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## Articles



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# Development of Climate Change Effects-Reducing Information Systems towards Sustaining Medicinal and Aromatic Plants

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***Abstract:*** Economic growth has been inextricably linked with accelerating negative environmental impact. Projections show that the full climate change mitigation potential of biotechnology processes and biobased products ranges from between 1 billion and 2.5 billion tons $CO_2$ equivalent yearly by 2030. MAPs' species are being destroyed at an unprecedented rate and are threatened with extinction by habitat loss, climate change, overexploitation, land conversion, and deforestation. Global projections of MAPs diversity loss show that the largest losses of habitat and diversity will occur in tropical ecosystems (forest, woodland, savannah), accounting for a projected loss of 25,000 to 40,000 MAPs species by 2050. This paper presents the development of a framework for MAPs productivity and sustainability management. It establishes systems to closely monitor the change in MAPs resources and the recovery of habitats from the unprecedented effects of climate change. A logistic growth model was developed. A conservation program based on MAPs' intrinsic rate of increase and carrying capacity parameters was written in C++ programming language. The emerged system ran with varied data to ascertain population growth/harvesting and life span based on flexibility; scalability and modularity; and accuracy would reduce climate effects on MAPs; a framework that presents all the stakeholders and their respective responsibilities in the management of MAPs. The rapid loss of MAPs life has far-reaching consequences, and their loss will adversely affect future drug discovery. The increasing interest of people in MAPs commands a special attention to organize the actors and preserve the MAPs genetic resources against climate change. It is pertinent to improve access to technologies for use in a wider range of MAPs, fostering public dialogue and increasing support for the adoption and use of internationally accepted standards for life cycle analysis together with a range of other incentives designed to reward environmentally sustainable technologies.

***Key words*:** Biotechnology, Climate change, Informatics, Conservation, Medicinal and Aromatic Plants, Sustainability

## Introduction

Medicinal and Aromatic Plants (MAPs) are increasing sought worldwide for various purposes and continue to attract growing interest from farmers, traders, economists, teachers, professionals, health officials and various industries. The MAPs are natural biological resources that have a great potential to synthesize a huge variety of important secondary metabolites, also referred to as natural products, far more than animal and even microorganisms. These natural compounds are used as pharmaceuticals, agrochemicals, and cosmeceuticals. Recently, MAPs also are used as functional herbal food ingredients, nutraceuticals and health products. The supply of the source plants however, is often limited due to diseases, changes in climate, and changes in the development in the growing regions [1]**.** Climate change refers to long-term changes in the earth's average temperature, precipitation and other factors. Climate disruption refers to significant climate change over a short term and long-term harmful effects. Likely effects of climate change include (1) some areas getting colder and others hotter; some drier and some wetter, (2) forest dieback and ecosystem change, and (3) more frequent and devastating forest fires which will add more $CO_2$ to the atmosphere **[2}.** Climate change is a 'threat multiplier' – it increases a range of livelihood threats and vulnerabilities, rather than being an isolated specific risk. The impact of changing climate on long-term trends needs to be better understood **[3].** The sustainable use of natural resources has become an unavoidable necessity from both environment protection and socio economic points of view. Currently, between 4,000 and 10,000 MAPs are on the list of threatened and/or endangered species and this number is expected to rise. These problems could be overcome through MAPs selection and cultivation under agricultural conditions which also could respond to increasing demands in terms of plant security and traceability, socio-economic development, biodiversity conservation and sustainable use of genetic phyto resources as basic inputs for the future [1]**.**

## Degradation of the Natural Environment

Degradation of the natural environment and need for conservation measures are urgent concerns with ever more evidence of human activities despoiling the planet, exacerbated by current climate change predictions **[4].** Environmental degradation, mismanagement of natural resources, and unhealthy consumption patterns and lifestyles impact health. Ensuring environmental sustainability would demand encouragement of community level good practices on sustainable use as well as management of medicinal,

nutritional and cultural resources **[5].** Extinction is a natural phenomenon that is part of the evolutionary cycle of species. Some estimates indicate that endangered species encompass 11% of plants, 4.6% of vertebrates, 24% of mammals and 11% of birds worldwide. Anthropic activities and man's development is a major cause of resource depletion and weakened habitat. Some 15,000 of 50,000 medicinal species are under threat of extinction. Shortages have been reported in China, India, Kenya, Nepal, Tanzania and Uganda. Commercial over-harvesting does the most harm, though pollution; competition from invasive species and habitat destruction all contribute. Commercial collectors generally harvest medicinal plants with little care for sustainability. This can be partly through ignorance, but [happens] mainly because such collection is unorganised and competitive **[6].**

## Renewable Resources and Conservation

Renewable resources are under extreme pressure worldwide despite efforts to design better regulation in terms of economic and/or control instruments and measures of stocks and harvests. One focus of biodiversity economics and management is to establish an economic basis for preservation by pointing out the advantages it procures. Consequently, there is growing interest in assessing the value and benefit of biological diversity **[7].**

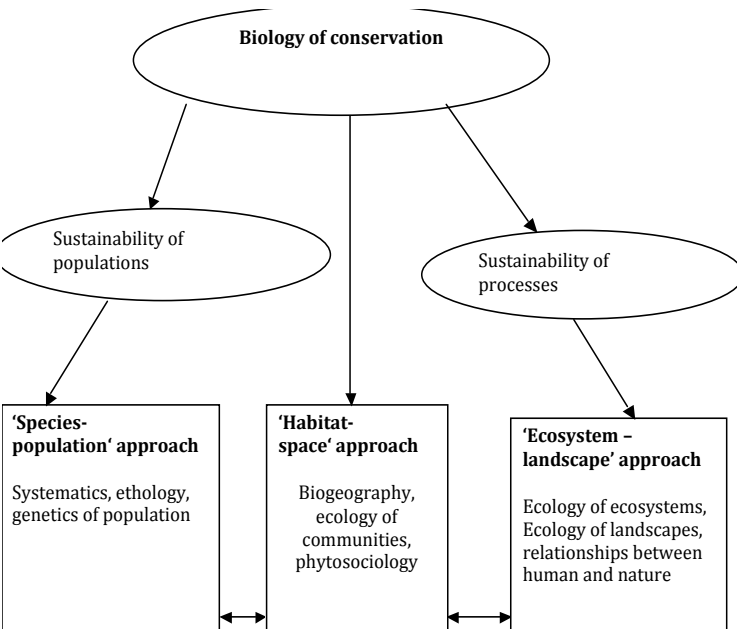


**Fig. 1. Biology of Conservation [9]**

To combat diminishing biodiversity and dwindling traditional knowledge, access to and exchange of information is crucial amongst researchers, scientists, policy makers and indigenous populations. To facilitate the exchange of information between these diverse users, significant information management systems are required. Information technology (IT) has dramatically changed the way scientific research is conducted, giving rise to multidisciplinary fields such as biodiversity informatics. Biodiversity informatics is a new discipline that integrates species level information from diverse domains using the scientific name of the organism as the linking thread **[8].** Biodiversity losses occur due to habitat destruction, over-harvesting, pollution, inappropriate and often accidental introduction of exotic plants and animals, etc. Biodiversity conservation is a paramount concern worldwide to preserve the natural habitats of vulnerable MAPs species and achieve sustainable exploitation in less vulnerable areas. Action should be taken now to conserve the MAPs base of traditional medicine, as well as safeguarding its potential for modem medicines in other parts of the world **[5]**. Human induced development activities are introduced with insufficient attention to their consequences for our living environment, even in cases where environmental assessments have been carried out **(Fig. 1)**. This apparent lack of attention to biodiversity in environmental assessment is rooted in the difficulties we have in adequately addressing biodiversity within the scope, time frame and budget allocated for assessments **[9].**

**Materials and Methods**

Logistic growth model among natural resources sustainable management models reviewed was selected for this work.

**(i)** ***Logistic growth model:*** To ensure populations recruit enough members to both make up for human-caused mortality and maintain a certain "safe" population level, a simple model that describes this density-dependent growth is a θ-logistic model:

$$N_{t+1} = N_t + rN_t[1 - (N_t/K)^{\theta}] \quad (2.1)$$

where $N$ = population size, $t$ = time, $r$ = maximum growth rate (near $N$ = 0), $K$ = carrying capacity (for illustrative purposes set at 10,000), and θ = shaping parameter that controls the level of maximum net growth. With density-dependent growth, the fastest growth rate is near $N$ = 0, and at $K$ the births are equal to the deaths [10]**.**

Density dependence is a central process for exploited species. The logistic model forms the basis of much theory of sustainable use. In principle, the strength and form of density dependence have a crucial influence on the impacts of harvest, and defining the process can therefore be very helpful **(Fig. 2)** in

understanding and predicting outcomes **[11].**

**Fig. 2 (**a) The per capita growth rate (number of new individuals added to the population for each existing individual) as a function of population size; (b) The total number of new individuals added to the population as a function of population size; and (c) Yield as a function of harvesting effort, with the maximum sustainable yield shown.

**(ii)** ***Bioeconomic System:*** This is a modified conceptual model of the dynamics of harvesting for a village of a fixed size exploiting a closed population of a single species. The decisions and impacts of village harvesters are shown in **Fig. 3.** The harvested population has logistic growth [11]**.**

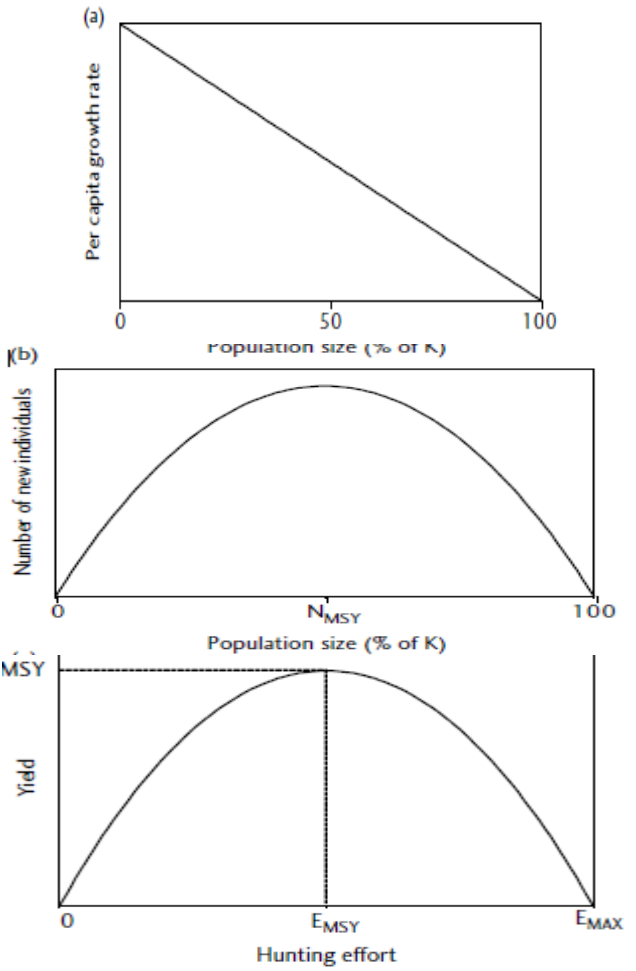


**Fig.2 .The logistic growth model [11]**

**(iii)** ***The Logistic Model*****:** The logistic growth model is the simplest model of density dependent population growth.

$$g(B) = B + rB\,(1 - B/K) \quad (2.2)$$

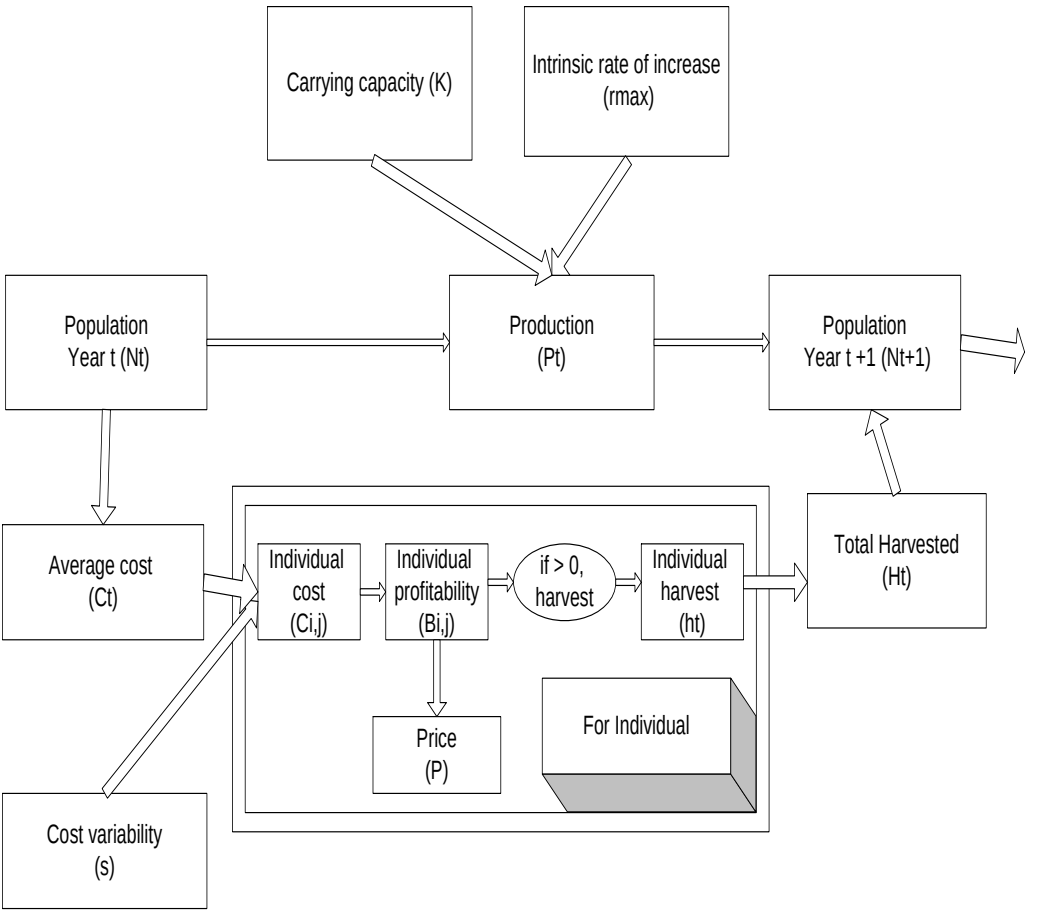


**Fig. 3. Summary of a simple bioeconomic system [11].**

where $B$ stands for the resource biomass and $g : R+ \rightarrow R+$ is taken to satisfy $g(0) = 0$,

$(r = R - 1)$ $r \geq 0$ is the *per capita rate of growth* (for small populations), and $K$ is the *carrying capacity* of the habitat. We shall also use the equivalent form

$$\mathbf{g(B) = (1 + r)B\,(1 - rB/((1 + r)K)} \quad (2.3)$$

Such a logistic model in discrete time can be easily criticized since for biomass $B$ greater than the capacity $K$ the biomass becomes negative, which of course does not make sense.

