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### From plant to drug: Galenic preformulation based on Phyllanthus amarus

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### Abstract

Conventional treatments for liver damage exist but not accessible to low middle-income countries (LMIC) populations. Due to this limitation, traditional healers use plants that they consider effective for the treatment of local diseases. One of the most popular herbal plants is *Phyllanthus amarus* used as a decoction, but without insurance of safety, efficacy and quality. The objective of this work was to develop a drug based on this plant according to the requirements of the international pharmacopoeia. Thus, tests of hepatoprotective activity, acute toxicity and galenic preformulation were carried out. Phytochemical screening showed the presence of polyphenols, flavonoids, cardiac glycosides, tannins and coumarins. The aqueous extract of the plant was administered to rats at different doses (100, 200 and 400) mg/kg/PC for seven days and paracetamol was administered on the fifth day at a dose of 2000 mg/kg/PC to induce disease; sylimarine was used as the reference standard drug. The results showed good protection of the liver at the dose of 400 mg/kg/PC. As for acute toxicity, the dose of 2000 mg/kg/PC of extract showed no signs of harm. An Improved Traditional Medicine (ITM) was preformulated as a capsule from the aqueous extract of this plant. The pharmaceutics controls carried out on these capsules made it possible to conclude that this Improved Traditional Medicine conformed to the production standards of the International Pharmacopoeia.

Keywords: Phyllanthus amarus, metabolites, hepatoprotection, preformulation, quality control

Headlines: Introduction, Experimental section, Results, Discussion, Conclusions, Know-How Transfer



# Introduction

Nowadays, many conventional medicines are put on the market as preventive or curative against many diseases. Unfortunately, they are not accessible to poor population because of their high costs and remoteness of the area. For health needs, it becomes imperative for them to resort to traditional medicine with local plants. Nevertheless, rush towards medicinal plants has unknown side effect which would be good to be careful. However, the data on the toxicity, dosage, efficacy and quality of traditional medicines are insufficient to ensure their safe use (Endougou, 2016). Improved Traditional medicines (ITM) developed on the basis of these four criteria would be an alternative for the management of patients with various disorders. Viral hepatitis is a major public health problem worldwide (Albano E. 2008; WHO 2008; Vaccine, 2012). They are the major cause of morbidity and death with 57% of cirrhosis and 78% of primary liver cancer, resulting from hepatitis B or C (Perz, 2006). The medicinal plants used in traditional medicine against liver damage are diverse (Blumberg BS, 1998; Wang BE, 2000; Jian Ping Liu et al. 2018). Related studies are scattered, most often limited to their activity or toxicity. To make MTAs, they must meet the conditions of safety (established acute toxicity), efficacy (established effective dose) and quality (guaranteed pharmaceutical form) in accordance with the standards of the international pharmacopoeia. In this study an improved traditional medicine (ITM) is developed and labeled "Hepathophyl capsules" based on Phyllanthus amarus for its hepatoprotective activity.

# **Experimental section**

# Material

# Plant material

The whole plant *Phyllanthus amarus* (picture 1), harvested in Akok in the Center region (Yaoundé); it is cleaned, cut and dried (humidity below 5%) in the open air protected from light. It is pulverized using a grinder. The botanical sample was identified



Picture 1. Phyllanthus amarus

by Ngansop T. Eric of the national herbarium of Cameroon as *Phyllanthus amarus* Shun & Thonn (phyllanthaceae) in comparison with the sample *of Phyllanthus amarus* Shum & Thonn from the collector Geerling C. n  $^{\circ}$  4784 of the specimen from the collection of herbarium n $^{\circ}$  36532 HNC (YA).

# Zoological material

The animals used were white albino rats of the Wistar strain (Rattus norvegicus) aged between two and three months. The average weight is 95 g for the hepatoprotective activity test and respectively 51.5  $\pm$  0.42 g and 51.6  $\pm$  4.80 g for males and females used in the assessment of acute toxicity.

The experiments were carried out in the pharmacotoxicology laboratory of the Faculty of Medicine and Biomedical Sciences (FMBS) of the University of Yaoundé 1, Cameroon. Ethical approval was obtained from the institutional review board of this faculty. Authorization was obtained from the administration of the FMBS, to work in the said laboratory of the faculty. The OECD Guidelines 423 for the use of animals in preclinical studies were observed. The experimental protocol and the maintenance of the experimental animals was done in accordance with the regulations of the OEDD guide, the EU parliament directives on the protection of animals used for scientific purposes, since in Cameroon, the ethics committee focuses only on clinical studies.

# Reference standard drug

Sylimarine (tablet) purchased in pharmacies is the reference standard drug used in this study against liver disorders

# Materials, devices and reagents

In addition to conventional laboratory equipment, the specific equipment used is as follows: dissection kit, operating table, force-feeding probe, sieve (2) with 200 and 100  $\mu$  mesh. The most representative devices are: CSP192 brand double boiler, clay adams brand centrifuge, sergent welch brand magnetic stirrer, UV spectrophotometer, oven, precision balances (sartoruis, explorer ohaus / 0.001g, mettler PE22 / 0.0001kg), a warning commercial grinder, an erweka AR 400 mixer, a erweka wet granulator. The reagents used are various, among which: Chronolab assay kit: ASAT,

ALAT, HDL, Fortress assay kit for protein, 10% ammonia, copper sulfate, lead acetate, Stiasny reagent, chloroform, 10% sodium acetate , nitric acid, hydrochloric acid, sulfuric acid, 2M sodium hydroxide, 0.9% sodium chloride, 10% potassium chloride, 10% formalin, 5% ferric chloride, 95% ethanol, glacial acetic acid.

### Methods

# Preparation of the aqueous extract of Phyllanthus amarus

500 g of *Phyllanthus amarus* powder are introduced into 5000 mL of distilled water and then boiled for 30 minutes. The cooled decoction is filtered on whatman no. 2 paper and evaporated in an oven at 55°C.

The extraction yield is calculated using equation below

$$yield = \frac{\text{mass of extract}}{\text{mass of plant powder}} \times 100$$
(1)

# Phytochemical screening of the aqueous extract of Phyllanthus amarus

of plant Periods Observations Parameters extracts was D1 (during Behavioral Appearance: Grooming, piloerection... done the two aspects Neuromuscular: hypoactivity, according to hours hypotonia, hypertonia, convulsions, myoclonus the protocol following Behavior when opening the cage: vocalization, of Odebeyi induction) aggression or abnormal flight, hyperactivity or stupor and J2 Respiratory Breathing: Abnormal ventilation, normal breathing, Sofowora criteria painful breathing and modified amplitude (1978). Weight: Animals J1-J14 Specifically, J1, J3, J5, j7, Weight: wet faeces, food consumed, water consumed Zootechnical the J9, J11, j13 criteria metabolites Table 1. Zootechnical parameters observed were:

The research of the different secondary metabolites

polyphenols, alkaloids, flavonoids, anthocyanins, tannins, saponosides, mucilages, steroids, resins, cardiac glycosides, quinones, betacyanes, coumarins, oxalates.

