Hevea latex and shea latex; Quainoo *et al.,* (2015) also reported that except for crude protein and moisture content, agro-ecological zones and land use did not have any influence on the quality of the proximate composition of the shea latex; Quainoo and Dugbatey (2016) also had results indicating that shea latex flow decreases with increasing age of the tree. The proximate composition of the shea latex with respect to the various ages analyzed among shea trees also revealed that crude fat and moisture were slightly higher in the younger trees than the mature trees. However, crude protein, crude fibre, ash and carbohydrate were found to be significantly higher in the mature trees.

Proximate composition for crude fat, moisture, ash and carbohydrate exhibited insignificant difference across different months of tapping. However, crude protein and crude fiber level were significantly variable within certain months (July and August). Furthermore, crude protein levels of shea latex with respect to time of tapping and location were low and significantly different.

Mineral ions such as K, Zn, Na and phytochemicals such as alkaloids, terpenoids, tannins and some reducing sugars are reported to be present in shea latex (Abdul-Aziz *et al.,* 2016).

2.14 Other economically important latex-bearing species

In a study conducted by Siler *et al.,* (1996) to demonstrate that serum IgE antibodies from individuals allergic to Hevea latex and IgG antibodies from Hevea latex-hyperimmunized mice do not recognize proteins from guayule latex, *Partheniurn argentatum* also known as guayule, which is a desert shrub native to the Chihuahuan desert of north-central Mexico and southwest Texas belonging to the sunflower family of plants (*Asteraceae*), a family with over 20,000 species, which also includes the ragweeds of the genus *Ambrosia* (West *et al.,* 1991). Guayule plants produce latex which contains natural rubber, which is known to be comparable in quality to that of *Hevea* (Hammond and Polhamus, 1965). However, unlike *Hevea*, which produces rubber particles in latex vessels that can be readily tapped, the latex of guayule is accumulated in parenchyma cells in the bark. A latex-like rubber particle suspension can be made from guayule bark, which is suitable for the production of rubber products including gloves, catheters, and other medical and consumer supplies (Cornish, 1998; Jones, 1948). According to West *et al.,* (1991) guayule proteins have the potential to induce IgE immune responses, hence should be considered potentially allergenic. On the contrary, Siler *et al.,* (1996) reported the absence of reactive protein indicating that guayule does not contain allergenic proteins that are structurally similar to those in Hevea-latex gloves and other latex products. According to Bonner and Galston (1947) the yield of rubber from Guayule is estimated at 0.027kgm^{-2} and 0.101 kgm^{-2}after one year and two years respectively. Supposedly, yields are estimated to of up to 0.168 kgm^{-2} and has been estimated that after 10 years the plants might yield 0.269 – 0.303kgm^{-2}.

According to Fisher (1980), *Carica papaya* commonly known as pawpaw, contain laticifers occuring throughout the mesocarp tissue, often paralleling vascular bundles. These produce latex which is white and is collected commercially and contains a proteolytic enzyme, called papain. The latex becomes granular and is not white or freely flowing as in the green, immature fruit as the fruit matures. Notably, latex is absent in ripe fruit (Griebel, 1928). The latex producing member of the family *Papaveraceae* are notable for their alkaloid content.

The Castilloa [*Castilla* (Castilloa) *elastica* Cerv.] of South America was exploited for rubber long before *Hevea brasiliensis* had ever been discovered or identified botanically (Metcalfe, 2018). The Assam Rubber (*Ficus elastica* Roxb.) is also a best rubber source in the family *Moraceae*. The rubber in both species is difficult to extract and is said to be variable in quality. Again, the latex of *Ficus elastica* is rich in magnesium salts and crystallizes upon exposure to the air.

Allium, the genus to which onions and their allies belong, have a taste which is attributed to the contents of the laticifers, which is in the nature of a resinous substance and said to be easily hydrolyzed to allyl sulphide (Hoffman, 1933;Mann and Stearn, 1960;Esau, 1965).

Funtumia elastica Stapf, a member of the family *Apocynaceae*, has stirred up special interest as a source of rubber. This tree can be cultivated to serve as shelter belts and also for weed suppression and reducing light intensity. Crushed bark from some rhizomes in this family are said contain 16% rubber of reasonably good quality. Among them is Carpodinus, a source of root rubber and Gutta Percha is obtained from *Dyera costulata* Hook. f. Vines of *Landolphia heudelotii*, although have small yields like 200 g/year from bark incisions. *L. thollonii* Dewevre. Evidently, rubber has been extracted from vines such as *Landolphia* spp (Mahlberg, 1993).

Cook (1943) reported the extraction of rubber from *Asclepias subulata* Decaisne, a Milkweed with reduced leaves, which can be grown as grass and is found in California and Arizona. *Cryptostegia grandiflora* R. Br., indigenous to Madagascar, is of significant interest in the family Asclepiadacea, from which about 79.4 kg of rubber per acre can be obtained (Snyder, 1955). The rubber is extracted from the cut ends of leafless stem is known as 'whips'. Bonner and Galston, (1947) also reported that rubber has also been extracted from the same species by retting the leaves with *Clostridium roseum*. Latex producing members of the genus *Caricaceae*, have contents that stain yellow with iodine and are of interest because it contains proteolytic enzymes (Metcalfe and Chalk, 1950).

The Ceara rubber plant (*Manihot glaziovii*), a member of the *Euphorbaceae* family, to which belongs also *Hevea brasiliensis* produces rubber. The latex from *Euphorbia* spp. has a high resin content, and that of *Excoecaria agallocha* L. is poisonous, causing skin blisters and irritation to the eyes.

The *Sapotaceae* family include species like the *Achras zapota* L. (Sapodilla) from the East Indies and Florida, an original source of chicle for chewing gum, and the *Manikara bidentata* (Balata tree) from French Guiana, which yields a substance related to rubber. Substances such as gutta-percha and chicle are produced in articulated laticifers throughout the family (Metcalfe and Chalk, 1950; Sassen, 1965). Chewing gums are highly enjoyed as confectionery preparation. The Greeks sweetened their breath and cleaning their teeth by using mastiche, a resin from the bark of mastic tree. Mayan Indians of Yucatan chewed chicle from the sapodilla tree. During the 1860's, Thomas Adams, realized the potential market for chewing gum products. He wrapped pieces of pure, flavorless chicle in colored tissue paper, packaged them in boxes, and public response to the product was very favorable (Shah and Mehta, 2014). It is reported by several researchers that gum

base which forms about 15-45 % of the whole chewing gum may be obtained from different latex sources including *Manilkara zapotilla* of family *Sapotaceace*, *Manilkara huberi* of family *Sapotaceace*, *Palaquium leiocarpum* of family *Sapotaceace*, Micropholis spp. of family *Sapotaceace*, *Dyera costulata* of family *Apocynaceae*, *Couma utils* of family *Apocynaceae*, *Couma macrocarpa*of family *Apocynaceae* and *Ficus platyphylla*of family *Moraceae*. These are made to constitute Vitamins, oral contraceptive, nicotine, minerals, analgesic, antacids, muscle relaxants, antihistamic, decongestants, anaesthetics, antitussive, antibiotics, etc. and used for Medicated chewing gums (MGCs) (Shah and Mehta, 2014).

2.15 Factors affecting latex flow and quality

Previous studies have reported two major factors that affect the yield of latex at each tapping; namely, internal factors such as clone and tree age and external factors such as tapping systems, stimulation, the depth, slope and length of tapping cut (Njukeng *et al*., 2011; Traore *et al*., 2011; Gunasekera *et al*., 2013). Also, Gunasekera *et al*., (2002) reports that initial flow rate, plugging index, and dry rubber content which form part of latex physiological processes involved in latex flow are closely linked to yield. Buttery and Boatman (1964, 1966)showed that the flow rate of latex slows down rapidly as a result of loss in turgor until the flow ceases eventually by the mechanism of latex vessel plugging (Milford *et al*., 1969). Additionally, the diameter and density of latex vessel can have a link with yield obtained (Mesquita *et al*., 2006). Jacob *et al*., (1989); Gunasekera *et al*., (2013); Pethin *et al*., (2015) report certainbiochemical parameters of latex especially sucrose to also relate to the yield, for the reason that latex biosynthesis occur within the latex vessels using sucrose (Tupy, 1973, 1985; Dusotoit-Coucaud *et al*., 2009). Latex exudation after tapping allows for rapidinitial latex-flow. The length oflatex flow is generally extended by

ethylene stimulation (Yeang, 2005) due to physiological changes observed in the drainage area of the cut after tapping (Gunasekera *et al*., 2013). The measurement of the latex vessel plugging rate is frequently used as the plugging index (Gunasekera *et al*., 2013; Yeang, 2005). Plugging index is related to many other clonal characters. It has a negative correlation with yield and incidence of dryness and a positive correlation with girth, dry rubber content of latex and magnitude of the response to yield stimulation (Waidyanatha and Pathiratne, 1971).