**(iv)** ***Harvesting*****:** When harvesting activities are included, the model above becomes the *Schaefer model*,

$$\boldsymbol{B(t + 1) = g(B(t) - h(t))\ ,\ 0 \leq h(t) \leq B(t)}\ , \quad (2.4)$$

where $h(t)$ is the harvesting at time $t$. Notice that, in the above sequential model, harvesting takes place at the beginning of the year $t$, hence the constraints $0 \leq h(t) \leq B(t)$ are right above, and regeneration takes place at the end of the year $t$ **[11].**

**Results**

**Fig. 4** presents all the stakeholders and their respective responsibilities in the management of MAPs. The key harvesting activity is affected by changes in business, environment, climate, MAPs population dynamics, and government policy / political scenarios as reflected in **Fig. 4**. This key activity requires strict regulation enforcement consistently in line with enumerated changes. The above are used in ascertaining the sustainability requirements. A conservation program based on plants' intrinsic rate of increase and carrying capacity parameters was written in C++ and run several times with varied values to ascertain the effect of these parameters on harvesting behaviour.

**Conclusion**

Climate change is a growing threat to species. Renewable resources are under extreme pressure worldwide despite efforts to design better regulation in terms of economic and/or control instruments and measures of stocks and harvests. The increasing interest of people in MAPs commands a special attention to organize the actors and preserve the MAPs genetic resources against climate change. It is pertinent to improve access to technologies for use in a wider range of MAPs, fostering public dialogue and increasing support for the adoption and use of internationally accepted standards for life cycle analysis together with a range of other incentives designed to reward environmentally sustainable technologies. Indiscriminate population growth, coupled with urbanization and overharvesting has led to erosion of precious genetic resources. In addition, extensive human intervention has resulted in global climate change, which is taking a heavy toll on natural biodiversity. To combat diminishing biodiversity and dwindling traditional knowledge, access to and exchange of information is crucial amongst researchers, scientists, policy makers and indigenous populations as demonstrated in the framework presented in this work. As Computer Science and Biotechnology communities join forces (bioinformatics and medical informatics disciplines in Health Informatics) to create new technologies for the advancement of medical science and improvement of medical service delivery, this might prove to be promising for enabling people to lead normal, healthy lives.

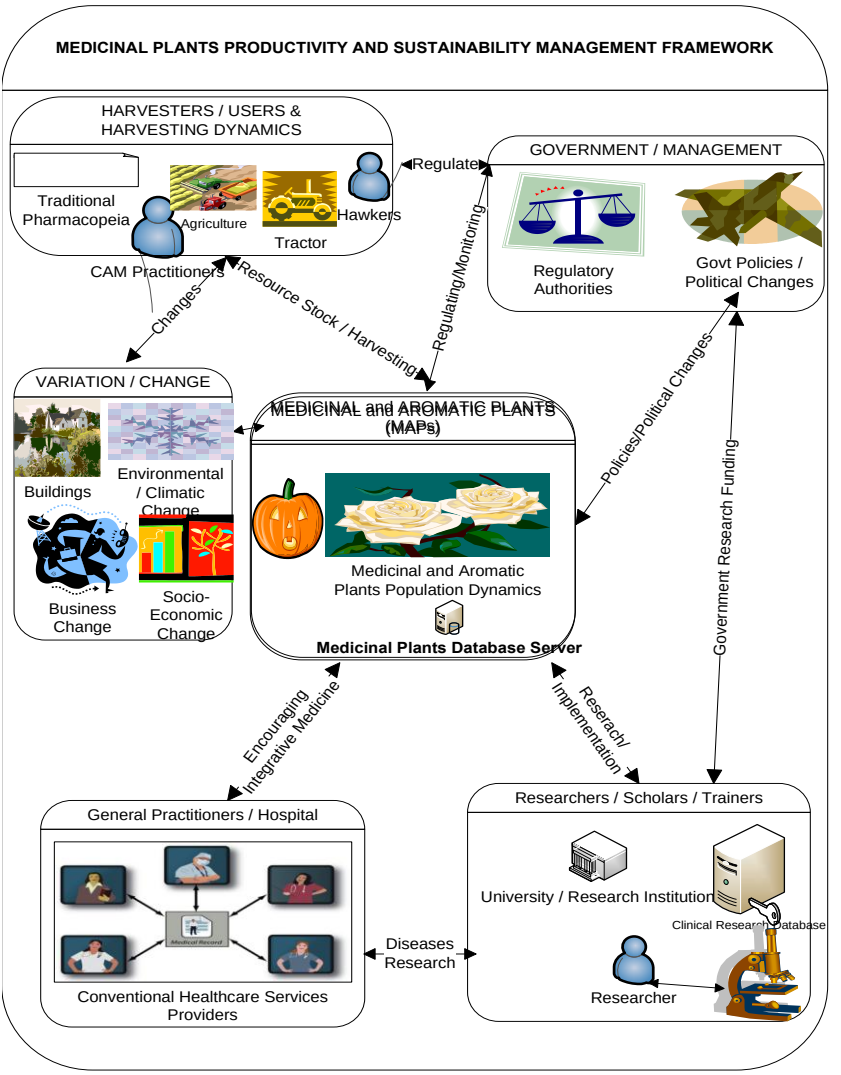


**Fig. 4 Medicinal and Aromatic Plants Sustainability Framework**

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# Herbosomes in the Delivery of Phytotherapeutics and Nutraceuticals: Concepts, Applications and Future Perspective

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***Abstract**:* Recent advances in natural products chemistry and phytomedicine research has been aimed at novel lipid based drug delivery systems. Herbosome technology is one of such systems that incorporate phospholipids into standardized active ingredients of herbal extracts, thus effectively enhancing the bioavailability of water-soluble bioactive constituents of phytomedicines such as flavonoids, phenolics and hydrophilic compounds. These phytoconstituents have been established to exhibit a variety of biological activities that have pharmacological benefits. However, poor absorption of these phytoconstituents limits their bioavailability. The poor absorption is principally due to the failure of these constituents to reach their site of action before being degraded as well as their inability to pass through the small intestine due to their multi ring structures and the lipid nature of the intestinal wall. This review chronicles the recent advances made in herbosome technology, highlighting the concepts, applications and future perspective of herbosome use.

***Key words**:* Herbosomes, Phytomedicine, Lipid based delivery systems, Phytosomes, Phyto-phospholipid complex.

## Introduction

Phytomedicines have been used for the treatment of various ailments since ancient times. In recent years there has been an increase in research output relating to natural products chemistry especially in Nigeria and Africa in general. Various plant materials have been observed to exhibit a variety of biological activity such as antilipidemic activity, hepatoprotective activity, immunomodulatory activity etc. Currently, as many as one-third to approximately one-half of all the drugs available are derived from plants or other natural sources [1].

The drug formulations of traditional systems of medicine like the African, Chinese and Indian systems usually contain crude extracts of different herbs which incorporate in them undesirable and many times, toxic

principles along with the active principles. With the developments in the field of phyto- and analytical chemistry, specific ingredients or a group of similar ingredients from plants are being extracted, isolated and tested for their different therapeutic applications [2]. The bioactive components of these herbs have been identified as mostly flavonoids, tannins, glycosides, phenolics and other hydrophilic molecules. Nevertheless, isolation and purification of individual components from whole herbal extracts often lead to partial or total loss of therapeutic activity. The chemical complexity of the crude or partially purified extract appears to be crucial for the bioavailability of the active constituents; hence standardization of herbal extracts has become imperative [3].

Although having excellent bioactivity *in vitro*, plant extracts often exhibit poor effectiveness *in vivo* or in animal models. The basic reasons for the low bioavailability of herbal extracts are that the bioactive components of these herbs possess multi- ring molecular structures which cannot be absorbed into the blood by simple passive diffusion and the bioactive phytoconstituents are mostly water soluble, hence, their poor lipid solubility limits their ability to pass across lipid biomembranes. This has restricted the use of pharmacologically effective polyphenolic plant actives for treating different disorders. Moreover, when taken orally, bioactive phytoconstituents are destroyed by or lost to the gastric environment or they may be rendered less effective by interaction with other drugs or nutraceuticals [4].

To counter these problems pharmaceutical research has been geared towards the development of novel lipid-based drug delivery systems to improve the bioavailability of drugs while maintaining the therapeutic activity of the drug. One delivery system designed to improve the in vivo solubility and hence bioavailability of poorly soluble herbal drugs involves the incorporation of standardized herbal extracts into phospholipids to form a ―lipid-friendly‖ complex called a herbosome or phytosome [5][6]. In view of their ampiphilic properties, herbosomes are more bioavailable (as demonstrated by pharmacokinetics and activity studies in animals), when applied topically or orally, as compared with simple herbal extracts owing to their enhanced capacity to cross into the blood through the lipid-rich biomembranes [3][6]. The active components of herbal formulation are also well protected from destruction by the gastric environment [7].

Lipid drug delivery systems have advantages over polymer based systems. The advantages include: heightened drug absorption, reduced side effects, controlled drug release and site specific targeting. Also,

most lipid formulations have high stability, high carrier capacity, feasibility of incorporation of both hydrophilic and hydrophobic substances, and feasibility of variable routes of administration [7].

**Properties and Morphology of Herbosomes**

The term ―phyto/herb‖ refers to plants while ―some‖ means cell-like. Herbosomes are lipid compatible molecular complexes. They are lipophilic substances with a clear melting point. They are freely soluble in nonpolar solvents (in which the hydrophilic drug moiety are not), and moderately soluble in fats. When treated with water, herbosomes assume a micellar shape forming liposomal-like structures. The size of the phyto- phospholipid complex molecules customarily varies from 50 nm to about 500 μm.

In herbosomes, phytomolecules are anchored through chemical bonds to the polar head of the phospholipids. Molecular imaging and NMR studies of phyto-phospholipid complexes show one or more phosphatidylcholine molecules effectively embedded with a polyphenol molecule and it has been shown that the main phospholipid-substrate interaction is due to the formation of hydrogen bonds between the polar head of phospholipids (i.e. phosphate and ammonium groups) and the polar functional groups of the substrate [6]. Some researchers had also suggested the formation of Van der Waals forces between the two moieties. It has been proposed that the aqueous head of phosphatidylcholine molecule i.e. the choline binds to the water-soluble compounds and the phosphatidyl portion being lipophilic encloses the choline bound structure. [2].

Pharmacokinetic and pharmacodynamic studies in experimental animals and in human subjects have been used to demonstrate the biological behavior of herbosomes [8]. The increased bioavailability of the phytosomes over the non complexed botanical derivatives has been evaluated from these studies. The studies suggest that most of the drugs having crystal structure when complexed with phospholipids get transformed into molecularly dispersed or amorphous form. The X-ray diffraction pattern of drug and drug–phospholipid physical mixture in almost all the studies has shown crystalline peaks, which disappear in the diffraction pattern of the drug–phospholipid complexes. The improved lipid solubility of the herbosome complexes has been attributed to this change of crystalline state. The herbosome complexes of different drug molecules have been shown to be slightly spherical in shape with a rough surface morphology and good flow properties [2]. Herbosomes are different from liposomes, and are not to be confused with them. In liposomes the active principle is dissolved in the medium in the cavity or internal pocket or floats in the layer membrane, while in

phytosomes the active principle is anchored to the polar head of phospholipids, becoming an integral part of the membrane [3].

**Merits of the Herbosome System**

1. Enhanced Bioavailabilty, Systematic Absorption and Assured Delivery to the Target Site: herbosomes provide an actual means of improving the use of herbal formulations as herbosomes are liable to get transported across the lipophilic environment of cell membranes, into the cell and eventually into the blood. There is improved intestinal lumen absorption of the extracts as well as better permeation of the extracts through the skin for dermal and transdermal delivery.


Fig. 1. Lipid Based Drug Delivery Systems [7].

Due to enhanced bioavailability, the dose of phytoconstituents required to produce a desired effect is reduced. The duration of action is also increased. Herbosomes are more stable in the gastric environment and resist the action of gut microbes. Furthermore, drug entrapment is not a problem with herbosomes, as the complex is biodegradable [3][9].

