Biochemical And Phyto –Chemical Characteristics Of Rubber Latex (Hevea Brasiliensis) Obtained From A Tropical Environment In Nigeria.

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ABSTRACT: The biochemical and phyto-chemical characteristic of rubber latex (*Hevea brasiliensis*) was investigated under laboratory conditions. Proximate composition, mineral contents, non-metallic constituents, metallic constituents, phyto-chemical constituents and n-hexane extract components of the latex were analysed. The proximate composition consisted of 60.17 ± 0.01 % moisture, 2.523 ± 0.01 % crude fat, 2.966 ± 0.01 % ash, 1.8 ± 0.1 % crude fibre, 2.133 ± 0.05 % crude protein and 30.47 ± 0.01 % carbohydrate while mineral contents consisted of 0.016 ± 0.001 mg/l Cu, 0.082 ± 0.00 mg/l Zn, 0.001 ± 0.001 mg/l Pb and 0.018 ± 0.001 mg/l Mn. Non-metallic constituents included 2.21 ± 0.001 mg/l nitrate, 0.613 ± 0.001 mg/l nitrite, 0.100 ± 0.00 mg/l Chloride, 0.616 ± 0.001 mg/l sulphate, 0.740 ± 0.01 mg/l phosphate and 0.1 ± 0.00 mg/l ammonia, while phytochemical constituents included 3.492 ± 0.1 mg/l hydrocyanic acid, 0.635 ± 0.01 mg/l phytic acid, 0.433 ± 0.01 mg/l alkaloids with oxalate showing negativity in the latex and n-hexane having a value of 2.194 mg/l at 430 nm wavelength.

Key words: *Hevea brasiliensis*, Biochemical, Phyto-chemical, Tropical, Nigeria.

INTRODUCTION

The commercial source of natural rubber latex is the Para rubber tree (*Hevea brasiliensis*), a member of the spurge family, Euphorbiaceae. *Hevea brasiliensis* is a quick-growing tree, rarely exceeding 25 m in height in plantations, but wild trees of over 40 m have been recorded (Hobhouse and Henry, 2005). Bole usually straight or tapered, branchless for 10 m or more, up to at least 50 cm in diameter, without buttresses; bark surface smooth, hoop marked, grey to pale brown, inner bark pale brown, with abundant white latex; crown conical, branches slender (Hobhouse and Henry, 2005). Natural rubber, also called India rubber or caoutchous, is an elastomer (an elastic hydrocarbon polymer) that was originally derived from latex, a milky colloid produced by some plants. The plants would be tapped; that is, an incision made into the bark of the tree and the sticky, milk coloured latex sap collected and refined into a usable rubber. The purified form of natural rubber is the chemical polyisoprene, which can also be produced synthetically. Natural rubber is used extensively in many applications and products (Hobhouse and Henry, 2005). It is normally very stretchy and flexible and extremely waterproof.

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Chemically, latex consists of rubber, resins, proteins, ash, sugar, and water. *Hevea* latex is a biological product of a complex composition. The basic components of freshly tapped natural rubber latex, other than water which constitutes about 22 to 48 %, are dry rubber (20 to 45 %), proteinous substances (1.5 %), Resinous substances (2 %), carbohydrates 1 %, inorganic matter 0.5 % and other components (CHIN, 1979). Natural rubber consists of C_4H_8 (isoprene) units, each containing one double bond in the *cis* configuration. However, polyisoprene of *H. brasiliensis* contains in addition two *trans*-isoprene units in the terminal region (Tanaka and Sakdapipanich, 2001). The objectives of this study was to investigate the biochemical and phyto-chemical characteristics of *Hevea brasiliensis.*

Materials and Method

Study Area

Samples of *Hevea brasiliensis* was collected from the rubber research instititute, Calabar, Nigeria located within Calabar (08°22'10.583'E and 05°8'2.85''N). The climate of the area is tropical and is characterized by distinct wet and dry seasons. The study area is generally rainforest and is surrounded by two major rivers, the Calabar River and Great Kwa River. Human activities in the area include farming, hunting, fishing and sand mining. (Pers. Com). (Figure 1).