Tapping signifies a serious abiotic wounding stress for exploited trees - under regular tapping. It necessitates latex cells to fully regenerate their cytoplasm after latex expulsion (Dusotoit-Coucaud *et al*., 2010). Factors such as environmental and harvesting stresses, as well as the metabolic activity necessary for latex regeneration between two tappings, lead to the production of reactive oxygen species (ROS). Excessive production and accumulation of ROS may lead to laticifer dysfunctions such as Tapping Panel Dryness (TPD). The flow of latex stops as a result of Tapping Panel Dryness. Wounding and subsequent plant hormone stimulation could cause overproduction of reactive oxygen species (ROS) which oxidises proteins, lipids, carbohydrates and DNA (Gill and Tuteja, 2010).

Some specific organelles and subcellular components are the main sources of ROS, and these may include Frey-Wyssling particles, Lutoid membrane, Lutoids, and other cytosol inclusions (Zhang *et al*., 2017). Frey-Wyssling particles are globular double-membraned specialized chromoplasts, which are of 0.5–2 µm in diameter and contain lipids and carotenoids. These plastids have o-diphenol oxidase (ODP), a specific polyphenol oxidase (PPO), which are a source of ROS (Coupé *et al*., 1972). Single-membrane bound lutoids are lysosomal micro-vacuoles, 1–3 µmin diameter, and generally amount to 10–20% of the volume of fresh latex. These have been considered as the major source of ROS in latex cells (D'Auzac *et al*., 1989).

Oxidation-reduction (redox) reactions are known to have effects on the biology of latex production. This involves the transfer of electrons between two compounds. According to Mittler (2002); You and Chan (2015), redox reactions are common and vital to some of the basic biological functions such as stress response, development, photosynthesis and respiration. In maintaining a cell or compartment environment in favour of biological processes, redox homoeostasis is necessary. High levels of ROS are generated as a result of disturbances triggered in the basal redox state. Abiotic stress, biotic stress andcertain plant development processes, are known to be factors that trigger these disturbances in the basal redox state. A study by Zhang *et al*., (2017) revealed that redox reactions and tolerance of biotic and abiotic stress are key functions for latex and natural rubber production and quality. Several Peroxides and free radicals have adverse effects on all components of the cell, including proteins, lipids and nucleic acids. According to Baxter *et al*., (2014); Foyer and Noctor (2005) the ROS are also involved in plant development and are thus described as secondary messengers.

However, research has been focused on ROS-scavenging systems in laticifers, and also the supply of antioxidants to mitigate such effects. The ROS-scavenging systems play an essential role in maintaining redox homoeostasis. Redox homoeostasis is controlled by the biosynthesis and reduction of antioxidants and by ROS-scavenging enzymes. Latex contains three main antioxidants, that is thiol, ascorbate and tocotrienol. Phytosterols, phospholipids, phenols, betaines, proteins and amino acids are other molecules which possess antioxidant ability (Zhang *et al*., 2017). According to McMullen (1960), the main thiols in latex are Glutathione and cysteine. The entire thiol content is one of the many parametersused for latex diagnosis. A positive correlation exists between the total thiol contentand latex production and is used to

monitor the physiological status of trees under production (Eschbach *et al*., 1984; Prevot *et al*., 1984b; Sreelatha *et al*., 2009).

The quality of natural rubber maybe influenced by natural antioxidants in fresh harvested latex, and during rubber maturation and processing. During storage, hardening of raw rubber occurs due to Oxidative degradation (Morris, 1991). Natural antioxidants might hinder such oxidation, nonetheless they are not adequate in latex to protect the polymer. Vitamin E, phytosterols, phospholipids, phenols, betaines, proteins and some amino acids from the latex can act as antioxidants to prevent oxidation in raw rubber (Altman, 1948; Dunphy *et al*., 1965; Tirimanne *et al*., 1971; Musigamart *et al*., 2014). Among the latex antioxidants in raw rubber, vitamin E has been proposed as the chief natural antioxidant. Vitamin E can persist in raw rubber during processing (Liengprayoon *et al*., 2013) and has the ability to maintain antioxidant effectiveness in vitro(Kamal-Eldin and Appelqvist, 1996) due to its fat-solubility property.

Some enzymes known as antioxidant defense enzymes, are vital for breaking down the harmful end-products of oxidative modification. Connected with an increase in respiration, tapped trees also improved the enzymatic ROS-scavenging system in soft bark tissues(Annamalainathan *et al*., 2001).

Tapping Panel Dryness (TPD) is known to extremely affect the latex production of a rubber treeplantation. Tapping Panel Dryness refers to two conditions (Putranto *et al*., 2015). The first condition is linked to overproduction of Reactive Oxygen Species and subsequent cellular damage. However, this condition can be reversible after resting trees without tapping (Das *et al*., 2002). The second form, involves histological changes and senescence mechanisms and it is called brown bast (de Faÿ and Jacob, 1989). Tapping Panel Dryness susceptibility is influenced by both genetic and environmental factors. Overexploitation of rubber trees including a high

tapping frequency and ethephon stimulation can cause early TPD occurrence (Putranto *et al*., 2015).

Cretin and Bangratz (1983) first reported that the generation of reactive oxygen species and the subsequent peroxidation of the cellular membrane system were involved in latex flow stoppage. According to Chrestin *et al*., (1984), high NAD(P)H oxidase activity at the surface of lutoids was considered as the main source of Reactive Oxygen Species leading to peroxidative degradation of the unsaturated lipids of the lutoid membranes and subsequently, the release of factors involved in latex coagulation. A protein, Hevein was shown to be involved in the clumping of rubber particles as reported by Gidrol *et al*., (1994). Another Hevea latex lectin-like protein present on the lutoid membrane was reported to induce aggregation of rubber particles and lutoid membranes (Wititsuwannakul *et al*., 2008). Typical Tapping Panel Dryness symptoms show an abnormally high NAD(P)H oxidase and peroxidase activities, but also a very low activity in Reactive Oxygen Species - scavenging enzymes such as superoxide dismutase (SOD) and catalase (CAT)(Chrestin, 1989). A confirmation was made on the bark of trees over stimulated with a high concentration of ethephon, which can generate higher concentrations of free radicals and show lower SOD activity than in unstimulated tree (Das *et al*., 1998). According to Wang *et al*., (2015), the SOD and glutathione S-transferase (GST) protein contents decreased in latex after trees were stimulated with ethephon. This shows that a high concentration of ethephon is an ROS-related toxin for latex tissue. The expression of Reactive Oxygen Species - scavenging enzymes such as catalase can be stimulated by moderate ethylene treatment in a healthy tree but not in trees already affected by TPD (Kongsawadworakul *et al*., 1997). Generally, antioxidants and ROS-scavenging enzymes are linked to the preservation of latex and rubber production capacity (Das *et al*., 2002; Lacote *et al*., 1998).

A wide range of environmental factors including oxygen shortage is known to induce oxidative stress. In TPD-affected trees, the consumption of oxygen by NADH-cytochrome-c-oxidoreductase was particularly high and hypoxia condition was observed (Chrestin, 1989).

2.16 Ethylene Stimulation

Latex biosynthesis takes place in the cytoplasm of laticifers, which are specialized latex cells and needs sucrose as the unique precursor. Ethylene stimulation during latex production results in high sugar flow from the surrounding cells of inner bark towards the latex cells (Dusotoit-Coucaud *et al*., 2010). Although D'Auzac (1964) and Chow *et al*., (2007), presented different pathways, whichever the pathway, sucrose is the unique precursor of natural rubber and its passage into latex cells may be crucial to latex production. Latex cells are heterotrophic and need to be provided with photosynthetic products to meet their high carbon and energy requirements (Tupy, 1973; Eschbach *et al*., 1986; d'Auzac *et al*., 1989; Silpi *et al*., 2007). Results from cytological studies showed that laticifers lack plasmodesmata (de Faÿ *et al*., 1989; Hebant, 1981), and data from electrophysiological investigations (Bouteau *et al*., 1991; Bouteau *et al*., 1992; Bouteau *et al*., 1999) have shown the evidence of active sugar/H+ symporters on the latex cell plasma membrane.These supposed transporters were expressed in source and sink organs, including latex cells. Sucrose is known to be the key sugar transported into sink latex cells in Hevea trees; it is involved in latex generation, in addition to osmoregulation (Jacob *et al*., 1989). With regards to sucrose, electrophysiological data showed that exogenous glucose induced plasma membrane depolarization of isolated latex cells or protoplasts, signifying the presence of a putative glucose/H^+ symporter in these cells (Bouteau *et al*., 1992, 1999). In a case of this sort, glucose would directly enter glycolysis to produce pyruvate, then acetyl-CoA, the precursor of

cis-polyisoprene biosynthesis. Sucrose and hexose transporters expressed in most sink organs, indicates that sucrose released into the extracellular space can be taken up directly through sucrose transporters and/or through hexose transporters after being divided by a cell wall enzyme called invertase (Braun and Slewinski, 2009). There is a preference by these cells for the importation of sucrose rather than hexoses owing the high diversity of sucrose transporters in the latex cells, coupled with the central role of sucrose in rubber synthesis. Latex cells may control their sugar transport in relation to their carbon demand (Dusotoit-Coucaud *et al*., 2010).