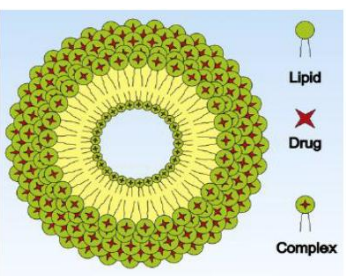


Fig. 2. The Herbosome complex [2]

2. Safety: the herbosome system is passive and non-invasive. The components of the herbosome are non-toxic and non-mutagenic and they have been approved for pharmaceutical and cosmetic use [2][6]. The conventional approaches for preparing the herbosome complexes using harmful organic solvents like tetrahydrofuran and dichloromethane have now been widely replaced by the hydrophilic solvents liethanol, thus increasing their safety and potential for use in clinical applications.

3. Other additional benefits: The process of manufacturing herbosomes is relatively simple, with no complicated technical investments required. Herbosome technology has no large drug development risk since the toxicological profiles of its component are well documented in scientific literature [6].

The phospholipids employed in herbosome formulation, apart from a physiologically compatible pharmacokinetic and toxicological profile, also possess some medicinally significant properties in human. Phospholipids add to the nutritional value of the plant extract like phosphatidylserine, which acts as a brain cell nutrient.

Phospholipids are a good source of phosphatidylcholine and choline, both of which liquefy the fat deposited in the liver as in case of hepatic steatosis or fatty liver. Soya phospholipids have been shown to be hepatoprotective in nature, preventing liver damage by alcohol drugs and other toxins thus providing a synergistic effect for liver protection. They have also been reported to
aid in clearance of serum cholesterol and increase circulating HDL levels in plasma. [2][10][11]. Phospholipids such as phosphatidyl choline show unique compatibility with biological membranes. phosphatidylcholine have shown to be incorporated in the cell membrane to replace cellular phospholipids and thus affect the fluidity of the membrane hence they maintain and nourish the skin.

Furthermore, the low solubility of herbosomes in aqueous media allows for the formulation of stable emulsions and creams.

**The Phospholipid Complexation Technique (Herbosome Formulation)**

Herbosome is a patented process, developed in the year 1989 by Indena, an Italian pharmaceutical and neutraceutical company. They patented the technology as PHYTOSOME®.This phytosome is a cell like structure, which is a combination of soy lecithin with standardized extracts containing polyphenolic compounds, which had vastly improved their absorption and utilization [2]. Herbosomes result from the chemical reaction of a stoichiometric amount of the phospholipid to the standardized herb extract or specific active phytoconstituents and are generally prepared by solvent evaporation or anti solvent precipitation techniques using alcoholic or organic solvents as reaction media. The supercritical fluid technique has also been incorporated into herbosome technology (for preparing puerarin–phospholipid complex) by researchers such as Li and coworkers [12]. In the more frequently used solvent evaporation technique the drug (standardized extract or isolated bioactive phytoconstituents) and the phospholipids are placed in the same flask containing a suitable solvent system. The reaction is carried out at suitable fixed temperature for a fixed duration of time to get maximum possible yield and drug entrapment [2]. The optimum ratio of phospholipid to drug is 1:1 although different molar ratios ranging from 0.5:1 to 3:1 have also been employed with success [13][14]. The herbosome complex thus formed can be isolated by precipitation with an aliphatic hydrocarbon or lyophilization or spray drying [15].

The common stages for the preparation of herbosomes are shown in Fig. 3. Usually, Aprotic solvents like acetone, methylene chloride, ethyl acetate, dioxane etc. are used as reaction media for formulating herbosomes, however they have been largely replaced by protic solvents

like ethanol. Other Solvents such as tetrahydrofuran, dichloromethane and n-hexane have also been used by researchers [13][16][17]. Most of the recent works have been carried out using absolute ethanol as the reaction medium.

The common criterion for selection of phospholipids for herbosome formulation was the ratio of phosphatidyl group present in them .The most commonly used phospholipids are those derived from soya bean containing higher proportions (that is about 76%) of phosphatidylcholine with a high content of polyunsaturated fatty acids like linoleic acid about 70%, linolenic acid and oleic acid. The phospholipids of soya bean have been the phospholipid of choice because of the higher content of phosphatidylcholine in them offers compatibility and similarity with the mammalian plasma membrane [2]. Soy lecithin, phosphatidylserine, and 1,2-distearoyl-sn-glycero-3-phosphocholine have also been used.

**Evaluation and Characterization of Herbosomes**

Factors such as size, membrane permeability, the amount and purity of preparatory materials, the percentage of entrapped phytochemicals and chemical composition, determine how a herbosome would behave in a biological system. A variety of techniques have been employed for the study and characterization of herbosomes.

Transmission Electron Microscopy and Scanning Electron Microscopy have been used to visualize the herbosome after formation to assess its size and shape. The formation of the phyto-phospholipid complex can be confirmed by FTIR spectroscopy, X-ray diffraction, NMR and Molecular imaging techniques while the drug content of the of the complex can be quantified used HPLC. Other techniques used include Dynamic light scattering (DLS) coupled with a computerized inspection system and Photon correlation spectroscopy, to determine the particle size and Zeta potential of the complex and Ultracentrifugation to determine the entrapment efficiency of an extract by a herbosome.

**Applications of Herbosomes**

Herbosomes formulations in solutions, emulsions, creams, lotions, gels etc., have gained importance in various fields like the pharmaceutical, veterinary, cosmetic and nutraceutical fields.

Companies involved in production and marketing of herbosomal products include, Indena in Milan, Italy; Jamieson Natural Sources in Ontario, Canada; Thorne Research in Dover, England and Natural Factors in Canada.

The herbosome process has been applied to many popular herbal extracts including Gingko bilboa, grape seed, hawthorn, olive fruits and leaves, green tea, ginseng, kushenin, marsupsin and curcumin [7]. These phytosomes are significantly more bioavailable and

hence therapeutically more effective than the standardized extracts or their conventional forms and are useful in various disorders.

Maiti *et al.* [18], have demonstrated the improvement in pharmacokinetic profile of curcumin on carbon tetrachloride-induced acute liver damage in rats by preparing its complexation with phospholipids. The antioxidant activity of the herbosome was significantly higher than that of pure Curcumin at all dose levels tested [2][6][18]

Maiti *et al.* [19], also demonstrated the improvement in pharmacokinetic profile of naringenin herbosome. The developed naringenin herbosome exhibited better antioxidant activity than the free compound with a prolonged duration of action. [6][19].

Yanyu *et al*. have evaluated the bioavailability of silybin–phospholipid complex against silybin-N- methylglucamine. The phospholipid complex showed prolonged plasma therapeutic level and increased bioavailability [20].

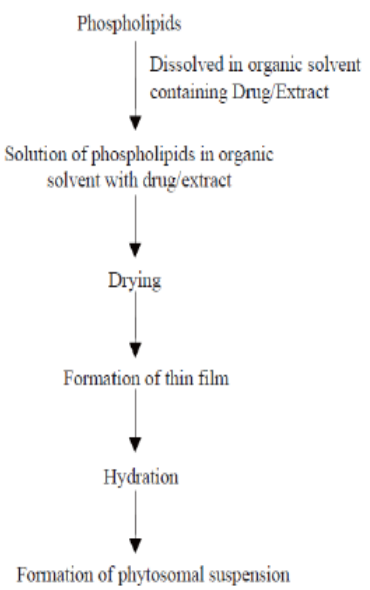


Fig. 3. Common stages for preparation of Herbosomes [6].

Chen *et al*. [21], have demonstrated the pharmacokinetic profile of quercetin,kaempferol and isorhamnetin present in *Ginkgo biloba* extract after oraladministration in rats by formulating its phospholipid complex. The results demonstrated an immense increase in bioavailability of the extract in its phospholipid complexed form [2][21].

Other therapeutically efficient phytosome complexes from different plant extracts/ active compounds developed in recent years are summarized in Table 1 along with the improvement in pharmacodynamic and pharmacokinetic profiles of the crude drug and their clinical utility.

**Herbosome Technology in Nigeria**

One of the most prevalent fields of research in Nigeria is ethnopharmacology and Natural Products Chemistry. There is a wealth of literature and research findings on the pharmacology of a wide variety of medicinal and lesser known plants found in Nigeria. Bioactive phytoconstituents are being discovered yearly, not only in new medicinal plants but in those that have already been investigated. Bioactive phytochemicals that have been identified include: flavonoids, glycosides, terpenes, lectins, alkaloids, tannins and saponins. Crude extracts of plant materials as

well as isolated bioactive derivatives have been studied and observed to have such exploitable biological properties including antihyperlipidemic, allelopathic, antisickling and hepatoprotective effect.

Still, in order for these plant products to be useful clinically, it is necessary to have appropriate formulations and delivery systems, which provide optimum delivery of the active ingredients. Unfortunately, in spite of the wealth of scientific data and literature available on the medical significance of a vast number of Nigerian plants, there is little or no data or research carried on ideal delivery systems for these herbal products. There is no data available on the use of herbosomes for herbal drug formulations in Nigeria, in spite of the recorded improvement in pharmacodynamic and pharmacokinetic profiles of herbal drugs and isolated bioactive components when complexed with phospholipids. Hence, plant extracts are still being administered in extremely large doses and the possibility exists that some herbal plants which have great clinical significance been judged ineffective due to their low bioavailability.

**Future Perspectives and Conclusion**

Herbosomes improve the *in vivo* bioavailability of herbal drugs, which in spite of positive *in vitro* results fail to deliver a similar response in vivo. The hydrophilic constituents of plants like flavonoid and others polyphenolic constituents have immense therapeutic potential but because of their inability to cross lipoidal barriers their application in treatment of several disorders and disease conditions. The incorporation of these phytoconstituents with dietary phospholipids has successfully solved this issue and has offered a preparation of herbal drugs with sufficient lipid penetrability, higher concentration and sustained therapeutic levels in plasma with a slower rate of elimination.

TABLE I. LIST OF PHYTO-PHOSPHOLIPID COMPLEXES PREPARED USING PLANT ACTIVE COMPOUNDS AND EXTRACTS ALONG WITH THE ADVANCEMENT IN THEIR PHARMACOLOGICAL PROFILE [2].

| Phytoconstituent | Source | Method of preparation | Improvements in Pharmacological Profile | | Biological Activity |
|---|---|---|---|---|---|
| | | | Pharmacokinetics | Pharmacodynamics | |
| Evodiamine | Plants of Tetradium family | Solvent evaporation | Enhanced solubility in water, relative bioavailability of complex increased to 218.82% compared to evodiamine | Expected improvement in biological activity | Stimulant and lipid lowering |
| Gallic acid | Amla, grapes | Solvent evaporation | Apparent solubility drug increased in water from 10.86 to 18.12 and from 6.63 to 11.66 in n-octanol. | Improved free radical scavenging activity | Anti-oxidant |
| Emodin | Rhubarb | Solvent evaporation | The water and n-octanol solubility of emodin was improved from 2.25 to 9.97 and from53.45 to 77.62 µg/ml, respectively | Expected improvement in biological activity | Anti-oxidant, anti-cancer |
| Curcumin | *Curcuma longa* | Anti-solvent precipitation | Micellar solubility in water, Cmax increased from 258.64 to 803.86 | Increased therapeutic efficacy | Hepatoprotective |
| Embelin | *Embelia ribes* | Solvent evaporation | Solubility in n-octanol increased from 4 µg/ml (pure embelin) to 38 µg/ml (phytosome) and from1 µg/ml to 40 µg/ml in water | Expected improvement in biological activity | Anti-tumor, anti-inflammatory and anti-diabetic |
| Glycyrrhizic Acid | *Glycyrrhiza glabra* | Solvent evaporation | Cmax of phospholipid complex 2.14 times higher than free glycyrrhizic acid, the AUC of GL-PLC found 1.74 times higher than that of free GL | Improved anti-inflammatory action | Anti-inflammatory |
| Paeonia emodi root extract | *Paeonia emodi* | Solvent evaporation | – | Enhanced antianxiety, antioxidant and Antidepressant actions | Antianxiety, antioxidant antidepressant |
| Ellagic acid | *Quercus alba* and others | Solvent evaporation | Improved Cmax of 0.21 µg/ml to 0.54 µg/mL with increase in duration of action in rats | Improved hepatoprotective action | Hepatoprotective, anti-oxidant |
| Salvianolic acid | *Salvia miltiorrhiza* | Solvent evaporation | Improved water and lipid solubility | Expected improvement in biological response | Antioxidative agent and free radical scavenger |
| Quercetin | Different fruits and vegetables | Precipitation | – | Enhanced therapeutic efficacy to almost 2 times the extract | Hepatoprotective |

To further justify herbosomes as a productive novel lipid-based drug delivery system, the scope of the research needs to be broadened to solve the issues of the preparation technique, complex stability after preparation, mechanism of absorption and actual clinical advantage of these drug delivery systems. The solvent evaporation technique, which is the frequently used technique for formulating herbosomes, involves a number of processing steps, which are time consuming. Furthermore, the quality of the end product in terms of particle size, morphology and hygroscopicity many times depends upon the method adopted for drying of the residue, which has not been optimized in any of the studies. To counter these problems, techniques such as the supercritical fluid technique, which has emerged as an effective tool for preparing particle sizes ranging from 5 to 2000nm and which has been utilized for improving the solubility profile of poor soluble drug molecules can be incorporated to overcome the drawbacks of conventional methods as the particle size and its distribution can be more precisely controlled at very mild temperature conditions. The uniformity in particle size further improves the systemic bioavailability [2]. The CO2 supercritical fluid is

nonhazardous and provides stable inert conditions for processing of sensitive drug molecules. Hence, further research work for formulation optimization using the supercritical fluid technique and its impact on in vivo parameters of herbal drugs need to be carried out.

The yield of the herbosome complexes obtained from various studies varied significantly ranging from about 25% to more than 90%. These variations have been attributed to different formulation factors like drug to phospholipid ratio, temperature and duration of processing. These aspects of the formulation have to be optimized in future research works to get the formulation of best quality. Furthermore, statistical tools can be used for optimizing the molar ratios of drug candidates with phospholipids, along with the temperature and other variables to get maximum entrapment efficiency and a superior drug release profile [2].