# Assessment of acute toxicity

The slightly modified OECD Guideline 420 (OECD, 2001a,b) which calls for a single dose of

extract and observation of animals for 14 days has been used.

### Preparation of animals

Twenty rats (ten males and ten females) were used for the acute toxicity study. The test group of five males and five females received plant extracts at a dose of 2000 mg/kg/PC according to their weight and the control group of 5 males and 5 females received distilled water.

### Preparation and administration of the plant extract

Animals fasted twelve hours before the study (no food and water). They were then weighed just before the administration of the extract. On the fourteenth day, the animals fasted again for 4 hours before being sacrificed by cutting the aortic vein. The organs were immediately isolated and weighed. The formula used to calculate the volume to be administered is:

$$Va (mL) = \left[\frac{Dose\left(\frac{mg}{Kg}\right) \times body mass(Kg)}{Concentration\left(\frac{mg}{L}\right)}\right] (2)$$

After administration of the extract, the behavior of the animals is observed and certain parameters were noted (Table 1).

### Method for assessing hepatoprotective activity

Rat acclimatization

The rats were distributed five per translucent polyethylene cage depending on the extract to be administered. It is lined with litter lined with white wood shavings. The cages were cleaned and the litter changed once every three days. Drinking water from the tap was supplied in ad libitum bottles.

#### **Preparation of animals**

For this study, six experimental groups of five rats each were formed

Group I: healthy control group receiving only distilled water;

Group II: negative control group receiving water and the disease inducer

Group III: positive control group which receives distilled water and the reference standard drug before the disease inducer

Groups IV, V and VI: test groups which receive distilled water, plant extract at different doses and the disease inducer

All animals were fed throughout the study period.

### **Preparation of solutions**

### Aqueous extract of Phyllanthus amarus

A solution of *Phyllanthus amarus* at a concentration of 1 mg/mL was prepared and then administered to the rats of the test groups according to their body weights at the doses of 100 mg /kg, 200 mg/kg and 400 mg/kg.

### Solution of the reference standard drug (Sylimarine)

A sylimarine solution at a concentration of 1 mg/mL was prepared and then administered at a dose of 10 mg/Kg.

### Disease inducer solution

1mg/mL Paracetamol was administered at a dose of 2 g/kg/PC on the  $5^{\text{th}}$  day to induce hepatotoxicity in rats.

# Evaluation of the hepatoprotective activity of the extract of Phyllanthus amarus

The search for hepatoprotective activity lasted 7 days. The solutions were administered by the buccalesophageal route from the first day of the study until the seventh day using an orogastric feeding tube. The healthy control group received water for the seven days and the other groups received paracetamol on the fifth day; the positive control group, in addition to water and paracetamol, received sylimarine (10 mg/kg/ PC); the test groups received the plant extract at different doses (100 mg/kg, 200 mg/kg, 400 mg/kg) throughout the study period. On the 7th day, the animals were sacrificed and blood samples were taken from EDTA vials for analysis of markers of liver function (ASAT, ALAT), total protein and glutathione.

# Determination of alanine aminotransferase (chronolab kit)

Alanine aminotransferase (ALAT) or glutamate pyruvate transaminase (GPT) catalyzes the reversible transfer of an amino group from alanine to alphaketoglutaric acid, which forms glutamate and pyruvate. The pyruvate produced is reduced to lactate by lactate dehydrogenase (LDH) and NADH.

Alanine + 
$$\alpha$$
-ketoglutarate  $\xrightarrow{ALAT}$  Glutamate + Pyruvate  
Pyruvate + NADH + H<sup>+</sup>  $\xrightarrow{LDH}$  Lactate + NAD<sup>+</sup>

The rate of disappearance of the coenzyme NADH, H<sup>+</sup> measured by photometry, is proportional to the catalytic concentration of the ALAT present in the sample. The ALAT concentration is calculated according to equation below:

$$[ALAT](U/L) = \frac{\Delta A}{t(min)} \times 1745$$
(3)

Assay of aspartate aminotransferase (chronolab kit)

Aspartate aminotransferase (ASAT), formerly known as glutamate oxaloacetate (GOT), catalyzes the reversible transfer of an amine group from aspartate to  $\alpha$ -ketoglutaric forming glutamate and oxaloacetate. The oxaloacetate produced is reduced to malate by malate dehydrogenase (MDH) and NADH:

Aspartate + 
$$\alpha$$
-ketoglutaric ASAT  
Glutamate + Oxaloacetate  
Oxaloacetate + NADH + H<sup>+</sup> MDH  
Malate + NAD<sup>+</sup>

. . . . .

The rate of disappearance of the coenzyme NADH, H<sup>+</sup> measured by photometry, is proportional to the catalytic concentration of ASAT present in the sample. The concentration of ASAT is calculated according to equation (4)

[ASAT] (U/L) = 
$$\frac{\Delta A}{t(min)} \times 1745$$
 (4)

### Glutathione dosage

The assay for reduced glutathione was carried out according to the method described by Ellman (1959): The 2,2-dithio-5,5'-dibenzoic acid (DTNB) reacts with the SH groups of glutathione forming a yellow colored complex which absorbs at 412 nm.

$$[glutathion] = A. V_t / \varepsilon. L. Vi. m_{org}$$
(5)

A: optical density at 412 nm,  $\varepsilon$ : molar extension coefficient, L: length of the tank, V<sub>t</sub>: total volume of homogenate, Vi: volume used for the assay, morg: mass of the organ

### Determination of total proteins (chronolab kit)

The assay was done according to the Biuret method ( $H_2N$ -CO-NH-CO-NH<sub>2</sub>, i.e. two molecules of urea); this reagent gives with the cupric ions Cu<sup>2+</sup> and in an alkaline medium a complex which strongly absorbs

at 540 nm (purple blue coloring). The peptide bond established between two amino acids is also capable, under the same experimental conditions, of forming a complex with cupric ions. This complex is assayed by absorption spectrophotometry at 540 nm. The amount of protein is calculated according to the formula below:

$$C = \frac{Ab \text{ sample}}{Ab \text{ standard}} \times C \text{ standard} \quad (6)$$

C: total protein concentration (mg/mL); Ab = absorbance

### Statistical analysis

The results were expressed in terms of mean  $\pm$  standard deviation. The comparison between the groups was performed using the variance analysis test (ANOVA) followed by the post hoc test of Turkey's Kramer using GraphPad Instat version 5.0 software.