A significant way to enhance rubber production is to provide the trees with exogenous ethylene using the ethylene generator, ethephon (D'Auzac and Ribailler, 1969). Treatment with ethylene generator both increases the volume of exported latex and kindles latex regeneration between tappings (Coupé and Chrestin, 1989). Quantitatively, latex production is said to increase by this method by 1.5- to 2-fold. The mechanisms of ethylene action are not fully understood. Physiological and biochemical evidence indicate that ethylene acts on membrane permeability, leading to extended latex flow, and also on general reformative metabolism (Coupé and Chrestin, 1989). Additionally, severa1 enzymic activities have been shown to be specifically tempered by ethylene in the rubber tree (Coupé and Chrestin, 1989) and other plants (Lieberman, 1979). Studies have shown that ethylene increases latex production by different mechanisms, including enhancing latex sucrose concentration (Low and Gomez, 1982; Tupy and Primot, 1976), enhancing the sink strength of latex cells, through stimulation of latex cytosolic invertase activity (Tupy and Primot, 1982), and both sucrose influx and H^+/ ATPase activity (D'Auzac *et al*., 1982; Lacrotte *et al*., 1985). The effect of the enzyme, polyphenol oxidase (PPO) has been reported by Li *et al*., (2014), that this enzyme plays a role in defense against pathogen, responsible for latex browning and is associated with latex coagulation and wound healing. From this study, the PPO

decreased in ethephon-treated latex serum showing that it was down-regulated by ethylene. A conclusion was drawn that decline in accumulation PPO in ethephon-treated sample may have hampered rubber particle aggregation which subsequently prolonged the latex flow.

According to Dusotoit-Coucaud *et al*., (2010), the hevea model used indicated that sucrose demand of latex cells could be much higher in exploited trees than in unexploited ones. Certainly, the frequently tapped rubber tree produces more latex than the virgin ones; that is 450 and 220 g tap^{-1} $tree^{-1}$ respectively and these exploited trees are exposed to repeated severe wounding, combined with constitutive high metabolic activity required for latex regeneration between successive tappings (from every 2 to 5 days, dependent on the plantations).

A key enzyme glutamine synthetase (CS; EC 6.3.1.2) involved in nitrogen metabolism has been studied relating to ethylene stimulation and there is found a specific and significant activation of the cytosolic glutamine synthetase (CS) in the laticiferous cells after ethylene treatment paralleling the increase of latex yield. Following ethylene treatment,the CS response to ethylene might be mediated by ammonia that increases in latex cytosol. Quantitatively, a moderately producing tree during one tapping may export 100 mL of latex, which is completely regenerated within 3 days. This parallels to the net synthesis of about 50 g of dry rubber and 1.2 g of protein (Pujade-Renaud *et al*., 1994). Accordingly, a very intense metabolic activity is required, in particular energy-generating catabolic pathways like glycolysis (Jacob, 1970), and also anabolic processes permitting reformation of the intracellular components. In this setting, nitrogen metabolism involved in protein and nucleic acid synthesis takes a prominent part.

Glutamine synthetase (EC 6.3.1.2) allows NH_4^+ integration into organic compounds. This enzyme, specifically, supplies the cells with the amino acids needed for protein synthesis through the pathway involving glutamate synthase. Results have shown that ethylene induces a substantial

increase of the Glutamine synthetase (GS) activity in the latex of rubber trees in the various genotypes tested. The progression of this stimulation is well linked with the kinetics of latex yield, along with with other physiological parameters (pH, mineral ions, saccharose content, etc.) realized in rubber trees after ethylene treatment under similar conditions (Primot *et al*., 1979). According to Pujade-Renaud *et al*., (1994), the fact that ethylene treatment had no effect on the concentration of total cytosolic protein of the latex although increasing the volume of exported latex indicates that ethylene triggers a general activation of protein synthesis or protein turnover in the latex of the treated tree. It can be resolved that the protein content of the latex is efficiently controlled, with an equilibrium between full regeneration of the proteins and loss through each tapping, even under conditions of ethylene-increased latex removal.

Ethylene treatment induced NH_4^+ accumulation in the cytosol showing a measurement of 18% increase within 24 hours after ethylene treatment and reached 40% on day 10. Notably, this stimulation was specific for ethylene generator and independent of bark scraping or palm oil application (Pujade-Renaud *et al*., 1994). The NH_4^+ level could possibly be an intermediate regulator of GS expression in latex, as it is in some other plants (Kosaki *et al*., 1991; Miao *et al*., 1991; Santos and Salema, 1992). This hypothesis is buttressed by the fact that ethylene treatment induces NH_4^+buildup in the cytosol of the laticifers. According to Miao *et al*., (1991) the process by which ethylene triggers this buildup is not known; it might be through alterations of the membrane features, which would activate ammonia uptake, or through the stimulation of hydrolases able to release NH_4^+ from nitrogenous substrates. Under ethylene stimulation in latex production, GS activation might be involved in avoiding toxicity for the cell by limiting NH_4^+buildup. Contextually, direct regulation of the GS gene by NH_4^+ would seem appropriate. However, extensive studies conducted to understand the mechanism of ethephon and its

consequences to the plant system by Khan *et al*., (2008) showed that ethephon influenced growth, photosynthesis and nitrogen accumulation of *Brassica juncea* depending on the amount of nitrogen in the soil. The nitrogen requirements for protein synthesis is important in ethylene-stimulated trees for which one tapping expels twice as much latex. In that case, 28 mmol of nitrogen have to be reincorporated into proteins. The less than 2-fold activation of GS by ethylene measured by Pujade-Renaud *et al*., (1994) would thus provide the increased capacity for nitrogen incorporation essential to recompense for the ethylene-increased protein removal in the same amount of time.

According to Abd-Rahman and Kamarrudin (2018), a study on the effect of ethylene generator - ethephon on latex serum proteome and its significance to latex flow was conducted using quantitative proteomic analysis namely Isobaric Tags for Relative and Absolute Quantification (iTRAQ). The results showed that ethephon greatly induced numerous latex serum proteins that are involved in many biological processes and molecular functions. Most of the identified proteins were found to be in the organelles, membrane and extracellular region. Under the molecular function class, most of the proteins took part in binding activity, followed by catalytic activity as well as antioxidant activity. Also, the analysisrevealed that carbohydrate metabolisms were major under the metabolic pathways affected by ethephon treatment. As reported by Archer *et al*., (1969), Hevea latex is made up of a variety of molecules such as rubber particles, proteins, carbohydrates and other cell contents. Application of ethephon on rubber tree connects several metabolic responses and biochemical processes including compartmental pH and nitrogen assimilation, carbohydrate transport and metabolism, sucrose and glucose loading and protein synthesis (Coupé and Chrestin, 1989; Wang *et al*., 2015).

2.17 Mineral Ions

According to Gan and Ting (1993), working with fresh latex is problematic as its properties are subject to clonal and seasonal variations. Clonal variation can be overcome by selecting a fixed number of trees of the same clone to obtain the necessary quantity of latex per tapping. Seasonal variation is however quite beyond control when fresh latex is collected in different periods. Fresh latex contains metal ions (Archer *et al*., 1963) and during the coagulation process, some of them might be retained in the rubber molecules if present. Burfield (1986) reports that, removal of these ions by dialysis is able to reduce hardening of rubber. This implies that the presence if these ions will promote hardening. Divalent cations essential for ionic crosslinks which contribute to hardening can come from latex which contains Mg^{2+} and Ca^{2+} (Archer *et al*., 1963). Latex also contains K^{+} and Na^{+} which are unable to form ionic crosslinks. Thus, monovalent ions such as Li^{+}, Na^{+}, K^{+} do not affect the incorporation of nitrogenous groups into the rubber chain and consequently do not inhibit crosslinking. Transitional metal ions such as $Co^{2+,}$ Cu^{2+}, Mn^{2+}, Fe^{2+}, Ni^{2+} and Ag^{+} promote oxidative degradation to such an extent that the chain scission become the predominant reaction during the storage.