Emphasis has been given on the characterization and evaluation of pharmacokinetic parameters of phyto-phospholipid complexes without going in to the clinical aspects of prepared formulations. More exhaustive studies establishing correlation between improvement of in vivo and in vitro pharmacokinetic parameters with the pharmacological efficacy of drug molecules in their phospholipid complexed forms are required to fill this gap and to correlate the improvement in bioavailability with clinical efficacy [2].

TABLE II. THERAPEUTIC APPLICATIONS OF DIFFERENT PHYTOSOMES WITH THEIR DOSE [6].

| Phytosomes | Phytoconstituent (complexed with Phosphatidyl Choline) | Daily dosage | Indication |
|---|---|---|---|
| Leucoselect® Phytosome | Procyanidolic oligomers (PCOs) from grape seeds | 50–100 mg | Systemic antioxidant. |
| Greenselect® Phytosome | Epigallocatechin 3-O-gallate from *Camelia sinensis* (Green tea) | 50–100 mg | Systemic antioxidant. |
| Ginkgoselect® Phytosome | 24 % ginkgo flavono glycosides from *Ginkgo biloba* | 120 mg | Protects brain and vascular lining |
| Silybin phytosome<br>Siliphos™ milk thistle phytosome | Silybin from silymarin (milk thistle) | 120 mg | Additional antioxidant protection for skin and liver. |
| Hawthorn phytosome | Flavonoids | 100 mg | Used in heart disease |
| *Panax ginseng* Phytosome | 37.5% ginsenosides from roots of *Panax ginseng* | 150 mg | As a Food Product |
| Mirtoselect® Phytosome | Anthocyanosides from an extract of Bilberry | — | Improves capillary tone, reduce abnormal blood vessel permeability & are potent antioxidants. They hold great potential for the management of retinal blood vessel problems and venous insufficiency. |
| Sabalselect® Phytosome | An extract of saw palmetto berries through supercritical $CO_2$ extraction | — | Prostate health. |
| Polinacea™ phytosome | Echinacosides and a unique high-molecular weight Polysaccharide from *Echinacea angustifolia* | — | It enhances immune function in response to a toxic challenge. |
| Oleaselect™ Phytosome | Polyphenols from olive oil | — | As potent antioxidants, inhibit harmful oxidation of LDL cholesterol, and also have anti-inflammatory activity. |

The stability of the herbosomal complexes are another area of which needs more attention and exploration. The data in support of the stability of the phyto-phospholipid complexes on storage are insufficient in terms of their market utility and survival. Such preparations are at risk of aggregate formation and chemical degradation on storage [2]. Preparations with more than 90% purity of phospholipids are considered to be more susceptible for oxidative changes, which may be a decisive factor in terms of stability of the final product. Zeta potential plays a key role in determining stability of solid dispersions and inter-particle interactions and so is temperature related hygroscopicity of the formulations [2]. Experimental work has revealed strong moisture absorbing potential of the phytophospholipid complexes when compared with pure drug and the formulation has also exhibited to become more viscous when kept in free air [14]. These parameters have remained more or less untouched in most of the studies and require more emphasis in the future to establish and improve the stability of the phyto-phospholipid complexes.

The exact mechanism of absorption of the drug– phospholipid complexes from the gastrointestinal tract should be an area of focus in future research. In conclusion, in order to be clinically useful, the vast number of plant products being isolated and studied in countries such as Nigeria, need to have appropriate formulations and optimum drug delivery systems for the polar bioactive ingredients. Herbosome technology aids to explore maximum potential of these polar phytoconstituents. The formulation technology is simple and can be easily upgraded to a commercial scale and the components of the herbosome are relatively safe. Hence, several plant extracts which have been reported to possess various pharmacological and health promoting properties but which show poor bioavailability can be standardized and formulated into herbosomes for further systematic studies and clinical application.

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# Microbiological and Biochemical Indicators for Anthropogenically Polluted Soils of the City Mednogorsk, Russia

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***Abstract:*** This study is a bio-indicative evaluation of anthropogenically-polluted soils of the city Mednogorsk in orenburg region, Russia. This work evaluated – the total number of heterotrophic microorganisms, the number of iron- and manganese-oxidizing bacteria in the polluted soil samples, the activity of soil enzymes (dehydrogenase, catalase, invertase), and also the magnetic susceptibility of these soils ($K_{mag}$) – an index which shows the concentration of iron (Fe) in soil. 10 samples were analysed which showed the highest coefficient of magnetism ($K_{mag}$ > 3) and also a reduced content in heterotrophic microorganisms compared to the control soil samples with $K_{mag}$ <1, which indicates the inhibitory effect of heavy metals on soil bacteria. It was discovered that soil samples with extremely high significance of magnetic susceptibility possessed high amount of iron-oxidizing bacteria in their soil microbial community. Also, based on the sensitivity to metallic pollution, the studied enzymes formed a decreasing order: dehydrogenase>invertase>catalase. This study reveals the possible use of these indicators as diagnostic tools for monitoring soils polluted with heavy metals.

***Key words*:** Heavy metals, Coefficient of magnetism, iron- and manganese oxidizing bacteria, Heterotrophic microorganisms, Dehydrogenase, Catalase, Invertase.

## Introduction

As a result of anthropogenic pollution, significant amount of different xenobiotics are released into the environment among which the most dangerous are heavy metals (HM) [1]. Heavy metals accumulating in soils reduce its biological potential: changes the number, species composition, biomass and productivity of soil microorganisms, represses the activity of soils enzymes, leads to the proliferation of phyto-pathogenic microorganisms and inhibits the growth of plants [2]. Soil contamination by HM need to be strictly controlled, since these toxicants can have long and dangerous impacts on living

organisms. As essential components of any ecological community, soil microorganisms can serve as indicators of changes in the state of the environment. The index of the fermentative activity of soils provides information of the biochemical processes, which occur in soil, and also provides information of the state of the microbial community during cases of anthropogenic disturbances [3,4].

This study is a bio-indicative evaluation of anthropogenically-polluted soils of the city Mednogorsk. The work evaluated: the total number of heterotrophic microorganisms, the number of iron- and manganese-oxidizing bacteria in soil samples, the activity of soil enzymes (dehydrogenase, catalase, invertase), and also the magnetic susceptibility of soils ($K_{mag}$) – an index which shows the concentration of iron (Fe) in soil.

The Objects for this research were soil samples obtained from a copper - sulphuric plant in the city Mednogorsk located at the region called Orenburg (*Fig.1*), which is among the five most difficult cities to live in based on environmental and sanitary living conditions in Russia and the major pollutants are copper, iron, manganese and sulfur compounds.

**Fig. 1. Copper-Sulphuric plant in the city Mednogorsk.**

## Materials and Methods

From the 70 samples obtained from the city Mednogorsk, 10 samples, which were characterized by an extremely high level of $K_{mag}$ (>3) were selected for microbiological analysis. And 3 samples (No К1, К2 и К3) with low levels of $K_{mag}$ (<1) served as control samples.

An estimation of the total number of heterotrophic microorganisms was carried out using a 10-fold serial dilution and subsequently plating dilutions $10^{-3}$, $10^{-4}$, $10^{-5}$ on nutrient

agar while observing conventional bacteriological methods [6]. Total number of iron- and manganese-oxidizing bacteria, was carried out by plating dilutions $10^{-1}$ , $10^{-2}$ , $10^{-3}$, $10^{-4}$ on selective media having the following composition, g/l: $(NH_4)_2SO_4$ – 0.5; $NaNO_3$ – 0.5; $K_2HPO_4$ – 0.5; $MgSO_4 \times 7H_2O$ – 0.5, citric acid – 10, glucose – 2, peptone – 1, agar – 20. To determine the iron-oxidizing bacterial content in the soil we added to the medium: 5.9 g/l of $FeSO_4 \times 7H_2O$; and for manganese-oxidizing bacteria – 4.72 g/l of $MnSO_4 \times 5H_2O$. Given that the number of neutrophilic bacteria was being analyzed, the pH of the media before sterilization was adjusted to 7.0 by titrating with 30% aqueous NaOH.

The plates were incubated at temperature (28±2oC) and counts were recorded from duplicate plates after 2-3 days for total heterotrophic bacteria and 5-7 days for iron- and manganese-oxidizing bacteria. The surfaces of the selective media showed characteristic colonies whose growth was accompanied by the accumulation of yellow-orange oxides of iron or brownish oxides of manganese.

Dehydrogenase activity in the soil was determined calorimetrically based on substrate recovery, and the substrate used was a 2.3.5-triphenyl-tetrazoliumchloride, which accepting mobilized hydrogen dehydrogenase is converted to 2.3.5-triphenylformazan having a red color [7]. Catalase activity in the soil was measured by titration method of R.S. Katznelson and V.V. Yershov [7], based on the measurement of the rate of decay of hydrogen peroxide when reacting with the soil and by the number of undecomposed peroxide determined by permanganometric titration. Invertase activity in soil was determined by the method of F.H. Khaziyev, Y.M. Agafarovoy and A.E. Gulko. 5% of sugar solution, incubation time – 3 hours incubation temperature – 30°C, reducing sugars were detected in the filtrate by using a 0.2% alkaline solution of ferricyanide, their contents were calculated based on the standard scale prepared for glucose [7].

All data on the number of microorganisms and enzyme activity of soil were calculated for air-dry samples.

**Results and Discussion**

In soil samples with high levels of magnetic susceptibility total number of heterotrophic microorganisms averaged from 18.4 to $71.5 \times 10^5$ CFU $g^{-1}$ of soil. A low content of heterotrophic microorganisms was observed in sample No 1 and 6, these samples were characterized by a maximum significance of magnetic susceptibility. It was observed that sample (No 2) was characterized by high numbers of heterotrophic microorganisms, and this may be associated with another type of soil pollution, for example, a high organic content. In control samples

of soil total number of heterotrophic microorganisms varied from 1.9 to $34.6\times10^5$ CFU $g^{-1}$ of soil (Table 1).

The number of cultured iron-oxidizing bacteria in soils investigated was at an average of 0.8 to 32.0×105 CFU g-1 of soil. Some samples had higher values as compared with other samples (No 3, 4, 9 and 10). The soil sample No 2 was observed to have the highest number of iron-oxidizing bacteria, as well as heterotrophic bacteria. In the control soil samples, the content of iron- oxidizing

bacteria was low, accounting for 0.6 to $4.8\times10^5$ CFU $g^{-1}$ of soil (Table 1). This is consistent with a number of published data on the decrease in the number of prokaryotic microorganisms in different soil types under the influence of pollution with heavy metals [5]. Manganese- oxidizing bacteria content in soils compared with iron bacteria was much less in the two samples (No 1 and 10) – less than 100 CFU $g^{-1}$ of soil and in four samples it ranged from 0.90 to $2.30\times10^4$ CFU $g^{-1}$ of soil. Sample No 2 was characterized by very high amount of manganese-oxidizing bacteria ($14.20\times10^5$ CFU $g^{-1}$ of soil). In the control soil samples, the content of the manganese-oxidizing bacteria was 0.13 to $2.40\times10^5$ CFU $g^{-1}$ of soil (Table 1).

**Table 1. Researched Indices of Soil Samples from the City Mednogorsk**

| Parameters | № of soil sample | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | K1 | K2 | K3 |
| Index ($K_{mng}$) χ | 6.49 | 4.10 | 3.82 | 3.15 | 4.97 | 5.60 | 3.16 | 4.64 | 4.02 | 3.18 | 0.33 | 0.57 | 0.37 |
| THM, CFU $g^{-1}$ of soil ($\times10^5$) | 6.2 | 325.0 | 71.5 | 24.7 | 26.5 | 0.2 | 26.5 | 18.4 | 44.5 | 68.5 | 14.9 | 1.9 | 34.6 |
| № of iron oxidizing bacteria, CFU $g^{-1}$ of soil ($\times10^5$) | 2.1 | 74.2 | 32.0 | 10.9 | 1.1 | <0.1 | 0.8 | 3.3 | 19.9 | 17.8 | 4.8 | 2.4 | 0.6 |
| № of manganese oxidizing bacteria CFU $g^{-1}$ of soil ($\times10^5$) | 0.01 | 14.15 | 0.12 | 2.30 | 0.88 | 0.01 | 0.31 | 0.001 | 0.67 | <0.001 | 2.08 | 0.13 | 2.89 |
| Dehydrogenase activity µl $H_2$ $g^{-1}$ of soil $h^{-1}$ | 0.176 | 0.352 | 0.380 | 0.260 | 0.210 | 0.187 | 0.380 | 0.230 | 0.420 | 0.493 | 0.610 | 0.587 | 0.751 |
| Catalase activity, ml of 0.1 N $KMnO_4$ $h^{-1}$ | 12.5 | 13.4 | 2.5 | 11.0 | 14.6 | 9.8 | 8.3 | 6.9 | 8.2 | 7.6 | 4.7 | 2.9 | 3.4 |
| Invertase activity, mg of glucose $g^{-1}$ of soil | 0.9 | 2.3 | 2.5 | 2.9 | 1.4 | 1.7 | 2.1 | 3.0 | 2.1 | 2.7 | 2.1 | 2.6 | 2.4 |

The activity of dehydrogenase varied between 0.176 to 0.493 μl $H_2$ $g^{-1}$ of soil $h^{-1}$. This was a low index for dehydrogenase activity, which could justify the presence of soil agents (most likely HM) inhibiting these enzymes. Minimal activity was observed in the samples No 1, 5, 6 and 8, which had the highest values of magnetic susceptibility justifying the very dangerous level of iron in the soil. In control soil samples where the index of magnetic susceptibility was within acceptable limits, dehydrogenase activity varied between 0.610-0.751 μl $H_2$ $g^{-1}$ of soil $h^{-1}$, i.e it was1.5 to 4 times higher than in anthropogenically-damaged soils.