### Galenic preformulation (capsules)

Galenic pharmacy (pharmaceutical formulation) is defined as the science of transforming active substances into drugs using appropriate excipients, suitable technology, packaging or even an administration device while ensuring their control according to the regulations in force. The preformulation of a drug requires several steps, namely: choice of pharmaceutical form, choice of excipients, choice of control methods (Wehrlé P, 2007).

# Step 1- Determination of the human unit dose (HUD) from the animal dose

From the animal dose, human dose was determined using the formula of Shannon Reagan-Shaw et al. (2007):

нир –	Animal dosa	x <u>Animal kinetic factor</u>	(7)
1100 -	Allillai uose	Human kinetic factor(adult)	()

Drug	Made of Phyllanthus amarus			
	Gelatin	4		
	parahydroxybenz	0.05		
Raw materials (%)	oate			
	talc	1		
	Corn starch	20.54		
	Phyllanthus	79.46		
	extract			

Table 2. Choice of percentages of raw materials

Animal dose is obtained after experimentation and depends on the nature of the extract.

### Step 2- Preparation of the starch extract of the plants

A certain quantity of plant powder (3 kg) is weighed and then introduced into a pot containing 50 liters of water which was boiled for 30 minutes. Double filtration is then applied to the mixture with two mesh screens 200 microns and 100 microns. Decantation was done after 4 hours and 2 hours; The solution obtained (filtrate) is distributed into previously weighed and numbered trays, each containing 50 g of starch; The starch extract is evaporated in an oven at 70°C for 48 hours; the trays are reweighed and the powder (extract + starch) recovered then crushed in order to obtain fine particles.

### Step 3- Granulation

The main stages of wet granulation are summarized as depicted in figure 1.



Figure 1. Main stages of wet granulation

 $94.95 \rightarrow 1000$  1%  $\rightarrow$  K K = 10.532

The quantity of each excipient to be used is obtained by applying the formula below:

Weight per 1000g = % x K (8)

Knowing that for 10,000 g of active ingredients (AI) you need 1.8 liter of water, we deduct the amount of water to add for 1000g of PA (180 mL).

# Development of the internal wet granulation phase

The starch extract is introduced into a mixer (ERWEKA AR 400); In a first pot 150 ml of water is heated to 55°C; The gelatin, previously weighed, is introduced therein and using a spatula the whole is mixed until a homogeneous phase is obtained. In a second pot, 30 ml of water is boiled and the parahydroxy benzoate added. After making the mixture homogeneous, the contents of the second pot are poured into the first and the mixture produced using the spatula; the contents of the first pot are then poured into the running mixer; the wetted mass of the mixer is collected using a ladle and then passed to the granulator on which a large mesh is previously mounted; the wet granules are then collected on a tray and dried in an oven at 65° C until obtaining a humidity level of 2%; The dried dough is reintroduced into the wet

Average weight	Authorized	LCS	LCI	LSS	LSI		
observed white	deviations						
	ELA						
Mmc < 300 mg	10%	Mmc x 1.1	Mmc x 0.9	Mmc x 1. 20	Mmc x 0.8		
Mmc >300 mg	7.5%	Mmc x 1.075	Mmc x 0.925	Mmc x 1.15	Mmc x 0.85		

Table 3. Method for calculating limit values applicable to the mass uniformity test

# Pellet manufacturing

The choice of the proportions of the raw materials (active ingredients and excipients) is a prerequisite. Table 2 gives the percentages of raw materials used for the manufacture of the improved traditional medicine. The mixing of the reagents leads to a dilution of the extract and the starch. To determine the mass of each constituent of the mixture, the quantity of starch extract to be used is fixed in advance. If we consider 1000 g of starch extract, knowing the percentages of excipients to take (gelatin 4%, parahydroxy benzoate 0.05%, talc 1% and mixture starch + extract 94.95%), we determine the value of the proportionality constant K as follows:

granulator for the external phase of the granulation.

# External phase of wet granulation

Talc is added to the granules and mixing is carried out for 15 minutes; the mixture is then recovered for the 4th step.

# Step 4: Capsule filling tests

10 empty capsules taken at random are weighed; Then 20 capsules are filled and 10 taken at random are weighed. The average mass, mG, of the granules in a capsule is then known; The mass of active ingredient mPA (plant extract) per capsule is calculated from the following formula:

mPA = % of plant extract desired in the formulation x mG (9)

The number of capsules per dose, NG, is obtained as

Secondary metabolites	Test or reagents used	Results
Alkaloids	Wagner	-
	Hager	-
	Valse Mayer	-
Saponosides	Foam index	+
Mucilages	Ethanol 96°	-
Coumarins	HNO <sub>3</sub>	+
Polyphenols	FeCl <sub>3</sub> 10%	+
	lead acetate	+
Flavonoids	NaOH / H <sub>2</sub> SO <sub>4</sub>	+
Tannins	CuSO <sub>4</sub>	+
	gallics	+
	catechics	+
Anthocyanins	H <sub>2</sub> SO <sub>4</sub> , NH <sub>4</sub> OH	-
cardiac glycosides	Keller-Killani essay	+
Steroids	Acetic anhydride/	-
	$H_2SO_4$	
Betacyanes	NaOH 2N	-
Oxalates	Ethanoic acid	-
Quinones	FeCl <sub>3</sub> /HCl	-

Table 4: Classes of secondary metabolites identified in the aqueous extract of Phyllanthus amarus

- Absence of metabolites + presence of metabolites

follows:

NG = daily therapeutic dose / mass of the extract in a capsule = D/mPA (10)

For the final filling, we most often have to make dilutions. The dilution coefficient, k' is calculated as follows:

k '= % of extract in the capsule / desired % of the extract in the capsule (11)

### Step 5- Pharmaceuticals controls of capsules

Mass uniformity

10 capsules, taken at random, are weighed using a precision balance and the average mass, Mmc, calculated. Table III summarizes the method for calculating the limit values applicable to the mass uniformity test., The upper and lower control limits (LCS and LCI) and the upper and lower monitoring limits (LSS and LSI) are the parameters of controls

sought, which depend on the authorized limit deviation (ELA) which in turn depends on the average mass (Mmc) of the capsules (Table 3)

### **Disintegration test**

6 capsules are introduced into the disintegrator equipped with a water bath which is brought to 37°C. We then note the time at the end of which all the capsules are completely disintegrated. The experiment is repeated three times. The mean disintegration time, the upper control limit and the lower control limit are calculated (Table 3).