Mukhopadhyay and Sharma, (1991) describe manganese as an essential trace element for higher plant systems and are absorbed mainly as divalent Mn^{2+}, which competes effectively with Mg^{2+} and strongly reduces its rate of uptake. The buildup of Mn particularly takes place in peripheral cells of the leaf petiole and spongy and palisade parenchyma cells. Mn is involved in photosynthesis and activation of different enzyme systems. Mn deficiency may be expressed as inhibition of cell elongation and yield decrease. However, Mn toxicity is one of the important growth limiting factors in acid soils. Toxic effects of Mn on plant growth have been credited to several physiological and biochemical pathways. Mn inhibits the uptake, transport and use of

several essential elements including Ca, Fe, Cu, Al, Si, Mg, K, P and N. Excess of Mn decrease the absorption of certain elements and increases that of others. pH plays a vital role in Mn uptake. Acidic pH causes a loss of considerable amount of nitrate as a substitute electron acceptor and leads to a high amount of Mn in leaves. The small quantitative requirement of trace elements such as Cu, Mn, Fe, Zn for plant life need not diminish from an assessment of their importance in the physiology of higher plants. Their importance cannot be underestimated. In that sense the fraction of a milligram of, for example, copper is just as indispensable as several hundred milligrams of a "major" element like potassium (Arnon and Stout, 1939).

Proteins and amino acids are the main sources of the nitrogen found in the dry rubber. According to (Coupé, 1978), there is an association between the increase in protein biosynthesis and increase in the rubber productivity. However, Larcher (1995) reports that before the shedding of the leaves, there is the confiscation of some mineral nutrients, including N, which is easily translocated. As the process of foliation, flowering and fructification begins, the N absorbed by the leaves is continuously used and the values of the N% decrease, reaching the least with the flowering process and maximum leaf area. The elements N, P, K, Na, Cl, and S are transportable, while Zn, Cu, Mn, Fe, and Mg are partially movable (Malavolta *et al*., 1989). When the absorbed nutrients are used again, ash % tends to decrease, resulting fromincreasing precipitations(Moreno *et al*., 2005).

According to George *et al*., (2014), results obtained for the non-metallic constituents of *Hevea brasiliensis* latex recorded low values of non-metallic constituents ranging from 0.1 – 2.0 mg/l, which is in agreement with those of a couple of authors contain (Devan *et al*., 2000; Tanaka and Sakdapipanich, 2001). It is explained that the low non-metallic constituent in the latex may be

attributed to the inability of the latex to absorb higher doses of the constituents due to its sticky and gummy nature though it exists in liquid form.

2.18 Environmental Stresses

Metabolite biosynthesis are affected by environmental stresses such that bio-synthates may either increase or decrease. A multitude of environmental factors work in synergy in the regulation of metabolite biosynthesis in plants. The need for this control of synthesis is to ensure that plants are able to regulate the production of metabolites according to altering factors so they can survive. Light is clearly a key aspect in the production of many compounds. It supplies the energy needed to fix carbon. Light intensity is key in the biological synthesis of medicinally significant metabolites. An outstanding example is the tree of joy (*Camptotheca accuminata*), where there is significant increase in the quantity of the anti-prostate cancer drug, camptothecin (an alkaloid metabolite), as the amount of light reaching the tops of the plants reduces.

Temperature isa significant factor that controls plant metabolism. At decreased temperatures around 0°C, most enzymes are sedentary but as the temperature rises, the rate of enzymatic activity increases up to about 40°C, beyond which most plant enzymes become inactivated and could even be damaged permanently. Many enzymes are all the time present in plant cells at a certain level, but specific temperatures can activate a dramatic change in these levels.

Carbon dioxide gas is the essential carbon source for all plant metabolites. Its levels can differ depending on the environment, and consequently causes changes in biosynthetic output. For example, higher carbon dioxide levels in the Earth's atmosphere, together with high temperatures owing to elevated levels of "greenhouse gases", are currently leading to increases in total photosynthate produced in temperate zone plants. This is particularly true for photosynthetic

plants which are C-4, and are adapted to higher temperature conditions and have no or little loss of carbon through photorespiration (Kaufman *et al*., 1999).

Moreno *et al.* (2005), reported that there was an affinity of increasing ash % prompted by variations of climate, clonal and phenological (senescence). In that duration, low precipitation resulted in smaller quantity of water accessible in the soil, which may have steered to minor dilution of the latex which, in relationship with senescence, caused the percentage of ash increase.

2.19 Bio-adhesives

Professor Joseph R. Robinson in the early 1980's, at the University of Wisconsin initiated the theory of bio-adhesion as a novel approach to extend the residence time of various drugs on the optical surface (Bernkop, 2005). Today, there are several reports by research groups on quite a lot of gastrointestinal mucoadhesive dosage forms, for example microspheres, discs, and tablets that have been prepared (Ahuja *et al*., 1997).

According to Castellanos *et al*., (1993), adhesion is the bond produced by contact between a pressure-sensitive adhesive and a surface. The American Society of Testing and Materials has defined it as the state in which two surfaces are bonded together by interfacial forces which may comprise of valence forces, interlocking action, or both (American Society of Testing and Materials, 1984). A bio-adhesive is described as a material that has the ability to interact with biological materials and being bonded on them or holding them together for prolonged periods of time. According to Castellanos *et al*., (1993), within a biological system, four forms of bio-adhesion can be identified. These are the bond of a normal cell on another normal cell,bond of a cell with a foreign substance, bond of a normal cell to a pathological cell and bond of an adhesive

to a biological substrate. Ahuja *et al*., (1997) also classified bio-adhesives are into three centered on phenomenological observation, instead of on the mechanisms of bio-adhesion.

Type I referred to adhesion occurring between biological objects without involvement of artificial materials for example Cell fusion and cell aggregation. Type II represented cell adhesion onto culture dishes or union to a variety of substances such as metals, woods, and other synthetic materials. Type III referred to sticking together of artificial substances to biological substrates such as bond of polymers to skin or other soft tissues.

Describing the essential factors affecting muco-adhesion, Zate *et al*., (2010) reported based on categories based on polymer associated factors such as molecular weight such that for low molecular weight polymers permeation is more than polymers with high molecular weight because entanglements are ideal in high molecular weight polymers. Also, based on environmental – related factors including pH which affects the charge on the surface of both polymers and mucus.

A report by Roy and Prabhakar (2010) identified certain polymers as showing the best adhesion properties. These include guar gum, karya gum, sodiumcarboxymethylcellulose, hydroxyethylcellulose, methylcellulose, sodium alginate, polyethylene glycol (PEG), tragacanth and retene. Among these, guar gum, karya gum and tragacanth are plant based. From an in-vitro drug release kinetic study by Kumar and Verma (2010), a conclusion was drawn that the use of natural polymer for the preparation of bio-adhesive preparations will surely be helpful in future since the topical gel prepared from the natural polymer (*Aegel marmelos* - plant Bale) released the drug from gel by following zero-order release kinetic model. Meaning natural polymer performs a significant role to controlling the discharge of drug from topical gel.

To be suitable to be used in a drug delivery system, an ideal muco-adhesive polymer should have the following characteristics. The polymer and its degradation products should be nontoxic and should not be absorbable from the gastrointestinal tract. It should not be irritating to the mucous membrane. Also, it should rather form a strong non-covalent bond with the mucin-epithelial cell surfaces. It should stick quickly to soft tissue and should own some site specificity. The polymer should allow some easy integration of the drug and offer no interference to its release. The polymer must not putrefy on storage or during shelf life of the dosage form. The polymer should not be expensive, so that the prepared dosage form remains competitive. And lastly, the polymer should not be an obstruction in drug analysis (Duchene *et al*, 1988; Khar *et al*., 1997).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The sampling was carried out in two different experimental sites in Ghana from October to December. The first, Cheyohi, Kumbungu District in the Northern region located on latitude 9^0

25’45” N and longitude 0^0 58’42” W. This experimental area found within the Guinea Savannah Agro-ecological zone with two separate seasons per year: a rainy season usually lasting from the month of June to November and a dry season for the rest of the year. Rainfall and temperature are extremely subject to change. Particularly, rainfall distribution is normally irregular, sporadic, and torrential. Annually, the average rainfall falls within the range between 900 mm and 1000 mm. The rainy season generally commences from April and may reach a maximum in August/September. Rainfall declines by the end of October, then after, the long dry season, which sets in by late March/April, and in recent times some communities received their first rains only in June (Kusakari *et al*., 2014). The highest temperatures are normally recorded in March and can rise as high as 45 °C during the day. The second is Abura, located on latitude $4^0$54’0’’ N and longitude $0^0$3’0’’ W Western Region. The relief of the Western region falls in the physiographic type classified by (Dickson and Benneh, 2001) as the forest dissected plateau. Much of the region is a plain between about 240 and 300 metres above sea level with isolated hills. In the North-West (covering about five districts) the topography is rugged and hilly. The region is the wettest part of Ghana. The rainfall map of the region, indicates plainly that rainfall decreases northwards and eastwards from the extreme south-west which is the wettest part of the region and the country. Rainfall distribution in the region is typified by two seasons; with the main one reaching its maximum in May/June and the minor one in October. Aside these, Boateng (1960) has reported that there is virtually no month deprived of rain. The region falls within two main climatic types: the south-western equatorial and the wet semi-equatorial (Dickson and Benneh, 2001). The south-western equatorial climatic type and the evergreen forest roughly coincides and the wet semi-equatorial climatic type with the semi-deciduous forest shown on the vegetation map of Ghana.