The activity of catalase in most of the researched samples was higher, than in control samples, varying from 6.9 to 14.6 ml of 0.1 N $KMnO_4$ $h^{-1}$ (Table 1). The index of catalase activity in control soil samples varied from 2.9 to 4.7 ml of 0.1 N $KMnO_4$ $h^{-1}$. Increased activity of catalase in anthropogenically disturbed soils perhaps could be as a result of exposure to contaminants and the accumulation of peroxides in soils, which served as substrates for catalase (Table 1). Based on our results, there was no significant negative effect of HM on the activity of invertase in the polluted soil samples (Table 1). In the experimental samples with increased magnetic susceptibility was observed a high, and also a low significance in the activity of invertase when compared with the control samples. The activity of invertase in the researched samples varied from 0.9 to 3.0 mg of glucose $g^{-1}$ of soil. Also some samples precisely No 1, 5 and 6 with very high values of magnetic susceptibility 6.49; 5.60, and 4.97 respectively, were characterized by very low significance of invertase activity- 0.9; 1.4 and 1.7 mg of glucose $g^{-1}$ of soil respectively (Table 1).

## Conclusion

Thus, from the researched samples of the anthropogenically polluted soils of the city Mednogorsk, two samples were identified which showed the highest coefficient of magnetism and a reduced content in heterotrophic microorganisms, which indicates the inhibitory effect of HM on soil bacteria. The results of the microbiological analysis showed also that the content of manganese-oxidizing bacteria in the soil samples was lower than iron- oxidizing bacteria and it varied irrespective of high or low significance of magnetic susceptibility in the soil. It was discovered that soil samples with extremely high significance of magnetic susceptibility possessed high amount of iron-oxidizing bacteria in their soil microbial community. The results of our study helps suggest that the index of the number of this physiological group of bacteria can be used for monitoring soils polluted with HM.

Also, based on the sensitivity to

metallic pollution, the studied enzymes form a decreasing order: dehydrogenase>invertase>catalase. These results justifies that the activity of dehydrogenases most significantly reflects the influence and impact of HM on the biochemical activity of soils and serves as a sensitive monitoring index for diagnosing soils polluted with heavy metals.

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# Biotechnology in Malaria Management: A Case Study in a Semi-urban Nigerian Clinic

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***Abstract:*** Until recently, in areas of high malaria transmission such as Nigeria, malaria treatment has been based mainly on clinical diagnosis which was presumptive, because malaria was considered one of the commonest causes of fever. With the availability of new tools such as parasite–based rapid diagnostic kits, a product of biotechnology, which complements the standard microscopy, it is imperative to provide targeted treatment on accurate estimation of true malaria cases. To proffer solution to the limitations of microscopy, this study was carried out to ascertain the reliability of SD Bioline HRP-2-Based RDTs in malaria case management. A parasite based diagnosis of malaria was carried out on the total 1,276 patients attending an out-clinic in a Semi-urban area (Amukoko) of Lagos State, South West Nigeria to evaluate specific performance characteristics of SD Bioline HRP-2 RDT in malaria case management using microscopy as a gold standard. Only 15.4% and 14.6% was positive for P. falciparum by HRP- 2-RDT (Rapid Diagnostic Testing) and microscopy respectively and the sensitivity, specificity, PPV and NPV are 94%, 98.5%, 91.4% and 98.2% respectively. Using RDT, fever has a sensitivity of 79.7% and specificity of 60.5%. Patients have fever but no HRP-2 in their blood (fever + Neg. RDT). Since symptoms of malaria are not only peculiar to *P. falciparum* infection, parasite based diagnosis must be performed. The performance characteristics of the SD Bioline HRP-2 RDT generated from this study indicated that the tool is reliable and fast in generating the test result. Similarly, parasite based diagnosis is required to eliminate other causes of fever symptoms.

***Key words*:** Malaria, Biotechnology, Microscopy, Case management.

## Introduction

Malaria case is defined as a person presenting to the clinic with a history of fever and concurrent parasitaemia. Malaria transmission in Nigeria takes place all year round in the south but

is more seasonal in the northern regions. About 25% of all estimated malaria cases in the WHO African Region occur in Nigeria [1]. Almost all cases are caused by *P. falciparum*, but only a small fraction is tested parasite based. Until recently, in areas of high malaria transmission such as Nigeria, malaria treatment has been based mainly on clinical diagnosis which was presumptive, because malaria was considered one of the commonest causes of fever. With the availability of new tools such as parasite–based rapid diagnostic kits, a product of biotechnology, which complements the standard microscopy, it is imperative to provide targeted treatment on accurate estimation of true malaria cases [2]. However, in cases where parasitological confirmation is not available, highly vulnerable groups (including children under five years and those suspected with severe malaria) can be treated on a clinical basis [2].

A new generation of easy to perform Rapid Diagnostic Tests (RDTs) has been developed to diagnose *P. falciparum* rapidly and reliably without the need of a microscope. This is based on the immunochromatographic detection of antigen histidine–rich protein II (HRP-2) or specific plasmodium lactate dehydrogenase (pLDH). Both HRP-2 and pLDH are produced by the parasite during their growth and multiplication in red cells. In Nigeria, Parasight TML ICT and OptiMAL became commercially available in 2000. More recently, others like SD Bioline were also introduced into the Nigerian market. Studies to evaluate the efficacy of these RDTs in Nigeria [3-5], have reported efficacy similar to expert microscopy.

In addition, RDTs have been shown to be cost effective in malaria case management in Nigeria [6] and potentially saves the cost and time wasted on presumptive treatment [3]. This of course is essential in effective malaria case management, which entails early diagnosis and prompt treatment with effective antimalarial medicines recommended for use in the Country. Unfortunately, this has received a major setback in the past years because of the high level of resistance to the first and second line antimalarial medicines; Chloroquine and Sulphadoxine–pyrimethamine [2]. Despite the observed changes in parasite sensitivity to artemisinins, the clinical and parasitological efficacy of artemisinin based combination therapies (ACTs) has not yet been compromised [7], until recently when the drug efficacy studies have detected resistance of *Plasmodium falciparum* to artemisinins in the South-East Asia. *P. falciparum* resistance to artemisinins has not been detected outside of the Greater Mekong sub-region [8].

Perceived fever is the sign most health workers use to diagnose clinical malaria. However, studies in areas of intense transmission have

found reported fever or a history of fever to be an unreliable indicator of clinical malaria. It is reported that clinical diagnosis of malaria has led to over–diagnosis and over–consumption of anti-malarial since malaria presentation is not specific [9-10] and have, therefore, not been helpful in improving malaria diagnosis. Hence, prompt parasitological confirmation by microscopy or with RDT is recommended for all patients with suspected malaria before treatment is started [7]. In addition, treatment solely on clinical suspicion should only be considered when a parasitological diagnosis is not accessible [2]. This then took us into the study of SD Bioline HRP-2-Based RDTs in malaria case management.

The objective of this work is thus:

(a) To validate the SD-Bioline HRP-2-based RDT diagnostic performance using microscopy as a standard.

(b) To ascertain the performance characteristics of clinical diagnosis using SD–Bioline HRP– 2–Based RDTs as a standard in malaria case management.

## Materials and Methods

***Study Area:*** The study site was at St. Matthew Primary Health Care Centre, situated at No. 3 Mayegun–Oro St., Amukoko in Ajiromi–Ifelodun Local Government Area, on the Longitude $6^0$27' 52"N and Latitude $3^0$20'44" E. It is 14 km from Lagos center. It is bounded on the North by Apapa Local Government Area and on the East by Amuwo–Odofin Local Government Area. Amukoko is one of the 50 communities found in Ajeromi–Ifelodun LGA of Lagos state and it is densely populated with population density of 687,316 (Nigeria Census, 2006). The laboratory work was carried out at The International Center for Malaria Microscopy and Malaria Rapid Diagnostic Tests Quality Assurance Programme, Department of Medical Microbiology and Parasitology, College of Medicine University of Lagos (CMUL), Idi–Araba. The "Standard for reporting diagnostic accuracy recommendations" was followed [11].

Ethical approval for this study was obtained from the Ethics Committee of the College of Medicine of the University of Lagos and Lagos University Teaching Hospital, Lagos, Nigeria.

**Study Procedures**

Participants were administered with the consent form and the intending patients were given case report form (CRF) for any history of febrile illness or malaria, anti malaria treatment in the past 2 weeks, taking temperature with mercury thermometer (Goshen $^{CE}$ 0483,), age and other relevant data were also recorded (Appendix A & B). Samples were collected from individuals of all ages from infants to the old who attended St. Matthew Primary Health Care Center.

Venous blood of 5ml was collected

from peripheral vein of each participant and shared into EDTA and plain bottles. The EDTA blood was diluted in the ratio 1:20 against turk solution for WBC count using "Neubauer ruled chamber (Germany(R)) and recorded.

***Study Sample:*** The criteria for inclusion included the complaints of fever and malaise at the time of survey or in the past 48 hours and other malaria related symptoms like headache, body weakness, chills, joint pains, diarrhoea, cough and vomiting. Patients on admission were excluded from the research. The study area is a rural settlement composed of artisans and traders that deal in trade ranging from Carpentry & Upholstery, Transportation to Site labour.

A total of 1,276 subjects, which cover all age groups, were selected for this study using random sampling method of the patient attending outpatient department (OPD) of the primary health center. The recruitment was based on the presentation of symptoms and previous clinical history as narrated by the patient. It was a prospective study in which data collection was done before reference standard were performed.

The patients' whole blood was collected by vein puncture to test for the presence of *P. falciparum* malaria antigen (HRP-2) and for microscopic identification.

**Description and Interpretation of MRDTs**

The procedure of the test was strictly adhered to according to manufacturer's instructions.

**Standard Diagnostic Bioline HRP-2 Based RDT:** The test device had a LOT number 082048 and an expiry date of 31st March 2012. Quality assurance testing was carried out on the RDTs used for the test by "The International Center for Malaria Microscopy and Malaria Rapid Diagnostic Tests Quality Assurance Programme. Department of Medical Microbiology and Parasitology, CMUL, Idi–Araba", before use. The device was seen to contain a cassette, an applicator loop stick, lancet, a buffer solution and desiccant. The cassette had two wells on the surface as shown in Picture 2. The labeling was done on the RDT cassette using glass marker and placed on a smooth flat dry surface. 5µl of blood (one loop-full) was added to the smaller sample well on the cassette with the aid of the applicator loop stick, 4 drops of the buffer was added into the second sample well, which is larger. As the test began to work, a pinkish–red color was seen moving across the result window in the center of the test device. Test result was interpreted within 15 minutes.

A RDT result was interpreted as positive when both the test line and control line showed pink (Picture 3), negative when only the control line showed pink or invalid when the control line did not appear regardless of the test line (Picture 4). Four independent readings were graded based on visual assessment as "3+ or high" if the test line is darker than

the control, " 2+ or moderate" if the test line was as intense as the control line, "1+ or light" if the result was a line that could only be seen in good light for reactive tests and " – or negative" for non-reactive ones, where no line is seen but for the control.

Grading was also used to determine the source of variability in results reported by two independent readers. Any invalid RDT test was repeated. Two scientists read RDT cassettes independently. Temperature and humidity for the storage conditions for RDTs during study period were maintained.

***Microscopy*:** This entails film preparation, staining process, and mounting of the slide on the stage as well as counting and identification.

**Malaria Blood Film (MBF) Preparation**

Two (2) malaria blood films (MBF) were prepared for each patient, one is marked "R" for "read" and "A" for "archive" on the glass slide. On the frosted end of the grease free slides, the identification number and date for each participant were written using a glassmaker. A 12 µl of blood was spread over a diameter of 15 mm for a thick blood film while 2 µl of blood was used for a thin blood film on the same slide. Another clean slide was used to spread the blood drops to get a thin film by placing at an angle of $45^0$ at the edge of the blood drop and then pulled forward to make an even spread with a tail end. The thin film was fixed in absolute methanol for 2 seconds to prevent lysis of the red blood cells and air dried on the rack [12]. The dried slides were kept in the incubator already set at $35^0$C for 48 hours, EDTA blood took longer time to dry.

The blood films were stained after 48 hours with 3% Giemsa stain solution at pH 7.2. The stain was left for 45 minutes and then gently rinsed off with buffered solution of pH 7.2 until the flooding became clear to sight [12]. The slides were then placed vertically in a slide rack and allowed to dry and later arranged into the slide box.

Microscopic examinations of stained blood films were done using X100 oil immersion objectives. A minimum of 100 fields was carefully examined horizontally down on the thick film to ascertain the slide negative for malaria parasites. A definitive diagnosis of malaria by microscopy was made when a reddish chromatin dot with a purple or blue cytoplasm of the malaria parasites are seen together.

Malaria parasites seen on the slides were counted against 200 WBC or 500 if the parasite count is less than 100 parasites/200 WBC. The number of asexual parasitic forms (trophozoites and schizonts) and sexual (gametocytes) present in these microscopic fields was recorded separately. The slides were adjusted to read the thin film for parasite identification, concentration and clarity was observed at the tail end of the film. The calculation of the total number of parasite/µl of blood

required the input of total white blood cell (WBC) count and the formula is computed as:

The stained slides were read by myself and a competent microscopist as Reader-1 and Reader- 2. The parasite density was calculated for each of the reader and the mean parasite density was obtained from the two readers by adding the two parasite density and divided by 2.