# Results

### **Phytochemical screening**

The extraction yield of *Phyllanthus amarus* is 12.74%. Qualitative phytochemical screening of the extracts revealed the presence of polyphenols, tannins, flavonoids, saponosides, cardiac glycosides, coumarins, and the absence of alkaloids, anthocyanins, oxalate, quinones and steroids (Table 4).

# Evaluation of the zootechnical parameters of acute toxicity

No deaths were noted during the study; Therefore the LD50 would be between 2000 and 5000 mg/kg/PC. A non-significant increase (p > 0.05) in food intake and water intake was observed in male and female test groups compared to the control groups (Table 5). The same is true for body weight gain. Furthermore, no significant difference (p > 0.05) in organ weights was observed (Table 6).

In order to know if the extracts could preventively protect against liver damage, an experiment was conducted with the extract of *Phyllanthus amarus* at the end of which the zootechnical parameters of the paracetamol-induced hepatotoxicity were re-evaluated (Table 7).







Figure 4. Effect of Phyllanthus amarus on the endogenous activity of the organism

No significant difference in organ weights between male and female test and control groups was observed (p > 0.05); however, taking paracetamol at 2000 mg/kg resulted in decreased weight gain in animals, which decrease was restored by the plant extract in a dose-

dependent manner (FIG. 2). Sylimarine (reference standard drug, positive control) has virtually no effect on weight loss induced by paracetamol

### Effect of *Phyllanthus amarus* on paracetamolinduced hepatotoxicity

### Assessment of biochemical parameters of liver damage

The liver damage is marked by the abnormal secretion of the ALAT and ASAT biochemical markers. Now is the question of how well the extract of Phyllanthus amarus protects the liver by keeping these markers normal when taken as a preventative. The results obtained on the ALAT parameter indicate a nonsignificant decrease in the activity of the reference drug and of the plant in a dose-dependent manner (p > 0.05). The results (FIG. 3) are 7.90  $\pm$  4.07 IU/L for the group having received no treatment,  $27.77 \pm 13.77$  IU/L for the negative control group,  $10.22 \pm 2$ , 09 IU/L for the positive control group having received Sylimarine as a pretreatment at a dose of 10 mg /kg, i.e. 63.20% protection. The plant extract led to the following ALT values:  $13.32 \pm 7.83$  IU/L at a dose of 100 mg/Kg, ie a protection percentage of 52.03%;  $11.48 \pm 10.29$  IU/L at a dose of 200 mg/kg, i.e. a protection percentage of 58.66%; 6.89  $\pm$  2.96 IU/L at a dose of 400 mg/kg, i.e. a protection percentage of 75.19%. As for the ASAT marker, the values are  $7.56 \pm 0.86$  IU/L for the group of untreated animals,  $28.50 \pm 3.38$  IU/L for the negative control group;  $8.58 \pm 5.98$  IU/L for the positive control group, i.e. a protection percentage of 69.89%. The plant extract administered at a dose of 100 mg/kg led to an ASAT value of  $7.71 \pm 1.26$  IU/L, ie a protection percentage of 72.95%; At the 200 mg dose, a value of  $2.04 \pm 0.25$  IU/L was obtained, i.e. a protection percentage of 92.84%, and at the 400 mg / kg dose the value of  $2.07 \pm 0.22$  IU/L was obtained, i.e. a protection percentage of 92.74%. It should be noted that the extract of Phyllanthus, administered at a dose of 400 mg/kg of weight leads to a lower concentration of ALT and ASSAT than that of untreated rats, hence its advantage in preventing liver damage.

### Analysis of the endogenous activity of the organism

This is to assess the ability of *Phyllanthus* to restore glutathione. Figure 4 above shows a restoration of glutathione activity by the plant; the values obtained are  $16.00 \pm 11.28 \,\mu mol/$ 

mL for the healthy group; 6.75  $\pm$  3.59  $\mu mol/mL$  for the negative control group; 16.00  $\pm$  1.94

 $\mu$ mol/mL for the group which received sylimarine at a dose of 10 mg/kg; 21.60  $\pm$  3.38  $\mu$ mol/ mL for the plant at a dose of 100 mg/kg; 10.80  $\pm$  7.73  $\mu$ mol/mL for the

plant at the dose of 200 mg/kg and 18.20  $\pm$  1.56  $\mu mol/mL$  for the plant at the dose of 400 mg/kg.

The protein assay gave for the group of healthy animals  $(18.91 \pm 3.11)$  g/L, for the negative control group  $(22.71 \pm 2.47)$  g /L, for the positive control group  $(24, 31 \pm 2.22)$  g/L, for the plant at the dose of 100 mg /kg  $(32.56 \pm 2.58)$  g/L, for the plant at the dose of 200 mg/kg  $(21.18 \pm 2.57)$  g/L and for the plant at a dose of 400 mg/kg  $(21.56 \pm 2.35)$  g/L. It appears that endogenous proteins are not affected by hepatotoxicity.

### Capsule pre-formulation test

#### **Raw material**

The aqueous extraction of Phyllanthus in the presence of starch led, after drying, to a powder with a mass of 1,216.8 g (Table 8) consisting of 20.54% starch and 79.46% Phyllanthus (active ingredient). This powder represents the main raw material for the formulation.

# Composition of ingredients for preformulation (active ingredients and excipients)

A medicine consists of active ingredient and excipients. The addition of excipients to the active ingredient (PA) slightly modifies its composition as shown in Table 9

After wet granulation, the amount of granular powder obtained is 989.3 g.

#### Human Unit Dose

The amount of extract that should be taken per day is 3.892 g for an adult weighing 60 kg.

#### • Filling test

The filling test carried out on 20 capsules led to the results shown in Table 10.

The following calculations are the applications of the pharmaceutical claims declined in the methodology part of this study:

Amount of extract in a capsule 
$$=\frac{75.45}{100} \times 0.299g = 0.225$$
 g

Number of capsules per day =  $\frac{3.892 g}{0.225 g}$  = 17,297 capsules = 18 capsules

At 18 capsules there is an overdose, hence the need to dilute in order to respect the daily dose. After dilution the amount of active ingredient in a capsule is calculated as follows:  $\frac{3.892 g}{18} = 0.216 g$  of *Phyllanthus amarus* 

the percentage of extract in a capsule becomes:

$$\frac{0,216 \ x \ 75,45}{0,225} = 72,43 \ \%$$
  
If 75,45%  $\rightarrow$  72.43  
 $1\% \rightarrow k'$   
 $k' = 0,96$ 

By applying the dilution factor k' to the previous formulation (% x k'), the new composition of the mixture is obtained after dilution (Table 11).