The south-western equatorial climate is described as the wettest in the country with rainfall patterns as described above. The maximum temperatures which mostly occur in March/April are approximately 30 ^{0}C whereas the lowest of temperatures are around 26^{0}C and occur in the month of August. Relative humidity ranges between 70-80 % annually. On the average, the wet semi-equatorial climate has a yearly rainfall distribution between 1250 and 2000 mm with sharp dry seasons. The vegetation types namely, evergreen or rain forest, semi-deciduous forest, Guinea savannah and coastal savannah are found in the region (Ghana Statistical Service, 2013).

3.2 Sampling

In a Randomised Incomplete Block Design, two locations (natural discrete divisions – blocks), a shea tree parkland and a commercial *Hevea* plantation were identified within the two experimental sites. Within each of the locations (block), 50 matured trees were randomly sampled. Those with a stem diameter equal or greater than 0.20 m were tagged and the 50 ramdomly selected. Within trees sampled in the *Hevea* plantation block, no provision was made for unstimulated trees since it is for commercial purposes.

3.2.1 First Phase

The trees sampled in the *Hevea* plantation were treated with an ethylene generating stimulant by spreading the stimulant just 1 cm stripe on the bark over the tapping cut and tapped after three days. However, those in the shea parkland were not treated with an ethylene generating stimulant. Tapping was done during early morning hours over a period of one month (rainy season – within October and November). Tapping cuts were made in the bark of the tree for latex to ooze out and was replicated three times.

3.2.2 Second Phase

The same trees sampled-tapped for shea latex in the first phase were treated with the ethylene generating stimulant (Hevetex) used to treat the trees in the *Hevea* plantation by spreading the stimulant just 1 cm stripe on the bark over the tapping cut (in the first phase) and tapped after three days over a period of one month (rainy season declining – within November and December) in three replications.

Samples obtained from the experimental locations were pooled together according to the Shea latex Stimulated (SHS), Shea latex Non-stimulated (SH) and Hevea latex (HV).

3.3 Latex Flow Rate Quantification

The flow rate of latex at tapping was measured by a graduated beaker and a stop clock and was expressed as the capacity of fluid stored in a given time.

$$Q = \frac{C}{t},$$

Where,

Q is flow rate,

C, is the capacity of fluid stored,

t, is the time taken to flow.

3.4 Analysis of Samples

The samples collected were prepared prior to determination of proximate composition, mineral composition, non- metallic constituents, and phytochemical constituents.

3.4.1 Moisture Content

Moisture determination was done using standard procedures. Empty porcelain crucibles were labeled and weighed. Using a high precision electronic scale of least count 0.0001 g, 3 – 3.5 g of shea latex samples were measured into crucibles and placed in an electric oven set at a temperature of 105 ^{0}C for a period lasting 4 hours to attain constant weights. The oven – dried weights of shea latex samples were taken and moisture contents computed using the formula as follows:

$$\% \text{ Dry matter} = \frac{e}{d} \times 100,$$

$$\% \text{ moisture} = 100\ \% - \%\text{ of dry matter}$$

where,

weight of crucible is a

weight of crucible + sample is b

weight of crucible + dry sample is c

weight of the sample, d = b – c

weight of the dry sample, e = c – a

A 5 – 5.5 g of pooled and homogenized same unit samples were analyzed to check reliability and accuracy of results on a digital electronic moisture analyzer (Sartorius, MA 45).

3.4.2 Crude Protein

Protein content of the latex samples were determined based on the Kjeldahl method. One (1) gram of dried latex from each latex sample was weighed on a piece of filter paper and placed in digestion tube and 15 ml of concentrated H_2SO_4 plus two tablets of Kjeldahl catalyst added. The tubes were placed on Kjeldahl digestion blocks (J. P. Selecta, Bloc Digest 6) and digested at 420 ^{0}C for two hours in four cycled programme lasting 30 minutes per cycle. Distillation was carried out after the digested samples were left to cool down sufficiently in a rack and 50 ml of deionized water added to dilute the content. The dilution was a precautionary measure taken to minimize risk of explosive reaction when the digest (conc. H_2SO_4 and sample) was added to an equally concentrated base (35 % NaOH) during distillation. Distillation proceeded on an automated distillation unit for nine minutes. Distillate from the samples were collected and titrated against 0.1N Hydrochloric acid. A paralleled blank determination was done to provide a correctional factor for any extraneous nitrogen from reagents and other sources that might add up to the nitrogen content of the samples. In addition, a control sample (ammonium sulphate) was used to check for the distillation system efficiency at 98 % recovery rate. The titre values of samples as well as blank were recorded and % nitrogen and % protein was calculated as follows;

$$\%\, Nitrogen = \frac{(T - B) \times N \times 1.4007}{Weight\ of\ sample\ (g)} \times 100$$

Where,

T is titre value

B is blank titre value

N is normality of Hydrochloric acid (0.1 N)

3.4.3 Crude Fat

Fat analysis was done using the Soxhlet Extractor. The solvent used was petroleum ether, a favourable substitute for diethyl ether for its environmental friendliness. Three grams of samples were measured into empty thimbles, one each per sample on a highly sensitive electronic scale (Sartorius, CP 224S) with least count of 0.0001 g. Empty aluminum extraction cups were similarly weighed (W_1) and the weights recorded, then 50 ml of petroleum ether was measured into each of the extraction cups in a fume cupboard, to minimize hazards of inhalation. Subsequently, thimbles and weighed samples were secured in cartridges and carefully mounted on the condensers of the Soxhlet apparatus, using the thimble holder. Then, the extraction containers were also placed on the hot heating mantle component of the system and a lock lever carefully lowered and clamped. The samples were then subjected to reflux process in a total time of 105 minutes through process stages such as 35 minutes [boiling]; 45 minutes [rinsing]; 15 minutes [ether recovery] and 10 minutes [drying]. After the extraction regime, samples were left to cool down for 30 minutes in the service unit, after which the cups with their extracted contents were finally weighed (W_2).

Percentage (%) crude fat was calculated using the formula:

$$Percentage\ (\%) C.\ fat = \frac{Weight\ of\ extract\ [W2 - W1]}{Weight\ of\ sample} \times 100$$

Weight of extraction (fat) = $W_2 - W_1$, where

W_1 – Weight of empty extraction cup

W_2 – Weight of extraction cup + extract

3.4.4 Ash Content Determined

Ash content analysis of latex samples was determined in accordance with the procedure as follows. Ash content analysis gives an estimation of the total mineral composition of samples and determination was based on the gravimetric principle. As a standard practice, crucibles were pre-ignited and samples thoroughly dried at 550 ^{0}C and 105^{0}C respectively. Weights of latex samples in the range of 3 -3.5 g, were variously measured and transferred into pre-weighed crucibles and ignited again in the muffle furnace at 550 ^{0}C for 8 hours, and the obtained ash, which was without traces of carbon that would normally manifest as black spot in samples indicated successful ashing process. Furnace was switched off and cooled to below 200 ^{0}C before crucibles were removed into desiccators to cool further. The crucible with their ash contents were weighed and the percentage (%) total ash, calculated as;

$$\%\ Ash = \frac{(Weight\ of\ crucible + ash) - (Weight\ of\ empty\ crucible)}{Sample\ weight\ (swt)} \times 100$$

3.4.5Analysis of Crude Fiber

As a general principle, thoroughly dried defatted latex samples were treated by acid and alkaline hydrolysis using dilute acids and alkaline solutions respectively, with water treatment in between. The residue collected after the hydrolytic process was treated to an acetone bath, and subsequently dried and ashed. Weight of latex before and after ashing were recorded and used to calculate the value of crude fiber.