Microscopy was considered the gold standard reference level for positivity or negativity for the RDT device and SD Bioline HRP –2–Based RDT as the standard for the clinical symptoms. The age grouping was done to the nearest whole number, no gap is included nor any group excluded. All data were entered and analyzed using Statistical Package for Social Sciences for Windows V 17 (SPSS Inc., Chicago, IL). The comparism was done at 95% confidence intervals.

$$\text{Sensitivity} = \frac{\text{True Positive}}{\text{True positive + False Negative}}$$

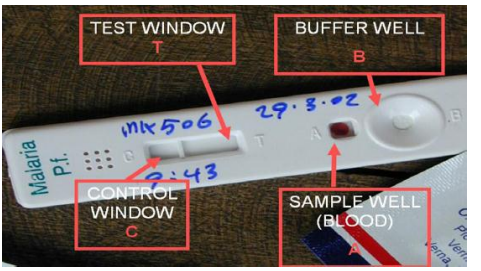
$$\text{Specificity} = \frac{\text{True Negative}}{\text{True positive + False Positive}}$$

$$\text{PPV} = \frac{\text{True Positive}}{\text{True Positive + False Positive}}$$

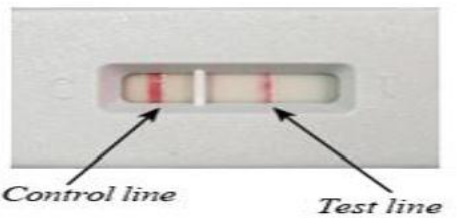
$$\text{NPV} = \frac{\text{True Negative}}{\text{False Negative + True Negative}}$$

## Performance Characteristics

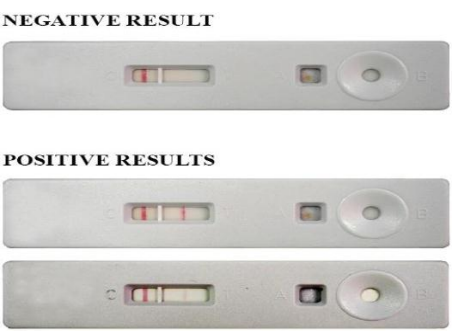
The sensitivity, specificity, PPV, NPV and test efficiency of clinical symptoms was calculated using 1273 whole–blood samples and SD-Bioline HRP-2 RDT as the reference test. The formula is as follows [13]. Sensitivity was calculated as:




**Picture 2: The pictorial view of the labelled SD Bioline HRP-2 RDT cassette.** *(T, test window; C, control window; A, sample well; and B, buffer well)*




**Picture 3: The RDT cassette with the result Lines**




**Picture 4: The pictorial view of the cassette indicating the test result after 15 minutes of applying the sample.**

## Results

This research was done between the month of November 2010 and August, 2011. The demographic characteristics of the study participants are shown in Table 1. A

total of 1,276 people were included in the analysis, which is composed of 60.2% female, and the overall mean age was 26.30 years with the range from 0.083 to 80 years.

Table 1. The malaria parasite prevalence by blood slide microscopy was 14.6% (186/1273) and 15.4 % (197/1273) by SD Bioline HRP-2-Based RDT. This put the HRP-2 RDT to the sensitivity value of 94%, specificity value of 98.5%, PPV of 91.4% and NPV of 98.2%. The older children age group "6-11years" presented the highest prevalence of 19.3% followed by adolescent by children ≤5 years with 13.7% (Table I).

Table 2. The distribution of symptoms with respect to age is represented on Table 2, fever is higher in children ≤ 5years (11.1%) than in older children "6–11" (6.8%) and in adolescent "12–16" (3.2%). Also high in the younger children ≤5 are chills, headache and febrile fever compared to older children and adolescent. The rate of vomiting is the same in the younger children and older children (19.6%) than in the other age groups. Adolescent presented the highest rate of loss of appetite (17.6%) but lowest in the adult group age "17 years and above". Adult group presented more diarrhoea than adolescent, followed by children ≤5years, older children have no diarrhoea. Children ≤5 years rated highest for cough (21.8%) followed by adult (14.3%), adolescent (13.4%) and older children (12.5%). Sleeplessness and dizziness are for adult while older children "6–11 years" presented highest level of yellow urine (51.8%) followed by adult (47.6%), adolescent has 41.8% and children ≤5 years have 39.7%. Diagnosing with febrile fever (tempt. $\geq 37.5^0$C) gave 18.1% prevalence and fever complaints by the patient rated highest even among other symptoms at 63.5%. Only 12 patients had had convulsion before attending the health center and are found negative by RDT. 18.3% of total male and 13.5% of total female were positive for HRP-2. There is statistical significant difference between the age and infection ($P<0.05$).

The relationship between various symptoms and infection using RDT and microscopy are also captured on Table 3.

Table 3. There was no much difference in the percentage occurrence of the symptoms in the two diagnostic methods. It is observed that 79.7% of the patients was having HRP-2 in their peripheral blood and presenting fever at the same time (Pos RDT + Fever) which is higher than (Pos. microscopy + fever) at 74.7%. So also (Neg. RDT + No fever) at 25.3% is higher than (Neg. microscopy + No fever) at 20.3%. (Pos. microscopy + joint ache) at 46.2% is higher than (Pos. RDT + Joint ache) at 40%. (Fever + Neg. RDT) was at 60.5%.

45.8% of the patients recruited for this study had access to medicines. Of the total 584 patients having access to medicine (ATM), 52% had

taken herbal remedy "Agbo", 43% took paracetamol/pain reliever (PCM) and 36% took ACT. The drug chart is represented in Fig. 1 and 2.

**Table I: The prevalence of malaria by HRP–2 RDT in different age categories**

| | HRP2 RDT | | |
|---|---|---|---|
| Age Group | Neg. (%) | Pos. (%) | Total (%) |
| 0-5yrs | 152 (14.1%) | 27 (13.7%) | 179 (14.0%) |
| 6-11yrs | 74 (6.9%) | 38 (19.3%) | 112 (8.8%) |
| 12-16yrs | 45 (4.2%) | 22 (11.2%) | 67 (5.3%) |
| 17yrs and above | 808 (74.9%) | 110 (55.8%) | 918 (71.9%) |
| Total | 1079 (84.6%) | 197 (15.4%) | 1276 (100.0%) |

**Table II: The distribution of the signs and symptoms with respect to age**

| Clinical signs &Symptoms | "0 – 5" years (%) | "6 – 11" years (%) | "12 – 16" years (%) | 17 Years & above (%) | Total (%) | Mean value |
|---|---|---|---|---|---|---|
| ***Fever:*** | | | | | | |
| 1. Body hotness. | 141 | 87 (6.8%) | 41 (3.2%) | 541 (42.4%) | 810 | 0.63 |
| 2. Febrile fever | (11.1%) | 39 (3.1%) | 12 (0.9%) | 115 (9.0%) | (63.5%) | 1.1813 |
| (≥37$^0$C) | 63 (4.9%) | | | | 229 | |
| | | | | | (17.9%) | |
| ***Chills (feeling cold & rigors)*** | 57 (4.5%) | 41 (3.2%) | 23 (1.8%) | 299 (23.4%) | 420 (32.9%) | 0.33 |
| ***Headache*** | 55 (4.3%) | 36 (2.8%) | 23 (1.8%) | 329 (25.8%) | 443 (34.7%) | 0.35 |
| ***Joint weakness*** | 36 (2.8%) | 37 (2.9%) | 30 (2.4%) | 446 (35.0%) | 549 (43.0%) | 0.43 |
| ***Digestive problems:*** | | | | | | |
| 1. Vomiting | 35 (19.6%) | 22 (19.6%) | 12 (17.9%) | 77 (8.4%) | 146 | 0.11 |
| 2. Nausea | 6 (14%) | 5 (11.6%) | 1 (2.3%) | 31 (72.1%) | (11.4%) | 0.03 |
| 3. Diarrhoea | 2 (11.8%) | 0 | 3 (17.6%) | 12 (70.6%) | 43 (3.37%) | 0.01 |
| 4. Loss of | 37 (20.7%) | 22 (19.6%) | 15 (22.4%) | 148 (16.1%) | 17 (1.3%) | 0.17 |
| appetite | 17 (9.5) | 9 (8.0%) | 8 (11.9%) | 161 (17.5%) | 222 | 0.15 |
| 5. Stomach ache | | | | | (17.4%) | |
| | | | | | 195 | |
| | | | | | (15.3%) | |
| ***Respiratory problems:*** | | | | | | |
| 1. Cough | 39 (21.8%) | 14(12.5%) | 9 (13.4%) | 131 (14.3%) | 193 | 0.15 |
| 2. Chest pain | 2 (7.1%) | 2 (7.1%) | 3 (10.7%) | 21 (75.0%) | (15.1%) | 0.02 |
| | | | | | 21 (1.6%) | |
| ***Itching*** | 1 (6.3%) | 4 (25.0%) | 1 (6.3%) | 10 (62.5%) | 16 (1.25%) | 0.01 |
| ***Sleeplessness*** | 3 (1.7%) | 3 (2.7%) | 1 (1.5%) | 42 (4.6%) | 49 (3.8%) | 0.04 |
| ***Dizziness*** | 10 (5.6%) | 8 (7.1%) | 3 (4.5%) | 86 (9.4%) | 107 (8.4%) | 0.08 |
| ***Yellow urine*** | 17 (39.7%) | 58 (51.8%) | 28 (41.8%) | 437 (47.6%) | 594 (46.4%) | 0.47 |

## Discussion

The prevalence of malaria from this study is 15.4% with a highly sensitive SD Bioline HRP-2- Based RDT (sensitivity= 94%, specificity=98.5%, PPV=91.4% and NPV=98.2) when compared with the gold standard microscopy.

This result is consistent with other published studies showing that the sensitivity and specificity of HRP-2-based tests usually are > 90% for *P. falciparum* [14-15].

Comparing the two diagnostic methods, prevalence by RDT is higher than microscopy. This is contrary to the report by [16}, where the prevalence of malaria parasites by slide microscopy was higher than prevalence by RDT though the different was not statistically significant. This is further confirmed in reports by [17] that RDTs have shown a comparable level of accuracy to microscopy in clinical setting. This is not surprising as the quality assurance studies have been conducted on all the RDTs used for this research work by "The International Center for Malaria Microscopy and Malaria Rapid Diagnostic Tests Quality Assurance Programme, Department of Medical Microbiology and Parasitology, College of Medicine University of Lagos, Idi–Araba".

**Table III: Relationship between clinical symptoms and infection by RDT and microscopy**

| S/N | SYMPTOMS | HRP-2 RDT | | MICROSCOPY | |
|---|---|---|---|---|---|
| | | POS. (%) | NEG. (%) | POS. (%) | NEG. (%) |
| 1 | Fever | 157 (79.7%) | 653 (60.5%) | 139 (74.7%) | 670 (61.6%) |
| | No Fever | 40 (20.3%) | 426 (39.5%) | 47 (25.3%) | 417 (38.4%) |
| 2 | Chills | 84 (42.6%) | 336 (31%) | 71 (38.2%) | 349 (32.1%) |
| | No Chills | 113 (57.4%) | 743 (69%) | 115 (61.3%) | 738 (67.9%) |
| 3 | Joint ache | 79 (40%) | 470 (43.6%) | 86 (46.2%) | 462 (42.5%) |
| | No Joint ache | 118 (60%) | 609 (56.4%) | 100 (53.8%) | 625 (57.5%) |
| 4 | Headache | 76 (38.6%) | 367 (34%) | 69 (37.1%) | 373 (34.3%) |
| | No headache | 121 (61.4%) | 712 (66%) | 117 (62.9%) | 714 (65.7%) |
| 5 | Febrile fever (Temp.≥37.5$^0$C) | 62 (31.6%) | 167 (15.7%) | 52 (28%) | 177 (16.5%) |
| | Temp. <37.5$^0$C | 134 (68.4%) | 900 (84.3%) | 134 (72%) | 897 (83.5%) |
| 6 | Body weakness | 52 (26.4%) | 269 (24.9%) | 52 (28%) | 269 (24.7%) |
| | No Body weakness | 145 (73.62%) | 810 (75.1%) | 134 (72%) | 818 (75.3%) |
| 7 | Vomiting | 24 (12.2%) | 122 (11.3%) | 21 (11.3%) | 124 (11.4%) |
| | No vomiting | 173 (87.8%) | 957 (88.7%) | 165 (88.7%) | 963 (88.6%) |
| 8 | Cough | 22 (11.2%) | 171 (15.8%) | 23 (12.4%) | 170 (15.6%) |
| | No cough | 175 (88.8%) | 908 (84.2%) | 163 (87.6%) | 917 (84.4%) |

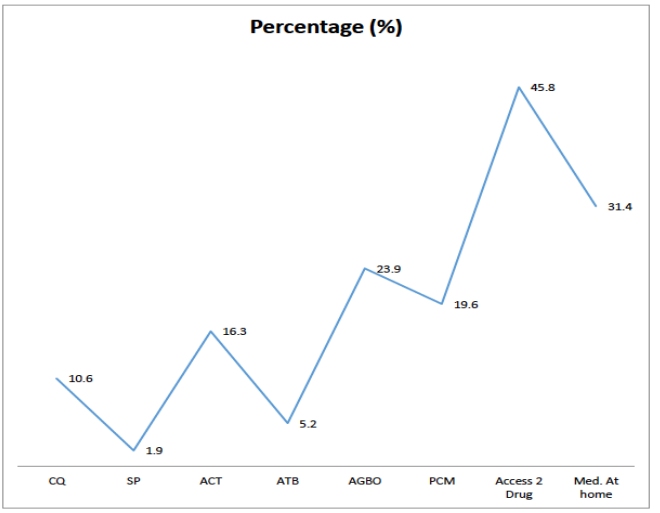



**Fig. 1 (Right): Chart indicating percentage patient and disposition to drug before attending Clinic** *(CQ-chloroquin; SP-sulphadoxine pyrimethamine, ACT-artemisinin-based combination therapy; ATB-antibiotics; AGBO-herbal remedy for malaria; PCM-paracetamol*

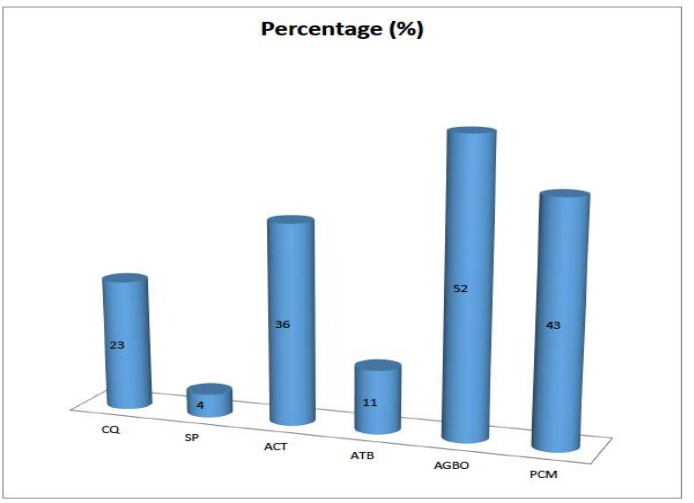



**Fig. 2: The percentage of drug taken by those having access to medicine only**

The prevalence of malaria in this study contrast sharply with the prevalence reported in the North Western Nigeria of prevalence rate of 27.29% [18], this is attributed to the fact that most of the sampling collection was done in the early part of the year when the transmission is usually low due to dry season period of the year.