The proper proportion of water is 4.17%. The corresponding mass is calculated as follows:

95.83 % 
$$\rightarrow$$
 weight of powder collected.  
mass of diluent added =  $\frac{powder weight}{95.83\%} \times 4.17$   
mass of diluent =  $\frac{989.3}{95.83} \times 4.17$  =

43.049 *g* 

Table 12 gives the final composition of the capsules

#### Pharmaceuticals controls of the capsules produced

### **Disintegration test**

The average disintegration time (15.33 min, Table 13) is between the LCI (13.80 min) and the LCS (16.86 min). Therefore, the capsules produced are conformable from the point of view of disintegration.

Parameters	food intake (g)	food intake	Weight change				
		(mL)	1 <sup>st</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day		
Groups							
Female control	$144.17 \pm 67.45$	$98.70 \pm 33.74$	$98.20 \pm 29.07$	$121.00 \pm 39.18$	$144.40 \pm 46.09$		
Female test	$159.17 \pm 4.83$	$104.92 \pm 17.97$	$102.20 \pm 28.22$	$132.00 \pm 37.82$	$149.60 \pm 44.39$		
male control	$152.25 \pm 49.02$	$133.45 \pm 16.20$	$103.80 \pm 42.17$	$138.40 \pm 54.48$	$164.20 \pm 62.63$		
male test	$175.67 \pm 42.81$	$139.75 \pm 14.10$	$100.60 \pm 31.74$	$144.40 \pm 43.19$	$171.60 \pm 50.43$		

Table 5. Effects of the aqueous extract of Phyllanthus amarus on water and food intake

Groups	Male control	Male Test	Female control	Female test
brain	$1.48 \pm 0.09$	$1.67\pm0.44$	$1.63 \pm 0.44$	$1.61 \pm 0.43$
Lungs	$1.39 \pm 0.21$	$1.23 \pm 0.37$	$1.16 \pm 0.36$	$1.25 \pm 0.35$
liver	$8.03 \pm 1.40$	$8.14 \pm 2.22$	$5.73 \pm 2.22$	$6.33 \pm 2.10$
spleen	$0.63\pm0.07$	$0.56\pm0.16$	$0.57 \pm 0.17$	$0.64 \pm 0.17$
left kidney	$0.60 \pm 0.05$	$0.74\pm0.23$	$0.56 \pm 0.21$	$0.60 \pm 0.19$
right kidney	$0.61 \pm 0.05$	$0.73 \pm 0.23$	$0.58 \pm 0.20$	$0.60 \pm 0.19$
left testicle / ovary	$0.81\pm0.24$	$0.85\pm0.25$	$0.06 \pm 0.03$	$0.06 \pm 0.03$
right testicle / ovary	$0.78 \pm 0.27$	$0.85\pm0.25$	$0.05 \pm 0.02$	$0.06 \pm 0.03$
Heart	$0.57 \pm 0.05$	$0.66\pm0.18$	$0.54 \pm 0.17$	$0.56 \pm 0.17$

Table 6. Effects of the aqueous extract of Phyllanthus amarus on the weight of the organs (g) after administration at a dose of 2000 mg/kg/ PC

Organs	Healthy	negative	Sylimarine	100 mg/Kg	200 mg/Kg	400 mg/Kg
	animals	control				
Brain	$0.93\pm0.23$	$0.79\pm0.04$	$0.86\pm0.03$	$0.89 \pm 0.09$	$0.94\pm0.11$	$0.88\pm0.09$
Lungs	$0.66 \pm 0.22$	$0.51\pm0.07$	$0.57\pm0.12$	$0.58 \pm 0.14$	$0.62 \pm 0.21$	$0.57\pm0.10$
Liver	$3.65 \pm 0.80$	$3.33 \pm 0.60$	$3.07 \pm 0.26$	$3.01 \pm 0.33$	$3.10 \pm 0.53$	$2.95 \pm 0.25$
spleen	$0.56 \pm 0.19$	$0.29 \pm 0.04$	$0.35 \pm 0.16$	$0.26 \pm 0.09$	$0.27\pm0.08$	$0.30\pm0.05$
left kidney	$0.27\pm0.08$	$0.26 \pm 0.03$	$0.29 \pm 0.03$	$0.27 \pm 0.02$	$0.28\pm0.02$	$0.25 \pm 0.01$
right kidney	$0.30\pm0.06$	$0.26 \pm 0.03$	$0.27\pm0.02$	$0.26 \pm 0.02$	$0.28\pm0.01$	$0.24 \pm 0.01$
Heart	$0.33 \pm 0.14$	$0.24 \pm 0.01$	$0.25 \pm 0.03$	$0.24 \pm 0.03$	$0.25 \pm 0.02$	$0.22 \pm 0.02$

Table 7. Effects of the aqueous extract of Phyllanthus amarus on organ weights after induction of hepatotoxicity

NT1	Tt.	A	<b>D</b> :	1	W	6		Incredients		Dereentees	Dereentees offer
Number	Empty	Amount	F1	nai	weight (g) o			ingredients		Percentage	Percentage after
of trays	weight	of starch	weig	,ht (g)	extract +					before dilution	dilution (%)
	(g)	(g)			starch					(%)	
P1	1672.4	50	191	16.5	244.1			Phyllanthu	s amarus	75.45	72.301
P2	1659.1	50	188	88.4	229.3			extract			
P3	1679.1	50	191	13.3	234.2			Starch		19.50	18.689
P4	1668.3	50	190	00.4	232.1			Gelatin		4	3.833
P5	1675.2	50	195	52.3	277.1			Parahydrox	xybenzoate	0.05	0.048
Total		250			1216.8			Talc		1	0.958
Table 8.	Main raw r	naterial for g	galenica	al form	ulation (starch	L		Totals		100	95.83
extract)					Table 11. (	Composition	of the granules aft	er dilution			
active ing	redients	Percentag	e 🚺	Weight	(g) per 1000	Ro	ole				
(PA) and	excipients	(%)	l g	g of star	rch extract						
	1	Ì	(	(% x k)							
starch		19.50	2	205.403		dis	integrating				
Phyllanth	us extract	75.45	7	794.611		her	patoprotective	e (PA)			
gelatin		4	4	42.128		bin	nder		Ingredients Percer		Percentage
parahydro	oxybenzoate	0.05		0 527		ant	timicrobial pr	eservative			(%)
talc	onjoenzoute	1		10 532		flo	w agent	eservative	Phyllanth	us amarus extract	72,301
			- 1	141.552	-1	110	Starch		Starch		18,689
Table 9. Percentage of raw materials and their roles in the caps			suie	;		Gelatin		3,833			
Mass of 10 full Mass of 10 empty Mass of powder in		A	Average mass of the Parahydr		Parahydro	oxybenzoate	0,048				
capsules	(g)	capsules (g)	1.12	caps	ules (g)	c	contents of a capsule (g) Talc			0,958	
3.739		0.749		2.990	)	0	.299	1 (8)	Diluent		4,17
Table 10 Cancule filling test						Table 12.	Final capsule form	ula			

### Mass uniformity test

The average observed mass (Mmc) of a capsule is 398.9 mg (Table XIV). The upper and lower control limits are 428.8 and 368.9 mg respectively; the upper and lower monitoring limits of 458.7 and 339.1 mg. The Mmc being within the required intervals, the capsules produced are conform from the point of view of mass uniformity.