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Analysis of Samples

Samples of *Hevea brasiliensis* was taken to pure and applied chemistry laboratory, University of Calabar, Calabar, Nigeria. The sample collected from Cross River Rubber Plantation was prepared prior to determination of proximate composition, mineral composition, non- metallic costituents, phytochemical constituents and the n-hexane extract of the latex.

Determination of proximate composition Hevea brasiliensis

Proximate composition was determined following the standard method of AOAC (2000).

Determination of Inorganic Metallic ion constituents of Hevea brasiliensis

The AAS method was used for the determination of inorganic metallic ions. 1 g of sample was weight into a crucible and ignited in a muffle furnace at 300 °C for 24 hours. It's was

allowed to cooled, then 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.

Determination of Inorganic Non- Metallic ion constituents of Hevea brasiliensis

Inorganic Non- Metallic ion constituents of *Hevea brasiliensis* was determined following the standard method of AOAC (2000).

Determination of Phytochemical constituents of Hevea brasiliensis

Phytochemical constituents of *hevea brasiliensis* was determined following the standard method of Vogel (2000).

Determination of n-hexane extract of Hevea brasiliensis

The concentration of organic extract of Hevea brasiliensis was determined using spectrophtometirc measurement of the latex extract at 430 nm wavelength using a scanning model direct reading (DR) 6405 spectrophotometer (Stuermer *et al*., 1981), and the measurement was taken as displayed.

RESULTS

The proximate composition of *H. brasilensis* is shown in Table 1. Moisture content ranged between 60.15 - 60.18 % with a mean of 60.1 ± 0.01 %, while crude fat ranged from 2.52 - 2.53 % with a mean of 2.523 ± 0.01 %, ash 2.96 - 2.97 % with a mean of 2.966 ± 0.01 %, crude fibre ranged between 1.7 - 1.9 % with a mean of 1.8 ± 0.1%, crude protein 2.10 - 2.20 % with a mean of 2.133 ± 0.05 % and carbohydrate between 30.47 - 30.48 % with a mean of 30.473 ± 0.01 %. Table 2 shows the mineral composition of the *H. brasilensis* used for the experiment. Copper ranged between 0.015 - 0.017 mg/l with a mean of 0.016 ± 0.001 mg/l. Zinc maintained a value of 0.082 mg/l with Lead ranging between 0.001 - 0.002 mg/l with a mean of 0.001 ± 0.001 mg/l and Manganese ranging between 0.018 - 0.019 mg/l with a mean of 0.018 ± 0.001 mg/l. The non-metallic constituent in *H. brasilensis* are shown in Table 3. Nitrate (NO_3^-) ranged between 2.209 - 2.211 mg/l with a mean of 2.21 ± 0.001 mg/l, nitrite (NO^-_2) ranged between 0.612 - 0.614 mg/l with a mean of 0.613 ± 0.001 mg/l, Chloride (Cl^-) maintained a value of 0.100 mg/l, while Sulphate (SO_4^{2-}) ranged between 0.615 - 0.617 mg/l with a mean of 0.616 ± 0.001 mg/l, Phosphate (PO_4^{2-}) ranged between 0.740 - 0.741 mg/l with a mean of 0.740 ± 0.001 mg/l and Ammonia (NH_3^-) maintaining a concentration of 0.1 mg/l. The phyto-chemical constituents of *H. brasilensis* are shown in Table 4. These included hydrocyanic acid, phytic acid, oxalate and alkaloids. Hydrocyanic acid ranged between 3.456 - 3.564 mg/l with a mean of 3.492 ± 0.1 mg/l, phytic acid ranged between 0.634 - 0.636 mg/l with a mean of 0.635 ± 0.001 mg/l, Alkaloids ranged between 0.43 - 0.44 mg/l with a mean of 0.433 ± 0.01 mg/l with oxalate showing negative appearance in *H. brasiliensis* latex. The n-hexane extract component in *H. brasilensis* at the wavelength of 430 nm is shown in Table 5. This ranged from 2.192 -2.196 mg/l with a mean of 2.194 ± 0.002 mg/l.