Children are still more vulnerable, but there is now a shift from the ≤5 years which was at 16% in Nigeria as reported in the World Malaria Report [7] to 19.3% of the older children of age group "6-11years" as

observed from this study. The drop in the prevalence is not surprising because of the various intervention strategies adopted for the Country in the last one decade that focuses on all age group in the country which include the disbursement of insecticide treated nets/ long lasting insecticide nets (ITNs/LLINs) in 2009 [1].

The prevalence is observed to be higher by RDTs than microscopy basically due to the fact that some patients are fond of self-medication as indicated in the case report form (CRF). Some of the patients had taken anti-malaria, which has the ability to bring down the level of parasitaemia, but *P. falciparum* antigens HRP-2 still persist in the peripheral blood. In Fig. 1, 45.8% of the patients had access to drug, 31.4% actually kept the medicine at home. 16.3% had taken

ACT and 23.9% had taken "Agbo" before coming to the clinic. From Fig. 2, 52% of those having access to medicine had taken herbal remedy "Agbo" before attending the clinic while 32% had actually taken artemisinin based combination therapies (ACTs). Among many plants used for herbal remedy "Agbo" for malaria are lemon grass, bitter leaf, brimstone leaf, pawpaw leaf and mango tree bark which have been proved effective (Adodo, 2008), this is the reason for low or no parasitaemia compared to HRP-2 positive.

There was no statistical significant different between the symptoms and age (Table 2) except for Nausea and Diarrhoea ($p<0.05$). From this study, it can be said that 157 (79.7%) of the total patients having HRP-2 in their peripheral blood and presenting fever are actually regarded as having malaria case, this is higher than what is observed with microscopy 139 (74.4%), 653 (60.5%) patients have fever but no HRP- 2 in their blood (fever + Neg. RDT) as represented on Table 4, meaning there are other aetiologies of fever other than *P. falciparum* which may include influenza, shigella, salmonella, cholera etc, which could have been the causative agents in these negative results. This is therefore in agreement with the report of a work done in a similar endemic region that stated that clinical diagnosis has little utility in malaria case management [19].

18.1% prevalence by febrile fever (tempt. $\geq 37.5^0$C) is lower compared to the findings reported by [20] in Senegal at 49.7% though theirs was calculated from $38^0$C and above. The fever complaints in this study were 63.5%, which is also lower, compared to 80.4% found in Senegal. The twelve (12) patients that had had convulsion before attending the health center were cleared from malaria by presenting a negative RDT result. The severe presentation of convulsion may be because of the poor ventilation of the residence houses of the patients. Based on the high sensitivity coupled with the rapid availability of the test

result which is practicable in twenty (20) minutes, SD Bioline HRP-2-Based RDT is hereby preferred to microscopy this is in line with the requirements documented by [21] which also include technical simplicity of the test and training needs, ease of interpretation and absence of any need for electricity to operate the assay. Further criteria essential for selecting appropriate RDTs by an operational manual on universal access to malaria diagnostic testing [22] are the supplier's production capacity and lead times, storage conditions, delivery schedules, shelf-life as well as registration and budget requirements. With the availability of the quality assured sole distributors in Nigeria such as Codix Pharma Ltd. and others, SD Bioline HRP-2-Based RDTs should be advocated for use in every level of health care facilities.

**Conclusion**

The performance characteristics of the SD Bioline HRP-2 RDT gotten from this study indicated that it is reliable in the parasite based diagnosis of malaria, but clinical symptoms are not reliable yet, the parasite based diagnosis must always be done to eliminate other causes of the symptoms. Based on the high sensitivity coupled with the rapid availability of the test result which is practicable in twenty (20) minutes from this study, SD Bioline HRP-2-Based RDT is hereby preferred to microscopy. The low prevalence rate from this study indicated the effect of the intervention put in place by the national malaria control programme (NMCP).

With the availability of the quality assured distributors of SD Bioline HRP-2-Based RDTs in Nigeria like Codix Pharma Ltd. and others, I hereby recommend that the continuous effort by national and international organization on the malaria control should be strengthened with a continuous advocate for the use of HRP-2-Based RDT in the malaria case management for all level of health facilities, so that Nigeria can also be enlisted one day with malaria-free Countries.

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# Comparative HPLC Evaluation of the Effect of Roasting and Deep Frying Cooking on Vitamins Content of Unripe Plantain (*Musa x paradisiaca*)

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***Abstract:*** *Musa x paradisiacal* (plantain) is among the major tropical staple foods. This study was designed to compare roasting and deep frying method cooking plantain. The fat soluble vitamins constituent of unripe plantain was determined fried with three different oils (*canola* oil*, soya* oil and *vegetable* oil). The vitamins content were determined using High Performance Liquid Chromatography. Roasted plantain samples gave a higher value of vitamins A (0.08 ± 0.01mg/g), D (0.02 ± 0.01mg/g), E (0.01 ± 0.00mg/g) and K (0.05 ± 0.00mg/g) compared to plantain samples fried with canola oil which gave low level of vitamin D (0.03 ± 0.00mg/g), E (0.25 ± 0.00mg/g) and K (0.03 ± 0.00mg/g). However, canola oil gave a very high level of vitamin A (0.72 ± 0.02mg/g). The result showed that roasting plantain in oven retains the vitamin contents compared to deep fat frying. *Canola* oil is recommended for frying although *soya* oil can also be used.

***Key words***: Plantain, Roasting, Deep-frying, Fat soluble vitamins, *Canola* oil, *Soya* oil.

## Introduction

Plantain is among the major tropical staple foods [1]. It is known that cooking methods or processing techniques of foods often lead to losses of vitamins and other nutrients such as vitamin up to 90% depending of the cooking or processing used [2]. In Nigeria, steaming, roasting and frying are the most commonly used cooking methods for plantain. Cooking of foods leads to the improvement of microbiological and organoleptic qualities, destroy toxins and anti-nutritional factors, increase digestibility and nutrients bioavailability [3], unfortunately these procedures cause the loss of some of the micronutrients in foods [4], micronutrients such as the water soluble vitamin and the fat soluble vitamins though thermo sensitive are important in the stimulation of immune system, liver disorder, fight

against cancer and cardiovascular diseases [5]. In view of their importance in human health, this work was initiated to evaluate the effects of frying in boiling oil bath on the fat soluble vitamins (A, D, E and K) contents of plantains.

**Materials and Methods**

***Plant Material:*** Plantain fruits were purchased from Covenant University farm, Ota, Nigeria. Fig. 1 shows the picture of *Musa x paradisiaca*.

**Fig. 1: The exterior view of the plantain (*Covenant University farm)***

***Preparation of Sample*:** The Plantain fruits were peeled and washed in distilled water. After which they were cut into thin circular slices just like the locally sold "Plantain" chips. Three different oils: *canola* oil, *soya* oil, and vegetable oil, were used in the frying of the chips. A deep fryer was used for this, and the plantain fruits were fried at a temperature of 190$^{o}$C for 10 minutes. After cooling, the samples were grinded into powder form using mortar and pestle. After grinding the different plantain fruits powder samples that were fried with the different oils were kept in Ziploc bags and labeled accordingly.

***Analysis of Fat Soluble Vitamins*:** This was done using the HPLC method and had been described previously by [6].

***Statistical Analysis*:** All the experimental results were the mean (±standard deviation) of three parallel measurements. Data were evaluated by using Excel 97 as a tool for the analysis.

**Results and Discussion**

***Vitamin Content:*** Fig. 2 shows that there are low levels of fat soluble vitamins in the varieties of samples with vitamin A levels in plantain dry sample ( 0.08) was very low, plantain with canola oil (0.725) has the highest level of vitamin A with plantain with soya oil (0.06), plantain with vegetable oil, (0.064) showing no significant difference. Vitamin D levels in the varieties of samples were relatively low with little or no significant difference. Vitamin E level on the plantain dry sample was higher (0.11), plantain with canola oil and soya oil was lower with no significant difference. Vitamin k was relatively low with no significant difference although the values were very low Vitamin A is more present in any of the cooking methods or techniques, it can also be stated that cooking with canola oil is an ideal way of keeping Vitamin A which is an important vitamin in the body system. [7], reported that steamed fruits had the highest concentration of β-carotene and fruits roasted at the semi- ripe stages had the highest concentration of nutrients.

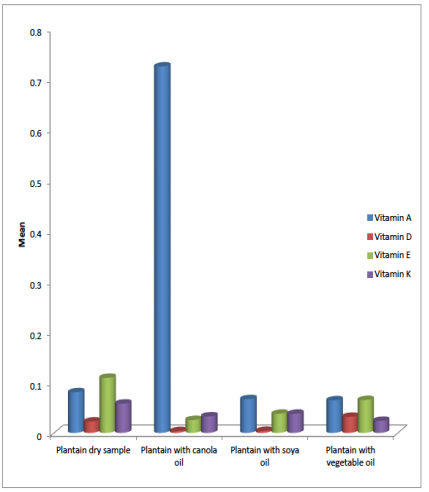


**Fig. 2: Graphical representation of vitamin contents of unripe Plantain**

## Conclusion

The results of the study suggest that most vitamins are lost during the frying processing of plantain. Vitamin A was present in most of the cooking methods although statistically it can be said that some of its nutrient and other vital vitamins were lost due to the cooking method used and oven-drying method retained more nutrient. In conclusion, roasting of plantain in oven retains the vitamins A, D, E, and K content of plantain compared to deep fat frying. Canola oil is recommended for frying although vegetable oil can also be used.

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# Morphology, Biochemical and Genomic Diversity of Hexaploid Wheat (*Triticum aestivum* L.) Varieties in Ethiopia: A Prospective Study

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***Abstract*:** Hexaploid Wheat (*Triticum aestivum* L.) is one of the most important food cereals grown in many areas worldwide. World population is to increase by 2.6 billion in 2050. Ethiopia is one of the world's nine countries needed to increase food production. Since few studies on genetic diversity & in adequate evaluation of bread wheat varieties; my objective the project is to study genetic diversity of bread wheat varieties using morphological, SSRs, good baking quality, grain quality performance across environments, and compare and contrast all the mentioned characters. 121 bread wheat varieties, arranged in 11x11 simple lattice design, will be studied at 3 agro ecological regions of Ethiopia for 2013/14 using 25 morphological/phenotypic parameters as a preliminary genetic diversity study from the field; 10 baking and milling quality parameters of the varieties, seed storage protein using SDS electrophoresis banding patterns, and DNA finger printing microsatellite markers of each varieties in laboratory. Hence, the extent and nature of genetic diversity, grain quality, environmental effects on seed storage proteins, genetic variation using SSRs markers, and potential varieties for further breeding and improvement of nutritional and baking quality suggested. Genetic diversity, plant breeders rely on during selection in cultivar development, is one of the key factors for the improvement of many crop plants including wheat. This research is of great interest and is in line with the current Global Wheat Program, will contribute to the increasing of food security, improve productivity and profitability of wheat farming and sustain natural resources in the developing world.

***Key words:*** Genetic diversity, SSRs, *Triticum aestivum* L., Varieties.

## Introduction

Wheat is one of the most important cereals worldwide and it is grown in many areas [1}. A rapid increase in global wheat production has taken place during the last five decades,

mainly due to increased productivity rather than an expansion of the cultivated area, with average global yields having risen from 1 t/ha in the 1950s to about 2.5 t/ha at the turn of the century [1].

In global terms, wheat is the leading source of cereal proteins in human food, having higher protein content than maize or rice. In total, 16% and 26% of total dietary calories in developing and developed countries, respectively, come from wheat [2]. In Sub-Saharan Africa, Ethiopia ranks 2nd next to South Africa in terms of total wheat area and production. According to CSA [3], wheat in Ethiopia is an important cereal crop and it ranks third in total production next to teff and maize. It is largely grown in the highlands of the country and constitutes roughly 20-30% of the annual cereal production and plays an appreciable role of supplying the production with carbohydrates, proteins and minerals [4].

Within the total area of wheat under cultivation in Ethiopia, it has been reported that hexaploid and tetraploid species each occupy approximately 50% of the area [5]. However, a change in the relative proportions of wheat types grown has been reported more recently, with e.g. hexaploid and tetraploid species occupying approximately 70% and 30%, respectively, of the total wheat area under cultivation [3].