# Discussion

# Phytochemical screening

In Phyllanthus amarus extract, saponosides, tannins, polyphenols, flavonoids, cardiac glycosides and coumarins have been highlighted. Polyphenols, metabolites of interest, have been found in abundance, which would justify its use in traditional medicine. These results corroborate those of Adiko et al. (2013) who found the same compounds in Phyllanthus muelleranus (Euphorbiaceae), plant of the same family harvested in Ivory Coast. In addition to these compounds, some authors have highlighted the presence of alkaloids, sterols and polyterpenes in the ethanoic extract of Pyllanthus amarus (Eka et al., 2013; Nguessan et al., 2009). This difference is related to the chemical composition of the soil and the extraction solvent.

# Acute toxicity

Acute toxicity of aqueous extracts of the whole plant of Phyllanthus amarus administrated to rats has been assessed according to OECD Guideline 420.

One of the major criteria in assessing the toxic effect of substance is the evolution of body weight. Gaining or losing excessive weight is a clinical sign of appreciation for the general condition. The extract resulted in an increasing weight change in animals of both sexes compared to the control groups. This growth is very well observed and significant from day 6. The same observation is made in terms of water and food intake which did not decrease during the study.

Data obtained from the acute oral toxicity test in animals can be used to meet needs for hazard classification through the LD50, and for the evaluation of risks to human health and / or the environment. After sacrificing the animals, the isolated vital organs showed no sign of apparent toxicity after administration of the extract at a dose of 2000 mg /kg. Therefore, the LD50 would be

once a day for seven days has shown a significant protective effect on the hepatic damage caused by paracetamol. At the dose of 400 mg/kg/PC, remarkable effect was observed compared to the other doses. This extract prevents the elevation of serum ALAT and ASAT levels in a dose-dependent manner. Another proof of the restoration of liver functions is the increase

T1 (min)	T2 (min)	T3 (min)				
16	15	16				
Average time $(T1 + T2 + T3)/3$						
		Tm = 15.33 min				
Upper control limit	LCS = Tm x 1.1	LCS = 16.86 min				
Lower control limit	$LCI = Tm \ge 0.9$	LCI = 13.80 min				
Conclusion	Disintegration time compliant					
Table 13 Cansule disintegration test results						

Criterion	Formulas	Results				
Average mass	$Mmc = (m_1 + m_2 + m_3 + \dots + m_{10}) / 10$	Mmc = 398.9 mg				
observed (mg)		C				
Authorized limit	According to the choice tables	ELA = 7.5 %				
deviation						
Upper control	LCS = Mmc x 1.075	LCS = 428.8				
limit						
Lower control	$LCI = Mmc \ge 0.925$	LCI = 368.9				
limit						
Upper monitoring	$LSS = Mmc \ge 1.15$	LSS = 458.7				
limit						
Lower monitoring	$LSI = Mmc \ge 0.85$	LSI = 339.1				
limit						
Table 14 Results of the cansule mass uniformity test						

glutathione in and protein levels observed after administration of the extract. These results are consistent with those of Vidhya and Bai (2009) who highlighted the hepatoprotective effect of the extract of **Phyllanthus** emblica administered at the dose of 100 to 200 mg/kg/PC to rats victims of а paracetamol-induced liver damage. Similarly, Syed et al (2012)showed protective activity of the liver in response to hepatic the attack induced by carbon tetrachloride, this with the methanoic extract

between 2000 and 5000 mg /kg/PC. This LD50 value made it possible to classify the toxicity of this extract in category 5 of the globally harmonized classification system for chemical substances, a category characterizing slightly toxic substances. These results corroborate those of TUO et al. (2005). Furthermore, Awuku et al. (2011) found the same results with the aqueous extract of the leaves of Phyllanthus niruri.

### Assessment of hepatoprotective activity

Experiments were carried out to assess the hepatoprotective effect of the aqueous extract of Phyllanthus amarus Schum and Thonn against liver damage induced by paracetamol. Administration of a high dose of paracetamol (2 g/kg/ PC) to normal rats caused liver damage, causing the level of ALAT and ASAT in blood to increase. Furthermore, the administration of the aqueous extract of Phyllanthus amarus at different doses (100, 200 and 400) mg/kg/PC

of Phyllanthus amarus at a dose of 250 mg/kg/PC.

# Galenic preformulation

Drug in the form of capsule (HEPATOPHYL) has been developed. This liver protective drug is made from an aqueous extract of the whole plant of Phyllanthus amarus, following the rules of good preparation practice. The granules were filled in capsules N<sup>0</sup> 1. The intake of the drug is 06 capsules three times a day. The pharmaceuticals checks (mass uniformity and disintegration time) which were carried out made it possible to conclude that the capsules produced complied with the standards of the international pharmacopoeia.

# Conclusions

The objective of this study was to convert a medicinal plant (Phyllanthus amarus) very popular in traditional medicine into a hepatoprotective drug which respects the clauses of the international pharmacopoeia: active ingredients, excipients (disintegrant, stabilizer, binder, flow agent), efficacy, safety, pharmaceutical form and quality were established. Phytochemical screening carried out on plant extracts showed the presence of a certain number of bioactive compounds derived from the secondary metabolism of this plant, which would justify its use in traditional medicine. These are polyphenols, flavonoids, saponosides, cardiac glycosides, coumarins, tannins. The hepatoprotective effect of Phyllanthus amarus is mainly due to its richness in polyphenol, especially lignans (phyllanthine, hypophyllanthine) which act as an antioxidant by trapping free radicals. The best activity was observed at a dose of 400 mg/kg in wistar strain rats intoxicated with paracetamol, dose which was used for the preformulation of the capsules. Aqueous extract of the whole plant of Phyllanthus amarus showed no signs of toxicity at the dose of 2000 mg/kg body weight. This preclinical study led to the development of an improved traditional medicine with a good safety and quality profile and potentially effective for the treatment of liver diseases.

# **Know-How Transfer**

This study shows that it is possible to have a traditional medicine that meets the standards of the international pharmacopoeia. Thus, the following technical platforms are essential:

- Extraction platform;
- Pharmaceutical platform for putting the drug in a durable form (capsule in our case);
- Quality control laboratory or at least devices to carry out the mass uniformity test and the disintegration test.