A 1 mg (0.001 g) of each sample was weighed (W_0) into porous glass crucibles and mounted onto a 6-unit fibertec equipment (Dosi fiber 6 units 400623) in low position. Acid hydrolysis was started after 100 ml of pre-heated H_2SO_4 solution and 5 drops of foam-suppressant were added to each column of the unit. The cooling circuit was engaged to ensure the condenser is kept cool; heating element was switched on to the 90 % power mark to start with. Power was reduced to 30 %, immediately boiling started and timed for 30 minutes. After the time had elapsed each column valves were opened to drain off the acid followed by a copious rinse with deionized water to ensure samples were no longer acidic. The same process was followed for the alkali hydrolysis using KOH to substitute H_2SO_4. The samples were treated to an acetone bath in a washing sink within a fume hood, and 30 ml of acetone was poured over each sample. The samples, following the acetone bath were transferred to dry in the oven at 150 ^{0}C, for 2 hours, cooled in a desiccator unit and weighed (W_1). Samples were transferred to a muffled furnace set at 550 ^{0}C, for incineration for 4 hours and again left to cool in the muffle furnace and desiccator respectively and weighed (W_2).

Computations were done by using the formula:

$$\%\ Crude\ fibre = \frac{W1 - W2}{W0} \times 100$$

Where;

W0 is initial weight

W1 is weight before ashing

W2 is weight after ashing

3.4.6 Analysis of Carbohydrates

Carbohydrates computation was by difference after analysis of all the other proximate parameters as;

Carbohydrates = 100 – (% moisture + % crude protein + % crude fat + % crude fiber + % ash)

3.5 Determination of Inorganic Metallic Ion Constituents

The AAS method was used for the determination of inorganic metallic ions. 1 g of each sample was weighed into a crucible and ignited in a muffle furnace at 300 ^{0}C for 24 hours. It was allowed to cool, and 20 ml 4 M nitric acid and 60 % of per-chloric acid solution was added. The mixture was heated to digest the ashed samples into solutions. The digested sample solution was diluted to 100 ml volume with distilled water. This was used for the analysis of various metals (Mn, Zn, Cu, Pb) in the Atomic Absorption spectrophotometer at their respective wavelength.

3.6 Determination of Inorganic Non-Metallic Ion Constituents

Inorganic Non- Metallic ion constituents of the latex samples were determined following the standard method of AOAC (2000). The latex samples were analyzed for nitrate, sulphate, phosphate, nitrite, chloride and NH_3N.

3.7 Phytochemical Screening

3.7.1 Alkaloid Test

About 10 mg of the extracts was boiled with Ammoniacal alcohol which was prepared by adding 3ml of strong ammonia and 27ml of 95% ethanol. It was filtered and the filtrate brought to evaporated to dryness on the water bath. The residue was extracted with 1% H_2SO_4. This was also filtered and the filtrate made distinctly alkaline by adding dilute NH_3 solution and shaken with

chloroform in a separating funnel. The chloroformic layer was evaporated to dryness and the residue dissolved in 1% H_2SO_4.

To about 1ml of the 1% H_2SO_4 extract, 1 drop of Dragendoff's reagent was added and an orange-red precipitate formed indicated the presence of alkaloids.

3.7.2 Flavonoids Test

About 10 mg of the extracts was taken and a small amount of water added. It was then filtered and a strip of filter paper dipped into the filtrate and air dried. The strips were then exposed to strong Ammonia.

3.7.3 Glycosides Test

About 10 mg of each extract was taken and heated over the water bath with dilute H_2SO_4 for 5 minutes. It was then filtered and about 2-10 drops of 20% NaOH was added to make the filtrate completely alkaline. Fehling's solution and B was added and heated on the water-bath for about 2 minutes. A brick-red precipitate formed showed the presence of glycosides.

3.7.4 Triterpenoids (Salkowski Test)

About 10 mg of the extracts was taken and chloroform added and brought to boil over the water bath for about 2minutes. This was then filtered and cooled. To about 3ml of the chloroformic extract, concentrated H_2SO_4 was added down the side of the test tube wall. A reddish-brown ring formed at the interface indicated the presence of triterpenoids.

3.7.5 Phytosterols Test

To about 1 ml of the chloroform extracts, 1ml Acetic Anhydride was added and concentrated H_2SO_4 added along the walls of the test tube and the rings formed observed.

3.7.6 Saponins

About 10 mg of the extracts was taken dissolved in water and shaken vigorously. The formation of a persistent froth on the bench for a while indicated the presence of saponins.

3.7.7 Reducing Sugars

One ml each of extract was added to 5 ml of equal volumes of Fehling's solution A and B and heated over a water bath for 2 minutes. Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars.

3.7.8 Anthroquinones

About 2 ml of each extract was taken into 10 ml of benzene and was agitated and then filtered. Following that, a 5 ml volume of 10 % ammonia solution was added to the filtrate and agitated again for 5 minutes. Appearance of a pink, red or violet colour in ammonia solution phase was taken as a positive test for anthraquinones.

3.7.9 Soluble Starch

One ml of each extract was boiled with 1 ml of 5 % KOH, cooled and acidified with H_2SO_4. A yellow colouration showed the presence of soluble starch.

3.8 Statistical Analysis

One-way analysis of variance (ANOVA) (GENSTAT) was used to indicate significant differences among samples. Significance testing was done using Fisher's Least Significant Difference (LSD) test; statistical differences at $P \leq 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Physical Properties of Latex

The three different latex samples as observed and studied over the experimental period showed the follow physical properties illustrated Table 4.1.

Table 4. 1: Physical properties exhibited by *Hevea brasiliensis* latex - HV, Shea latex (non-stimulated) - SH and Shea latex stimulated – SHS.

Sn.	Property	HV	SH	SHS
1	Smell	Unpleasant smell	No smell	No smell

2	Colour	Relatively Whiter to Pale Yellow during storage	White (changes to palepinkish-red upon contact with inner back during tapping). Browning upon storage	White (changes to palepinkish-red upon contact withinner back during tapping)
3	Flow duration	Relatively Longer period	Relatively shorter period	Relatively shorter period
4	Elasticity	Relatively High	Relatively Low	Relatively Low
5	Stickiness	Relatively Low	Relatively High	Relatively High
6	Coagulation	Relatively Longer period	Relatively Shorter period	Relatively Shorter period
7	Chewiness	Not chewed (due to smell/toxicity)	Very Good	Good
8	Thickness	Moderate	Moderate	High
9	Viscosity	Moderate	Moderate	High
10	Adhesiveness	Low	High	High
11	Cohesiveness	High	Moderate	Moderate

4.2 Flow Rate (L/min)

The rate of flow of latex (Table 4.2) from all three samples varied significantly (P< 0.05). HV recorded twice the quantity for SH. The stimulation had positive influence on the flow rate of SHS. However, quantity recorded was one part less compared to HV.

Table 4. 2: Mean flow rate for *Hevea brasiliensis* latex - HV, Shea latex (non-stimulated) - SH and Shea latex stimulated – SHS.

Latex Type	Mean flow rate (L/min)

HV	0.004 ±0.000109
SH	0.001±1.74E-05
SHS	0.003±4.61E-05
P-value	<.001
l.s.d (0.05)	0.001

Mean flow rate ± S.E.M.

4.3 Proximate Analysis

From Table 4.3, except for Crude Protein, mean values recorded for proximate composition for the three latex samples were significantly different. Percentage moisture for SH was approximately double of that for HV, whiles SHS was in-between. Percentage ash content recorded for SH was twice that recorded for SHS, HV recorded an intermediate value. The trend was the same for percentage fat content and percentage fibre content with either HV or SHS recording values in-between. For percentage carbohydrate, HV recorded the highest value which is approximately double for that of SHS and eight times that of SH. However, for Crude Protein, there was no significant difference between SHS and HV, where the value recorded for SHS was thrice that of SH.

Table 4 :entage (%) Proximate Composition of latex samples

Latex T	⅓ Moisture	% Ash Content	% Fat Content	% Fibre Content	Crude Protein	% Carbohydrate
HV	35.950 ±1.008	2.809±0.020	10.720±0.135	3.180±0.054	3.940±0.067	43.400±1.000
SH	66.240±0.589	4.187±0.081	15.920±0.469	6.420±0.310	2.220±0.022	5.020±0.556
SHS	48.820±0.076	1.650±0.071	14.420±0.203	4.750±0.088	4.950±0.568	25.400±0.401
P-value	<.001	<.001	<.001	<.001	0.003	<.001
l.s.d (0.	2.337	0.220	1.056	0.652	1.144	2.424

Mean P :e Composition ± S.E.M

4.4 Metallic Ion Composition

The Metallic ion composition (Table 4.4) from the three latex samples were significantly different ($P < 0.05$). Mean values recorded shows no consistent trend, as the samples were high in some elements but low in others.