TABLE 1: Proximate composition of *Heavea brasiliensis* . (in percentage)

Parameters	Readings			$\bar{X}$ (±)
	1	2	3	
Moisture	60.18	60.15	60.16	60.17 ± 0.01
Crude fat	2.52	2.53	2.52	2.523 ± 0.01
Ash	2.97	2.96	2.97	2.966 ± 0.01
Crude fibre	1.9	1.8	1.7	1.8 ± 0.1
Crude protein	2.10	2.10	2.2	2.133 ± 0.05
Carbohydrate	30.47	30.48	30.97	30.473 ± 0.01

TABLE 2: Mineral composition of *Hevea brasilienssis*.

Parameters	Readings			$\bar{X}$ (±)
	1	2	3	
Copper (Cu)	0.014	0.017	0.017	0.016±0.001 mg/l
Zinc (Zn)	0.082	0.082	0.082	0.082 mg/l
Lead (Pb)	0.001	0.001	0.002	0.001 ± 0.001 mg/l
Manganese (Mn)	0.018	0.018	0.019	0.018 ± 0.001 mg/l

TABLE 3 : Non-metallic ion constituents (mg/l) in *Hevea. brasiliensis*.

Parameters	Readings		$\bar{X}$ (±)
	1	2	
Nitrate (NO^-_3)	2.209	2.211	2.21 ± 0.001 mg/l
Nitrite (NO^-_2)	0.614	0.612	0.613 ± 0.001 mg/l
Chloride (Cl^-)	0.100	0.100	0.100 mg/l
Sulphate (SO_4^{2-})	0.615	0.617	0.616 ± 0.001 mg/l
Phosphate (PO_4^{3-})	0.741	0.740	0.740 ± 0.01 mg/l
NH^-_3	0.1	0.1	0.1 mg/l

TABLE 4 : Phyto-chemical constituents of *H. brasilensis*.

Parameters	Readings			$\bar{X}$ (±)
	1	2	3	
Hydrocyanic acid	3.456	3.564	3.456	3.492 ± 0.1 mg/l
Phytic acid	0.634	0.636	0.634	0.635 ± 0.001 mg/l
Oxalate	-ve	-ve	-ve	-ve
Alkaloids	0.43	0.44	0.43	0.433 ± 0.01 mg/l

TABLE 5: n-hexane extract component in *H. brasiliensis* used for the study

Component	Readings			$\bar{X}$ (±)
	1	2	3	
Organic extract (at 430 nm)	2.192	2.196	2.196	2.194 ± 0.002 mg/l