**Origin, Taxonomy and Distribution Of Hexaploid Wheat**

Wheat belongs to the genus *Triticum* and the tribe *Triticeae* of the family Poaceae (*Gramineae*) [6] and it evolved from wild grasses found in the Eastern Mediterranean and Near East regions. It was probably domesticated around 10,000 to 15,000 B.C. in the bordered areas of the countries known as Iran, Iraq, Syria, and Turkey; a mountainous hilly region in the upper reaches of the Tigris and Euphrates drainage basin [7].

There are fifteen recognized species within the genus *Triticum.* About 90% of the world's wheat production consists of three species: *Triticum aestivum* (common wheat), *Triticum compactum* (club wheat) and *Triticum durum* (durum or macaroni wheat).

The species of the genus *Triticum* and their close relatives can be divided into diploid, tetraploid and hexaploid groups, with chromosome numbers of 2n = 14, 28and 42 respectively, in which the basic chromosome number is x = 7. The wild species are diploids (2n = 2x = 14), e.g. with the genome designation AA (*T.monococcum*), DD (*T. tauschii,* syn. *Aegilops squarossa*), and SS (*T. speltoids*), or tetraploids (2n = 2x = 28), e.g. with the genomes AABB (*T. durum* or *T. turigidum*) or AAGG (*T. timopheevii).* The most common cultivated wheat (bread wheat) now a day is hexaploid, *T. aestivum*, AABBDD, (2n= 6X= 42) [8].

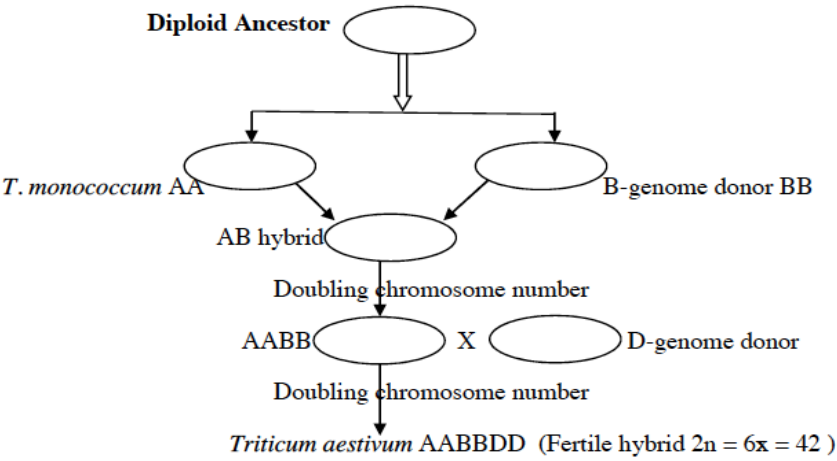


**Fig. 1. Possible origins of Triticum aestivum (after van Buren, 2001; Kerby & Kuspira, 1987).** ***(Each capital letter represents a genome composed of seven chromosomes).***

## Assessing Genetic Diversity

Genetic diversity is one of the key factors for the improvement of many crop plants including wheat. Plant breeders rely on the availability of genetic diversity during selection in cultivar development. The efficiency of genetic gain by selection can be improved if the patterns of genetic diversity within a population of breeding lines are known. Genetic similarity/distance estimates among genotypes are helpful in the selection of parents to be used in a breeding program. Varieties developed with wider genetic base may be helpful in enhancing the yield under various agro-climatic conditions. Diverse genetic base may also resist the spread of diseases, in approved varieties. Genetic diversity can be accessed from pedigree analysis, morphological traits or using molecular markers. However, diversity estimates based on pedigree analysis have generally been found inflated and unrealistic [9]. Genetic diversity estimates based on morphological traits, on the other hand, suffer from the drawback that such traits are limited in number and are influenced by the environment Molecular markers are useful tools for estimating genetic diversity as these are not influenced by environment, are abundant and do not require previous pedigree information [10].

## Morphological diversity

Agro-morphological characterization (phenotyping) is a first step towards diversity and conservation of plant genetic resources. When assessing genetic diversity, the use of agro-morphological variation provides greater complementary information to molecular markers characterization. Agro-morphological criteria such as the color and structure of seeds, glume

nature, spike density, days to maturity and heading, plant height, thousand kernel weight, etc. can be used to study the variation among the hexaploid wheat varieties. For successful diversity and conservation, knowledge on the nature and extent of the available variation is important. Different studies have dealt with the variability in the Ethiopian wheat landraces for agronomic and morphological characters [11-13]. In most cases the studies showed the presence of variation within and between populations and geographical regions.

**Biochemical diversity**

It is the investigation of variation that may exist within and between population in wheat by the use of biochemical markers that involves the nutritional contents of the wheat like hectoliter weight, thousand grain weight, grain protein percentage, zeleny sedimentation, gluten content, grain starch percentage, grain hardness, and the seed storage proteins and isozymes [14-15]. These techniques utilize enzymatic functions and are comparatively inexpensive yet power full method of measuring allele frequencies for specific genes.

Biochemical analysis can also be based on the separation of proteins into specific banding patterns. It is a fast method, which requires only small amounts of biological material. However, only a limited number of enzymes are available and thus, the resolution of diversity is limited.

This information can be used to measure population subdivision, genetic diversity, gene flow, genetic structure of species, and comparisons among species out-crossing rates, population structure and population divergence, such as in the case of crop wild relatives.

**Molecular diversity**

It is the divergence or similarity of crops based on the molecular markers work by highlighting differences (polymorphisms) within a nucleic acid sequence between different individuals. These differences include insertions, deletions, translocations, duplications and point mutations. They do not, however, encompass the activity of specific genes. In addition to being relatively impervious to environmental factor, molecular markers have the advantage of: (i) being applicable to any part of the genome (introns, exons and regulation regions); (ii) not possessing pleiotrophic or epistatic effects; (iii) being able to distinguish polymorphisms which not produce phenotypic variation and finally, (iv) being some of them co - dominant. The different techniques employed are based either on restriction-hybridization of nucleic acids or techniques based on Polymerase Chain Reaction (PCR), or both.

Criteria for the estimation of genetic diversity can be different: pedigree records, morphological traits or molecular markers. The use of molecular markers for the evaluation

of genetic diversity is receiving much attention. Many wheat scientists have studied genetic diversity in common wheat using different molecular markers [16-18]. However, most of these marker systems show a low level of polymorphism in wheat, especially among cultivated lines and/or cultivars [19].

Because SSRs are multiallelic, they have high potential for use in evolutionary studies [20-21] and studies regarding genetic diversity and relationships. At present, microsatellites are one of the most promising molecular-marker types able to identify or differentiate genotypes within a species. Their co-dominant inheritance, high level of polymorphism and easy handling make them extremely useful for many different applications [19].

**Seed Storage Proteins in Wheat**

Protein is considered the most important nutrient for humans and animals. The protein content of wheat grains varies between 10%-18% of the total dry matter. Wheat proteins are classified according to their extractability and solubility in various solvents. Classification is based on the classic work of T. B. Osborne [22] at the turn of the last century. In his procedure, sequential extraction of ground wheat grains result in the following protein fractions [23-24].

**Statement of Problem**

World population is expected to increase by 2.6 billion over the next 45 years, from 6.5 billion today to 9.1 billion in 2050. Ethiopia is one of the nine countries predicted to account for the 2.6 billion increases. There is a pressing need for an astonishing increase in food production to feed this population. Wheat is among the major cereal crops grown in Ethiopia. It grows on an area of about 1.69 million hectares, and ranks third in area and second in total production (FAO 2005). It is an important commodity crop, which could contribute a major part in achieving the country's agricultural objective of food grain self-sufficiency). Despite the country having potential environments for wheat culture and being the centre of diversity for wheats, the average national yield is low (1.8 t/ha). The national average of wheat productivity in the country is 1.4 tons/ha, which is still 24% and 48% that of the South Africa and the world’s averages respectively [25]. The major wheat yield limiting factors in Ethiopia which resulted in such low yield levels, compared to any other part of the world, are diseases, weeds, poor soil fertility, lack of cultivar choice, frost occurrence in the highlands, terminal drought stress and water logging in the intermediate altitudes, and drought stress in the lowlands. Moreover, many of the variability studies [12][26] conducted so far are based on morphological traits, which are largely influenced by environmental factors. The few studies performed using microsatellites [11][27], isozymes

[28], and glutenine and gliadine storage protein and AFLP [11] considered either few accessions or focused mainly in the central highlands of Ethiopia. Thus, it was felt that because wheat varieties have not been adequately evaluated, their genetic resource remains largely unexploited.

For a successful breeding program, the presence of genetic diversity and seed storage protein play a vital role. Genetic diversity based on morphological & biochemical, seed storage protein and SSR are essential to meet the diversified goals of plant breeding such as breeding for increasing yield, wider adaptation, desirable quality, pest and disease resistance.

Research on bread wheat genotypes for genetic and molecular diversity study based on seed storage protein and microsatellite markers is limited. Moreover, study in baking quality of hexaploid wheat varieties is lacking. Comparative study to show the inter and intra population variation in Ethiopian bread wheat using morphologic and biochemical characters, seed storage protein and DNA markers is not available. Hence, the objective of this study is to ascertain the genetic diversity of bread wheat (*Triticum aestivum* L.*)* varieties using morphological, grain quality, seed storage protein, and SSR markers in Ethiopia.

**Materials and Methods**

The experiments will be conducted in the field as well as in laboratory in the following manner:

**Experimental Materials**

121bread wheat varieties released at various times by different agricultural research centers will be used in the study. The varieties include advanced lines. These varieties will be obtained from Kulumsa and Adet Agricultural Research Centers, which are National Bread Wheat Research Coordination Center and Regional Research Center, respectively. Most released varieties are adapted to 1900-2800m.a.s.l.

**Field Experiments**

**Agronomic and morphological characters**

***Experimental site:*** The phenotypic characterization will be conducted at three sites viz., Adet Agricultural Research Center (west Gojam, Amhara), Debretabor sub-center (North Gondor, Amhara) and Kulumsa Agricultural research Center (Arsi zone, Oromia).

***Experimental Design*:** The treatments will be arranged in an 11x11 simple lattice design with plot size of 2 m with 4

rows of 2 m length, and 20 cm intra row spacing. Seed and fertilizer rates will be as recommended by respective research centers and testing sites. DAP will be applied at planting and urea will be splitted i.e., half at planting and half at late tillering stages.

Sowing will be done by hand drilling. The trial will be planted around mid of June, 2014 G.C.

**Morphological Data Collection**

The following data will be collected

based on two central rows to minimize boarder effect:

Plant height (PH), Maturity Date (MD), Number of tillers per plant (NTPP), Plant Stand (PS) or Stand percent (SP)%, Spike length (SL), Number of seeds per spike (NSPS), Grain yield(GY) or Grain Dry Weight (GDW) Kg/plot, Biomass yield (BY) or Total Dry Weight (TDW) Kg/plot, Harvest index, Spike density(5 samples), Days to flower, Number of spike lets per spike, Tillering capacity, Awn presence (awnedness) Characteristics, 1000grain weight(gm), (DH) days to heading is number of days from sowing to emergence of spike, Days to 50% germination, Glume Characteristics, Seed Characteristics, Grain Characteristics, Disease occurrence.

**Seed Storage Protein analysis**

In this analyses there are two possible characters to be determined, one is the determination of quality traits - characterization of wheat germplasm for composition of high molecular weight (HMW) ─ glutenin subunits, low molecular weight (LMW) ─ glutenin subunits and gliadins. Secondly determination of variation of bread wheat varieties as it can be done in molecular markers using band determination as given below in the data analysis part. Banding patterns of the varieties will be investigated for their variability using the gel documentation system (Damania et al., 1983), followed by Gel Documentation and - Analysis.

**Analysis using Simple Sequence Repeats (SSR)**

***Tissue harvest and DNA extraction***

Young leaves will be collected separately from 5 randomly selected individual plants per accession after four weeks of planting and dried in silica gel. Approximately equal amounts of the dried leaf samples will be bulked for each accession and ground with pestle and mortar. Total genomic DNA will be isolated from about 0.4g of the pulverized leaf sample using modified triple Cetyl Trimethyl Ammonium Bromide (CTAB) extraction technique as described by [29]; followed by Primer selection and optimization, PCR and gel electrophoresis, gel documentation and analysis.

**Statistical analysis**

***Morphological and Biochemical Data:*** Analysis of variance using SAS software (v. 9.1) will be used to determine all morphological and biochemical components.

***Protein Analysis:*** The similarity matrix generated will be converted to a dissimilarity matrix [30]. All analysis will be carried out using a statistical package NTSYS -pc, version 1.8 [31] and STATISTICA.

**SSR Profiles/Bands** will be scored visually for each individual accession from the gel photograph. The bands will be recorded as discrete characters, presence '1' or absence '0' and '?' for missing data if any. Based on recorded bands different software will be used for analysis. POPGENE version1.32 software [32] will be used to

calculate genetic diversity for each population as number of polymorphic loci, percent polymorphism, Gene diversity (H) and Shannon diversity index (I). Analysis of molecular variance (AMOVA) will be used to calculate variation among and within population using Harlequin version 3.01. NTSYS- pc version 2.02 [33] and Free Tree 0.9.1.50 [34] software will be used to calculate Jaccard's similarity coefficient which is calculated with the formula:-

**Expected Research Outcome**

a) The extent and nature of genetic diversity within Ethiopian bread wheat varieties analyzed.

b) The grain quality characteristics of the varieties determined.

c) Environmental effects on the quality of seed storage proteins determined.

d) Composition of high molecular weight (HMW) glutenin subunits, low molecular weight (LMW) glutenin subunits and gliadins characterized.

e) Genetic variation using SSRs molecular markers determined

f) Varieties of required potential for further breeding and improvement of nutritional and baking quality suggested

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