Table 4 eral (metallic) ion composition of latex

Latex T	Pb (mg/L)	Zn (mg/L)	Mn (mg/L)	Cu (mg/L)
HV	0.022±0.001	0.218±0.001	0.117 ±0.001	0.017±0.000
SH	0.028 ±0.001	0.056±0.001	0.146 ± 0.001	0.019±0.000
SHS	0.016 ±0.000	0.094±0.000	0.132±0.001	0.042 ±0.001
P-value	<.001	<.001	<.001	<.001
l.s.d (0.	0.003	0.003	0.004	0.002

Mean M (metallic) ion composition ± S.E.M

4.5 Non-Metallic Composition

Table 4.5 shows significant differences ($P < 0.05$) between latex samples on non-metallic constituent. Except for nitrite and NH_3-N, HV latex recorded the highest mean non-metallic ion values. These values are more or less double for those mean values recorded in the other latex samples. There was no significant difference in sulphate composition between HV and SHS. However, HV and SH latex samples showed no significant differences between both nitrite and chloride.

Table 4 eral (non-metallic) ion composition of latex

Latex T	Nitrate (mg/L)	Sulphate (mg/L)	Phosphate (mg/L)	Nitrite (mg/L)	Chloride (mg/L)	NH_3-N (mg/L)
HV	5.367±0.027	4.000± 0.000	2.477±0.003	0.007± 0.000	8.610±0.000	0.101±0.000
SH	3.567 ± 0.027	3.033 ± 0.027	0.032± 0.000	0.008± 0.000	8.617±0.007	0.501±0.000
SHS	4.400 ± 0.047	4.100 ± 0.047	0.028± 0.000	0.004±0.000	6.447± 0.003	2.610±0.005
P-value	<.001	<.001	<.001	<.001	<.001	<.001
l.s.d (0.	0.149	0.133	0.007	0.001	0.019	0.012

Mean M (non-metallic) ion composition ± S.E.M

4.6 Phytochemical Analysis

Table 4.6 shows the results for the phytochemical compounds tested for each latex sample. Generally, alkaloids, phytosterols and triterpenoids were present in all three samples. However, HV and SHS expressed high levels of alkaloids and triterpenoids respectively.

Table 4 tochemical composition of latex samples

Latex Types	ids	Anthroquinones	Flavonoids	Glycosides	Phytosterols	Reducing Sugars	Saponin	Soluble Starch	Triterpenoids
HV		-	-	-	+	-	+	-	+
SH		+	-	+	+	+	-	+	+
SHS		+	-	+	+	+	-	+	+

[+] posi negative

CHAPTER FIVE

5.0 DISCUSSION

Results from Table 4.1 showing physical properties of latex may be attributed to specie differences and ambient weather conditions peculiar to the different experimental sites. At the different specie levels, the growth stage and age of the tree may also contribute to the physical properties observed. According to Gomez (1982), the properties exhibited by hevea latex including the properties of size, shape of the latex particles and colour is dependent on the age or growth stages of the plant. In addition, colour is reported to differ in different parts of a single plant, and was observed in *Hevea brasiliensis* that the colour may change after the latex has oozed from the plant (Gomez, 1982). In line with this results, sections from all parts of hevea produced white milky latex and over time, turned pale to yellowish and coagulated at room temperature (Gomez, 1982). Also, Agrawal and Konno (2009) re-counted that both the stickiness and the characteristically white color of latex are often as a result of the presence of rubber particles distributed in the fluid. The implication is that, the more the rubber particles present, the whiter the colour and the more elastic the latex. This may explain why hevea latex was relatively whiter and elastic whereas the shea latex was gluey. This gluey nature of shea latex makes it a potential candidate for a bio-adhesive. The browning colour of HV upon storage may be associated with enzymatic reactions peculiar to the unstimulated sample. A report by Li *et al*., (2014), states the effect of the enzyme polyphenol oxidase (PPO), apathogen-defense enzyme, responsible for latex browning and also associated with latex coagulation and wound healing. From this study, it is realized that SHS did not show the brown colouration which may suggest that the presence of this same enzyme (polyphenol oxidase, PPO) may have been reduced as a result of the stimulation and since it is also involved in coagulation, its reduction will imply a prolonged flow of latex

which was evident between SH and SHS. This is in line with Li *et al*., (2014), who reported that PPO decreased in ethephon-treated latex serum showing that it was down-regulated by ethylene and a further conclusion that the decline in accumulation PPO in ethephon-treated sample may have hindered rubber particle aggregation which subsequently prolonged the latex flow. Regarding coagulation, Abd Razak (2000) reported the evidence of rubber particle coagulation in latex from hevea. Lutoids, which are present in latex are said to be directly linked in the coagulation process of latex which enables the ceasing of the flow of the latex (Southourn, 1968). Latex made to stand after tapping may have coagulated due to the presence of some fats which is able to solidify at room temperature. This is backed by Homans and Van Dalfsen (1948), who reported the coagulation of different fractions of latex after centrifugation and attributed it to the probability that all enzymatic reactions allow for an enhanced splitting of large-molecule complexes and the formation of free fatty acid which in the presence of some cations effect spontaneous coagulation. The varying flow duration may be attributed to the different abilities of water stores expelled by the trees which are affected by the ambient climatic conditions including rainfall and drought, age of tree, depth of cut and mode of stimulant application. In line with this, Gunasekera *et al*., (2013); Njukeng *et al*., (2011); Traore *et al*., (2011) reported that two major factors affect the yield of latex at each tapping which may be attributed to the flow duration; this include internal factors such as tree age and external factors such as stimulation, the depthand length of tapping cut. Quainoo and Dugbatey (2016) also had results indicating that shea latex flow decreases with increasing age of the tree. Among the sampled shea trees, stress from the environment and harvesting may have affected the flow duration due to the two successive tapping. According to Dusotoit-Coucaud *et al*., (2010), tapping signifies a severe abiotic wounding stress for exploited trees - under consistent tapping. It necessitates latex cells to fully

regenerate their cytoplasm after latex discharge. Factors such as harvesting stresses and environmental, as well as the metabolic activity necessary for latex redevelopment between two tappings, will further lead to the production of reactive oxygen species (ROS). Excessive production and accumulation of reactive oxygen species may lead to laticifer malfunctions such as Tapping Panel Dryness (TPD). The flow of latex stops as a result of Tapping Panel Dryness.

Significant differences observed in results from Table 4.2 showing flow rate of latex confirms the positive effects of ethylene stimulator on the flow rate of latex. According to Coupé and Chrestin (1989), treatment with ethylene generator (ethephon) both increases the volume of exported latex by acting on membrane permeability leading to extended latex flow and kindles latex regeneration between tapings through general reformative metabolism. Quantitatively, latex production is said to increase by this method by 1.5- to 2-fold which was evident between SH and SHS. This evidence may probably be due to the increase in water uptake as a result of sucrose loading in laticifers. This finding may be supported by the report that exogenous ethylene generator application induces several biochemical changes in laticifers, such as sucrose loading (Dusotoit-Coucaud *et al*., 2010) and water uptake (Tungngoen *et al*., 2009).Thus, it can be said that ethylene stimulator increased membrane permeability and improved latex regeneration metabolism. This implies that less time is required to acquire the same quantity of latex for SHS compared to SH.

Proximate analysis results in Table 4.3 shows that crude protein which has been the central focus for discussion in shea latex exploitation increased but was not significantly different from HV when stimulated. This may be attributed to increase innitrogen production and nitrogen being a precursor for protein metabolism will subsequently increase protein content of stimulated latex. A study by Pujade-Renaud *et al*., (1994) affirms that a key enzyme glutamine synthetase involved in

nitrogen metabolism has been found in the cytosol of laticiferous cells after ethylene treatment paralleling the increase of latex yield. It is reported that the enzyme's response to ethylene might be mediated by ammonia that increases in latex cytosol and quantitatively, the export 100 mL of latex, completely regenerated within 3 days will parallel a net synthesis of about 50 g of dry rubber and 1.2 g of protein. In this setting, nitrogen metabolism involved in protein synthesis takes a prominent part. However, Primot *et al*., (1979), suggests that the progression of this stimulation is well linked with the kinetics of latex yield, along with other physiological parameters such as pH, mineral ions, saccharose content, etc. which are realized in rubber trees after ethylene treatment under similar conditions. Pujade-Renaud *et al*., (1994) further resolves that, the protein content of the latex is efficiently controlled, with an equilibrium between full regeneration of the proteins and loss through each tapping, even under conditions of ethylene-increased latex removal.

Percentage carbohydrate expressed a significant increase in SHS over SH. This may be due to the increase in sucrose concentration which enhances the flow of latex after stimulation and may subsequently end up in the latex expelled from the trees. Ethylene stimulation during latex production draws high content of sugar molecules from the surrounding cells of inner bark towards the latex cells according to Dusotoit-Coucaud *et al*., (2010). Although D'Auzac (1964) and Chow *et al*., (2007), presented different pathways, both pathways present sucrose as the unique precursor of natural rubber, and its passage into latex cells may be crucial to latex production. Studies have confirmed that ethylene increases latex production by different mechanisms, including enhancing latex sucrose concentration (Low and Gomez, 1982; Tupy and Primot, 1976). Application of ethylene on rubber tree has been reported to connect several metabolic responses and biochemical processes including nitrogen assimilation, carbohydrate

transport and metabolism, protein synthesis as well as sucrose and glucose loading (Coupé and Chrestin, 1989; Wang *et al*., 2015). Percentage carbohydrate may have had an effect on the percentage moisture content due to the increase of solute molecules in latex from ethylene treated trees.