So, the mastery of the galenical formulation is a prerequisite. The same applies to the conduct of toxicity and activity tests. The main elements of the pharmaceutical platform are: mixer, granulator, capsule filler and compactor which make it possible to have the drug in the form of capsules or tablets.

Fortunately, Cameroon has a reference laboratory for the quality control of medicines (LANACOME), a research

institute on medicinal plants (IMPM) with a complete pharmaceutical platform within it, more than four public and private faculties of medicine and pharmaceutical sciences, some of which have a well-equipped toxicology laboratory (that of Yaoundé 1 in this case). All these achievements have been used to develop the ITM based on *Phyllanthus amarus*.

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### **Authorship contributions**

KT, Coordinator of research activities, wrote the first draft; TFG, carried out all the experiments and analysis (activity, toxicity, chemical screening, preformulation, quality control); NNE, bench work supervisor (pharmaceutical formulation, drug manufacturing); FC, supervision of work on the bench (animal experimentation: activity and acute toxicity tests)

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### Probability Distribution Modelling of Microwave Radio Refractivity over Selected Locations in Nigeria

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### Abstract

In this study, distribution models were used to model radio refractivity of some stations across the various climatic zones of Nigeria. Eleven years (January 2004 to December 2014) of Meteorological Data from ERA INTERIM Reanalysis data were used to calculate radio refractivity of each location. It was observed that normal distribution among the probability distribution functions, predicts the peak of the distribution curve and gives the best fit for radio refractivity in all the locations. Weibull distribution strength is built from a small sample sizes. The distribution of the mean value of refractivity in the coastal areas was observed to be weak.

**Keywords:** climatic zone, distribution models, era interim, meteorological data, normal distribution, radio refractivity.

Headlines: Introduction, Results and Discussion, Conclusions, Experimental Section, Know-How Transfer

# Introduction

The propagation of electromagnetic waves in the atmosphere is significantly affected by the composition of the atmosphere (Korak, 2003). This upset is due to the fluctuations of atmospheric parameters like temperature, pressure and relative humidity primarily in the troposphere and the troposphere is usually referred to as "the lower" part of the earth. The fluctuation of the atmospheric parameters results in the refractivity variation in the lower atmosphere that causes the refractive index of the air in this layer to vary from one point to the other (Dada, 2019). The refractivity that determines how radio wave propagates in the atmosphere depends on the physical structures of the atmosphere (Olasoji and Kolawole, 2011). The degree of accuracy of atmospheric parameters measurements is usually a function of care exercised by the observer and the sensitivity of the equipment used (Adedokun, 1978) because the calculation of the refractive index is based on a result created by the atmospheric parameters. The refractive index is responsible for bending of the propagation direction of the electromagnetic wave (Guanjun and Shukai, 2000).

Trajectory change of radio wave in the troposphere is as a result of the vertical variations of the refractive index and its gradient (Adediji et al., 2019). Multipath effects occur as a result of large-scale variations in atmospheric radio refractive index, such as different horizontal layers having different refractivity (Grabner and Kvicera, 2009). Refractivity is responsible for various phenomena in the wave propagation such as ducting and scintillation (Grabner and kvicera, 2003), refraction and fading of electromagnetic waves (Babin, 1996), range and elevation errors in radar acquisition (Lowry et al., 2002). In recent times, communication had advanced to the point in which people need to communicate with voice and video at any time (Duraimurugan and Jayarin, 2015).

Computer networking is mainly for data sharing and the ability of the user to communicate. This process is achievable through what is called media transmission which could either be a guided medium (Cable) or unguided medium (wireless). The wireless medium is through the atmosphere, and it makes the networking environment to be neater because less cable is required (Priya and Gurjot, 2016). The consideration of the refractive properties of the lower atmosphere is of great



### **Distribution related to the Central Limit Theorem**

The Theorem stated that sample means and sample sums approach normal distributions as the sample size approaches infinity. The Normal distribution also called the Gaussian distribution is ubiquitous in probability and statistics (Murray and Stephens, 2008). An actual normal distribution curve must conform to specific rules concerning its standard deviation, an expression of the

> amount of which the value of a frequency/functions is being distributed or concentrated. A small value of the standard deviation produces a sharp curve with a narrow peak and steep sides. A tremendous amount of it shows a large curve with less steep sides, and if it approaches zero, the curve becomes narrower while the curve becomes almost flat when the deviation standard becomes arbitrarily large (Ross, 2010).

> The Tlocation-Scale distribution is useful for modeling data distributions with heavier

Fig. 1. Map of Nigeria showing the location of the study areas

importance when planning and designing terrestrial communication systems mainly because of multipath fading and interference due to horizon propagation.

In this regard, the most studies that have worked on the statistical modelling tools is yet to provide the best modelling tools for predicting the maximum distribution value of refractivity. Taking into account the nature of radio propagation situation in some locations in Nigeria (Fig. 1), different probability distributions; Normal

distribution, Tlocation-Scale distributions, roomal distribution, Tlocation-Scale distribution and Weibull distribution were used to model the statistical behavior of the refractivity in Nigeria. The objectives of this study are to know the distribution pattern of radio refractivity of the locations and determine the best probability distribution for the prediction of peak radio refractivity spread in the selected area. tails (more prone to outliers) than the normal distribution. It approaches the Gaussian distribution as the degrees of freedom (v) approaches infinity, and smaller values of v yield the heavier tails (Murray *et al.*, 2001). The Weibull distribution fits well with experimental fading channel measurements, for both indoor and outdoor propagation environments. It is a continuous random variable that is often used to model the time until the failure of a physical system (Montgomery and Runger, 2010).



Fig. 2: Normal probability distribution curve for (a) Ibadan (b) Kano (c) Uyo (d) Jos

# **Results and Discussion**

One station was considered from each of the climatic region under consideration in Nigeria for a typical result that described the main observation of each of the standard probability distribution across the entire climatic region. The frequencies (number of occurrences) of the refractivity were plotted against the refractivity of each location, in this regard refractivity was observed to range between 250 to 400 N-unit across all the stations studied.

Figure 2(a - d) shows the Normal probability distribution of stations under consideration with a shape of symmetrical frequency curve assuming a fact of equidistant observation of the refractivity distribution from the central maximum having the same frequency. The degree of the peak of the refractivity distribution ranges from 100 to 550. The dumbbell across three stations (fig.2a-b, d) which is almost flat top and symmetrical shape (Mesokurtic) indicates the normal



Fig. 3: Tlocation-Scale probability distribution curve for (a) Ibadan (b) Kano (c) Uyo (d) Jos

distribution and arbitrarily large value of the standard deviation. This indication implies a proper spread out of the mean value of refractivity while the dumbbell at Uyo with a symmetrical shape like other stations give a narrow peak and steep sides (Leptokurtic), this observation of Uyo could be linked to the location of the station.