DISCUSSION

The proximate composition of *H. brasiliensis* are shown in the result. Moisture content of 60.17 ± 0.01 % was obtained, followed 2.523 ± 0.01 % of crude fat, 2. 966 ± 0.01 % of ash, 1.8 ± 0.1 % of crude fibre, 2.133 ± 0.05 % of crude protein and 30.47 ± 0.01 % of carbohydrate. Wititsuwannakul *et al.* (1998) reported the proximate composition of *Heavea brasiliensis* to consist of 2.0 - 2.7 % protein, 55.0 - 65.0 % moisture, 0.4 - 0.7 % ash. The results of the present study compares favourably with those of the authors under reference. Latex is a polydispersed colloidal system of rubber particles in an aqueous phase with varying quantities of the proximate composition (Gomez and Moir, 1979; subramanian, 1995; Wititsuwannakul *et al*., 1998). The variations usually observed in the proximate composition of substances have been limited to environmental conditions such as temperature and soil type (Petsonk, 2000). These might have given reasons for the variations in the proximate composition of the latex used in this study, as Pamol, Calabar, where the latex was obtained is typically a tropical environment when compared with those of Wititsuwannakul *et al.,* (1998) who conducted their studies using latex from *H. brasiliensis* obtained from a temperate environment of Northeast Brazil. Little wonder moisture content was higher in the report of Wititsuwannakul *et al.,* (1998) with a value of between 55.0 – 65.0 % than that of the present study which was recorded as 60.17 ± 0.01 %. Chin (1979) reported that freshly taped natural rubber latex in Malaysia contains 1.5 % of proteinous substances, 2.0 % resinous substances, 1.0 % carbohydrates, 0.5 % of inorganic substances which when compared the values obtained in this study show significant variations as previously stated by Gomez and Moir, (1979); Subramaniam, (1995); Wititsuwannakul *et al.,* (1998), which they respectively attributed to environmental factors and age of the plant itself. The mineral composition of *Hevea brasiliensis* recorded in this study included Copper (0.016 ± 0.001 mg/l), Zinc (0.082 mg/l), Lead (0.001 ± 0.001 mg/l) and Manganese (0.018 ± 0.001 mg/l). From available literature, few authors have reported on the mineral composition of *H. brasiliensis***.** These include those of Subramaniam (1995); Tanaka and Sakdapipanich (2001). Reasons advanced for this lack of information on the mineral composition and even some of the proximate components of the latex from *H. brasiliensis* may not be unconnected with the fact that the latex is never used as feed supplement or additive in feed making due to its toxic nature (Gomez and Moir, 1979; Subramanian, 1995; Wititsuwannakul *et al*., 1998; Muller, 2000; Tanaka and Sakdapipanich, 2001). Non-metalic constituents in the latex of *H. brasiliensis* used in this study included Nitrate (NO_3), Nitrate (NO_2), Chloride (Cl^-), Sulphate (SO_4), Phosphate (PO_4) and Ammonia (NH_3). Plant extract including latex are known to contain low values of non-metallic constituents (Devan *et al*., (2000); Tanaka and Sakdapipanich, (2001); Shivkar and Kumar, (2003). The results obtained for the non-metallic constituents of *H. brasiliensis* in this study, are in agreement with those of the authors under reference. The low non-metallic constituent in the latex has been attributed to inability of the latex to absorb higher doses of the constituents due to its (the latex's) sticky and gummy nature though it exists in liquid form (Subramaniam, (1995); Tanaka and Sakdapipanich, (2001). The non-metallic constituents are therefore not capable of becoming highly absorptive in the latex at a faster rate, hence the usually reported low non-metallic constituents in the latex (Shivkar and Kumar, 2003). Phyto-chemical are acids and related constituents which are known in plant extract and latex only but never found occurring in animals (Baker, (1971), Khalid (1982), Subramaniam (1995), Tanaka and Sakdapipanich, (2001), Udoh, (2006); Ayotunde *et al.*, (2011). In this study the phytochemical detected in the latex of *Hevea brasiliensis* included hydrocyanic acid, phytic acid, oxalate and alkaloids, a result similar to those of Subramaian (1995), Larhsin *et al.,* (1997) and Wititsuwannakul *et al.,* (1998). The occurrence of these phytochemicals in *H. brasiliensis* and in other plants has been attributed to the ability of plants generally to synthesis these substances and incorporate them in their tissues (Wititsuwannakul *et al*., 1998). Similar report was made by Fafioye and Adebisi (2004) when working on the effects of sub-lethal concentrations of *Parcia biglobosa* on haematological parameter of the African catfish *Clarias garienpinus* and Burkill (1985) when reporting on the useful plants of West tropical Africa. Although oxalate was not detected in the rubber latex, some plants species in the family Oxalidaceae are able to synthesize the acid a phenomenon which *H. brasiliensis* is devoid of (Subramanian, 1995), as the latex lack the enzyme oxalase (Khalid, 1982). The n-hexane extract component of *H. brasiliensis at* 430 nm ranged between 2.192 - 2.196 ± 0.004 mg/l. Organic extract of *H. brasiliensis* has been reported to fall within the range of between 0.4 -5.0 mg/l (Shivkar and Kumar, 2003; Wititsuwannakul *et al*., 1998; Subramainam, 1995). The quantity of the extract having been linked with the age of plant from which the latex for the extract is obtained (Tanaka and Sakdapipanica, 2001; Subramaniam, 1995).

Conclusion

The latex obtained from *Hevea brasiliensis* have been found to contained ammonia and phyto-chemical constituents such as Phytic acid, Hydrocyanic acid and Alkalliods. It is very imperative that ecologically friendly methods should be put in place in discharging the effluent by rubber companies producing latex as raw material for the production of various rubber-related products. These products are extensively used by pharmaceutical companies, paint companies, beverages companies, breweries and even as laboratory wares, foot wares and related wares.

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