The percentage fat content recorded for the samples may be attributed to enzymatic reactions which are able to break some large-complex molecules present in latex to enhance the formation of free fatty acids. In line with this, Homans and Van Dalfsen (1948) reported that the probability that enzymatic reactions allowed for an enhanced splitting of large-molecule complexes and the formation of free fatty acid.

Percentage fibre content revealed to be higher in SH may be attributed to the morphological and the physiological nature of the tree. This may have been influenced by the climatic demands of the area. This result may favour the use of SH in producing dietary fibre supplements as it may contain certain complex polysaccharides such as lignin, hemicellulose and cellulose. In agreement, Rao, (2003), describes dietary fibre not as a single entity but a wide-ranging complex polysaccharides for example, gums, cellulose, hemicellulose, mucilage and lignin with different physiochemical, chemical and physiological properties. The properties include its usefulness in minimizing blood glucose levels in diabetes, in minimizing blood cholesterol levels for treatment of cardiovascular disease and also in averting bowel cancer.

Percentage ash content showed results which were contrary to that ofMoreno *et al.*, (2005), who reported that there is a tendency of increasing ash % triggered by low precipitation resulting in smaller amount of accessible water in the soil, which may have contributed to insignificant dilution of the latex. This is so because, SHS which was sampled during the period of declining rainfall recorded the least value (Table 4.3). This may probably be due to the interaction of

several factors including mineral ions which were either present or absent in latex. As Moreno *et al*., (2005) reports that when the absorbed nutrients are used again, ash % tends to decrease.

Table 4.4 and 4.5 show the mineral composition of the latex samples as categorized as metallic and non-metallic respectively. It may be said that the small amounts of ions recorded in the three samples for the metallic ions relative to the non-metallic ions confirm that Cu, Zn, Mn and Fe are present in traces and for that fact are described as trace elements. However, it is noted that there are significant differences among the samples. This may be due to the differences in the trace mineral soil conditions in the two different experimental sites and also abiotic factors such as rainfall and temperature as well as biotic factors including translocation or mobility of the elements. It is explained in a report that fresh latex contains metal ions but seasonal variation which affects the constitution is quite out of control when fresh latex is collected at different periods of time (Archer *et al*., 1963).

Considering George *et al*., (2014), results realized for the non-metallic constituents of *Hevea brasiliensis* latex recorded low values for non-metallic constituents ranging from 0.1 – 2.0 mg/l, which is opposing to those recorded for the latex samples (Table 4.5).

Table 4.6 shows that SH and SHS both tested negative for flavonoids and saponins but positive for all phytochemicals tested. These are in line with reports by Mahajan and Badgujar (2008b), who reported that *Manilkara zapota* (L.) which has been exploited as gum base (Shah and Mehta, 2014) and belongs to the same family as the shea (*Sapotaceae*) also tested negative for Flavonoids and saponins. In view of this, it may be said that these latex samples may offer similar if not the same phytochemical potency properties (medicinal value) as reported by other researchers in other latex producing plants and when coupled with its physical properties could be beneficial for industrial use.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The application of an ethylene generator (Hevetex) positively influenced the flow rate of shea latex, confirming that the product enters the plant and migrates to tissues where it progressively

enhances ethylene decomposition and acts on the plant metabolism. Consequently, it also influenced the latex quality biochemically in terms of proximate composition, ionic composition (metallic and non-metallic) and phytochemical constitution. Most importantly, lower protein levels for unstimulated shea latex cited to be a possible comparative advantage over natural rubber latex for the manufacture of rubber products, for example gloves and condoms for reduced allergenicity increased after stimulation of shea latex with the ethylene generator.

Comparatively, SHS exhibited both physical and chemical properties which make it suitable for confectionery purposes over natural rubber latex. These properties such as good smell, appearance (colour) and chewiness, as well as testing negative for flavonoids and saponins for phytochemical composition which are similar for an already exploited gum base (*Manilkara zapota* L.) suggest a comparative advantage of shea latex over natural rubber latex when considered for gum making in the confectionery industry. Also, the relatively high stickiness and high adhesiveness of shea latex makes it a better option for bio-adhesive over natural rubber latex.

However, natural rubber latex showed a relatively longer flow duration which is an advantage over shea latex, even after stimulation. Also, natural rubber latex remained relatively more elastic and highly cohesive in comparison to shea latex suggesting the large presence of rubber particles which makes it more ideal for rubber products over shea latex, not considering the protein allergenicity.

6.2 Recommendations

To complement efforts in the drive to increase the knowledge base of shea latex, progressive research is recommended as follows;

1. To make certain the flow rate of latex for shea, different trees should be sampled and labelled as stimulated and unstimulated, then tapped for latex in the same season especially the rainy season.
2. Detailed protein analysis should be done to determine whether or not the proteins present in shea latex have the same allergic effect as those in *Hevea*.
3. Structural analysis of shea latex using a nuclear magnetic resonance equipment will be needful to confirm the amount of rubber particles present.
4. A study correlating the tapping of latex and fruiting of shea tress should be conducted to confirm or refute the negative perception held about tapping latex from shea trees on shea nut yield.
5. The mode of application of the stimulant as well as the concentration should be studied to reveal appropriate mode of application and concentration.

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APPENDIX

APPENDIX 1: Pictures of field latex tapping and laboratory analysis

Plate 1: A. *Hevea brasiliensis* tree tapped B. *Vitelleria paradoxa* tree tapped

Plate 2: A. *Vitelleria paradoxa* latex B. *Hevea brasiliensis* latex

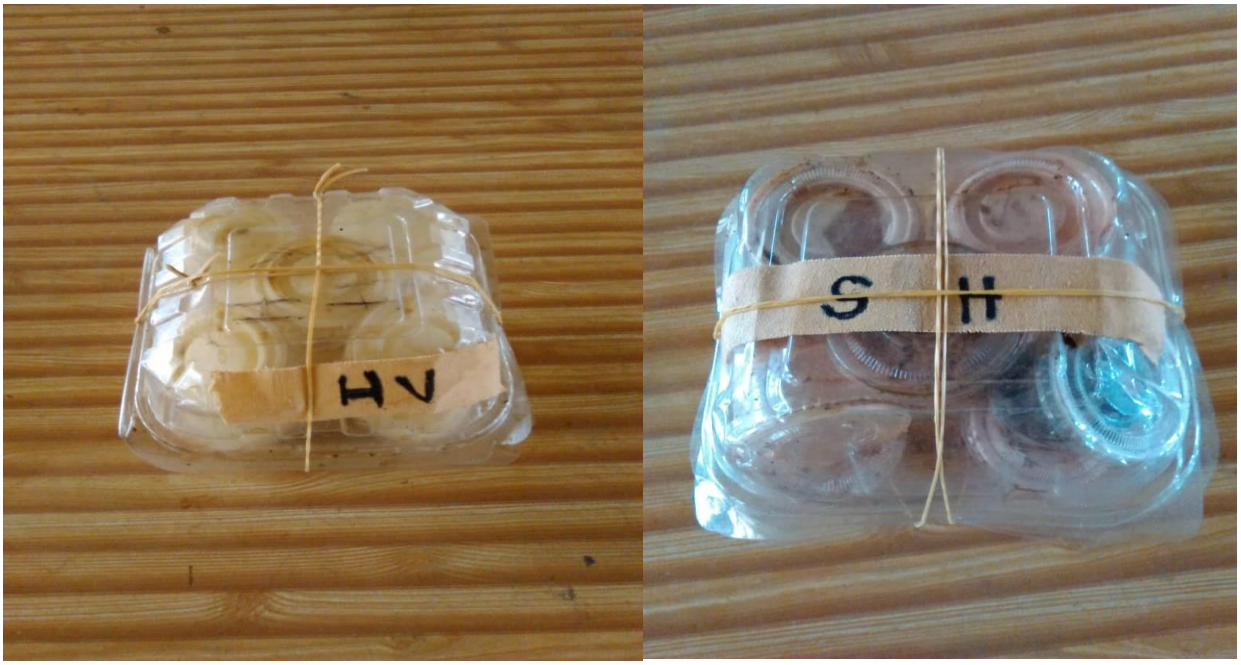

Plate 3: Packaged latex to be transported

Plate 4: Ashed latex sample

Plate 5: Student performing laboratory analysis

Plate 6: Equipment used during research