The frequency (number of occurrences) against the refractivity plot of the location (fig. 3a - d) describes the Tlocation-Scale distribution of the stations studied.

Asymmetric frequency curve was observed which approaches the Normal distribution of the locations with the dumbbell shape that skewed to the right (fat tail to the right). This curve defines a positive-departure of the distribution of the refractivity mean values across all the stations. The narrow dumbbell shape at Ibadan and Uyo (fig.3a & c) shows a standard deviation that approaches zero indicating a poorer distribution while at Kano and Jos it is relatively flattened (Mesokurtic) depicting large values of the standard deviation. The implication is a well spread out of the distribution around the norm mean value of refractivity

The Weibull probability distribution (Fig. 4a - d) assume the characteristic of normal distribution dumbbell curve that skewed to the right (fat tail to the right) depicting a progressive departure of the distribution from the refractivity mean values across all the stations. The degree of the peak of the refractivity distribution ranges from 100 to 600. Similarly to Tlocation distribution, the

**Conclusions** 

Refractivity has been modeled using distribution

models for two stations each from the climatic zones in

Nigeria. It was observed that the standard probability

distribution functions used, that is, Weibull, Normal and

Tlocation-Scale distributions are adequate for some

stations and inadequate for others, despite this fact, the Normal distribution function gives the best fit of the

Weibull probability distribution shows a dumbbell shape for Ibadan and Uyo with a narrow peak (Leptokurtic) and steep sides. The observation indicates a small value of the standard deviation, while at Kano and Jos it is relatively flattened (Mesokurtic) depicting large values of the standard deviation implying a well spread out of the distribution around the normal mean value of refractivity.

#### 700 250 Weibull Weibull Distribution curve Distribution curv Refractivity Distribution Refractivity 600 Distribution 200 500 150 400 Frequency Frequency 300 100 200 50 100 200 150 300 320 380 200 250 300 350 400 450 340 360 400 Refractivity Refractivity (a) (b) 700 500 Weibull Distribution curve Weibull Distribution curve Refractivity Distribution 450 Refractivity Distribution 600 400 500 350 300 400 Frequency Frequency 250 300 200 200 150 100 100 50 310 370 150 320 330 340 360 360 380 390 400 200 350 400 250 300 Refractivity Refractivity (c) (d)

Fig. 4: Weibull probability distribution curve for (a) Ibadan (b) Kano (c) Uyo (d) Jos

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distribution curve. The Tlocation-Scale tends to follow the Normal but has a heavier tail to the right. The standard deviation of the distribution at coastal zone is relatively small which is described by a narrow dumbbell shape by all distribution models used, and it implies weak distribution around the standard mean value of the refractivity. This implication could be associated with the seasonal variation in these locations that is characterized by the extensive rainy season and short dry season which is in agreement with (Emmanuel et al., 2013).

In accordance to (Todinov, 2009), the inability of Weibull model for the prediction of the peak of refractivity in this study could be linked to the fact that the Weibull is a mathematical formulation of the weakest-link concept. Also in support of (Danzer, 2006) and (Danzer et al., 2007) that in almost any case the Weibull distribution strength is built from limited/narrow sampling sizes compared to that used in this study (January 2004 to December 2014).

# **Experimental section**

Meteorological Data, temperature, pressure and dew point of Eleven years spanning from January 2004 to December 2014 for twelve locations were downloaded from ERA INTERIM and used to compute the radio refractivity of the locations. The equation 1 was used to convert Dew point to relative humidity by (Lawrence, 2005):

 $RH = 100 - 5(t - t_d)$  (1)

where t is ambient temperature,  $t_d$  is dew point temperature (°C), and RH is relative humidity in percentage.

Water vapour pressure e was determined from the relative humidity and saturated water vapour from (Adediji and Ajewole, 2008):

$$e = H \times \frac{6.1121 \exp(\frac{17.502 t}{t + 240.97})}{100}$$
(2)

The radio refractivity N is computed using (Adediji *et al.*, 2015):

$$N = 77.6 \frac{p}{T} + 3.73 \times 10^5 \frac{e}{T^2}$$
(3)

where: e = water vapor pressure (hPa), p = atmospheric pressure (hPa), T = absolute temperature (K), H = relative humidity (%), and t = temperature (°C).

The standard probability distribution functions used in the work that is Normal, Tlocation and Weibull, scale distributions are as follows:

*Normal Distribution*: The normal distribution is given as (Ross, 2010);

$$f(x) = \frac{1}{\sigma\sqrt{2\pi}} \exp(-\frac{(x-\mu)^2}{2\sigma^2}), \text{ for } \to x \in \mathbb{R}$$

$$\mu = \mu .. \sigma^2 = \sigma^2.$$

$$m(t) = \exp\left(\mu t + \frac{t^2 \sigma^2}{2}\right).$$
(4)

where the parameters  $\mu$  the mean and  $\sigma$  the standard deviation of the distribution are real numbers, being positive with positive square root  $\sigma$ .

Tlocation-Scale Distribution : (Murray et al., 2001):

$$f(x|\mu,\sigma,v) = \frac{\tau(\frac{\nu+1}{2})}{\sigma\sqrt{\nu\pi}\tau(\frac{\nu}{2})} \left[\frac{\nu+(\frac{x-\mu}{\sigma})^2}{\nu}\right]^{-(\frac{\nu+1}{2})}$$
(5)

where *v* is degrees of freedom,  $\tau$  is a gamma function.

Weibull Scale random variables: (Montgomery and Runger, 2010).

$$f(x|a,b) = \frac{\beta}{\delta} \left(\frac{x}{\delta}\right)^{\beta-1} e^{-(x/\delta)^{\beta}} \qquad ; \quad x > 0$$
(6)

where  $\delta$  is the scale parameter stored as a positive scalar value and  $\beta$  is the shape parameter stored as a positive scalar value.

# **Know-How Transfer**

This study has been able to establish the best distribution pattern of radio refractivity and the probability model for the prediction of peak radio refractivity spread in the selected location in Nigeria. Equation 1 - 3 has been employed to convert Dew point to relative humidity, determine water vapor pressure from relative humidity and saturated water vapor, and compute radio refractivity respectively while the standard probability distribution functions used in the work which include the Normal, Tlocation scale and Weibull scale distributions has been fully described in equation 4 - 6 of the study. This work will help radio engineer to be able to know the distribution pattern and the predict peak of radio refractivity spread in the selected location.

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