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Discovering Thoughts, Inventing Future

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Musca Domestica: A Vector of Pathogenic Microorganisms and Biocontrol Approaches

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I. Introduction

Because house fly lives close with human, it finalizes its entire life cycle in human houses and their domestic animals. *Musca domestica* Linnaeus can be found in human residences, hospitals, food processing factories, food markets, butchery, food centers or restaurants, poultry and livestock farms, and different domestic areas or buildings. House flies can be a cause of decreasing the production of milk in dairies. Therefore, recently significant emphasis has been given to fly control measures (Crespo *et al*., 1998).

Repeated interaction of the fly with different animals and wastes provides an occasion forthe mechanical transmission of diseases to both human and animal (Davari *et al*., 2010; Fisher *et al*., 2017). Places with vast quantities of dung or manure, such as animal raising houses and sites without human cleanliness practices, represent favorable conditions for the dissemination of house flies and simultaneous procurement of bacteria (Meerburg *et al.*, 2007). Though, the concentration, possibility, and species dissemination of bacteria in animal excrement or compost differ broadly among places and within hosts (Himathongkham *et al.*, 1999). Therefore, flies might confront and eat highly varying quantities of bacteria throughout their connotations with animal trashes (Ahmad *et al.*, 2011).The feeding habits of house fly are one of the most harmful characteristics because it is exposed to decaying plant and animal matter, this put fly in contact with pathogenic organisms found in various environments, garbage, and animal waste (Park *et al.*, 2019).

Flies carrying pathogens are usually found with human and animal wastes and waste management then propagates to human dwelling and activity (Sulaiman *et al*., 2000; Mian *et al*., 2002). House fly, *Musca domestica*, and stable fly, *Stomoxys calcitrans* can transmit injurious pathogens to humans and animals in urban and rural regions. These species can cause irritation to farmers and affect animal health causing a decline in the production of cattle and rooster. They breed in organic matter causing problems in places where organic waste is stored such as waste management facilities (Malik *et al.*, 2007; Taylor *et al.*, 2012 and Weeks *et al.*, 2017). As a result of its life and conduct, flies have been involved as a vector of pathogenic microbes by mechanical and biological route (Graczyk *et al.*, 2001; Zurek and Ghosh, 2014).

Park *et al*.(2019), investigated the inner and outer microbial fauna in 400 samples of house flies from three different environments (cow farm, homes, and clinics) in Belgium and Rwanda. They reported that whatever was the nation or territory, house flies ported a high potential of various bacterial microbiota and that bacterial communities on the external body were much more various than the internal populations from the intestinal gut. Various researches reported the effect of house fly in transmitting different pathogens including bacterial, viral, rickettsial, and helminthic diseases(Sanchez-Arroyo and Capinera, 2014;Shah *et al*., 2015), which causes infections such as enteric infections (dysentery, diarrhea, typhoid, cholera, and certain helminth infections), eye infections (trachoma and epidemic conjunctivitis, poliomyelitis), skin infections (yaws, cutaneous diphtheria, some mycoses, and leprosy) (Bahrndorff*et al*., 2017; Baharet*het al*., 2018).

Hulten *et al*.(1996), indicated that there are three different possible modes of bacterial transmission by flies. A confirming study by Thomas *et al.* (1992) and Kelly *et al*. (1994), reported the isolation of viable

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bacteria from feces. Thus, suggesting that transmission through the fecal-oral route seems possible. In Malaysia, Tan *et al*.(1997), performed their study on how house fly could transmit rotavirus on their different body parts.

When flies feed on bacteria, they can keep these bacteria in their guts for several days, then propagate them in the ecosystem. Kobayashi *et al.* (1999), observed many bacteria in the foregut of the flies (crop) until four days after feeding it on *E. coli* O157:H7. Zurek *et al.* (2001), mentioned that the bacteria persisted in the house fly digestive system for 36 hours after feeding it on *Yersinia tuberculosis. Aeromonas caviae* was replicated in house flies for about 2 days and endured for up to 8 days post digestion, and a large number of viable bacteria were shed in vomitus and feces (Nayduch *et al.*, 2002). Similarly, *Pseudomonas aeruginosa* proliferated and persisted in house flies, and has been discarded in excreta for at least 24 hours post-ingestion (Joyner *et al.*, 2013).

Control procedures are normally established on the use of chemicals, insect pesticides have been widely utilized for house fly control (van Emden and Peakall, 1996). These chemical pesticides hold prospective dangers for both the environment as well as human health and continuously lead to the development of resistance to most used insecticides (Asaeedi *et al.*, 2017).Various pesticides used to control flies showed harmful effects on non-objective organisms, involving those that are natural control agents, such as predators and parasitoids (Scott *et al.*, 1991). To diminish harmful effects on health, environment and to prevent pollution of the ecosystem, research for new highly efficient alternative strategies for pest control such as biopesticides has increased (Rodrigues *et al.*, 1988; Zimmer *et al.,* 2013). Besides, insecticides and insect growth regulators, attention has been given to biological control of flies especially in livestock units where predators and parasites may be used to control fly populations (Noorman, 2001). Among bioinsecticides, efforts focused on pathogenic organisms such as nematodes, fungi, bacteria, and viruses (Geden, 2012; Ruiu *et al.,* 2013). The application of different procedures in house fly control is necessary to limit and suppress this pest and to prevent the transmission of infectious diseases to humans and animals. For that, health education, appropriate environmental cleanliness, and personal sanitation are reassured (Issa, 2019). Because of their high dispersion in the ecosystem, bacteria could develop different interactions with insects such as symbiosis (Feldhaar, 2011).

While several bacterial species occupy insect bodies and create various degrees of reciprocal relationships, only a small number of them act as insect diseases, developing several strategies to enter the host, conquer, influence, and destroy its immune responses (Vilcinskas, 2010).

II. Biology of House Fly

House fly *M. domestica* has a full metamorphosis including clear egg, larval, pupal, and adult stages (Cossé and Baker 1996). House flies can live from 15-30 days, females become sexually mature within 2-3 days post-emergence and mate once, while males usually mate several times from the day of their emergence (Saccà, 1964). Oviposition takes place four days after copulation and the female lays several batches of 100 to 155 eggs for 3-4 days, during its lifetime. Females deposit eggs in a humid medium such as cracks and crevices to protect them from dryness, their main breeding areas are usually manure and spilled food (Kelling, 2001; Weeks *et al.*, 2017). Usually, warm summer conditions are ideal for their development as they can complete their life cycle within 7-10 days. While under undesirable conditions life cycle may need two months. In temperate regions, around 10 generations may occur annually, while more than 20 generations may occur in subtropical and tropical regions (Weeks *et al.*, 2017).

Whitish 1 mm long eggs hatch after 8-20 hours post oviposition. Saprophytic larvae, white and legless grow through three instars for 4-13 days (Sarwar, 2016). Each of the first and second larval stages lasts around 1-3 days, the third in star larva develops in 3-4 days to a creamy white 8-11 mm long maggot, tapering from the front and thicker behind to a shortened back end, where two apparent black spiracles are placed through which the tracheal system is attached with the exterior air (Kelling, 2001). At optimum temperature (32-37°C), pupae could finalize their growth for 2-6 days. Thus, the entire life cycle from egg to adult laying eggs ranges from 14-18 days under ideal conditions (25°C). Numerous generations could grow up during the warm season, but in unfavorable conditions, it could be slow down to nearly six weeks giving emergence to abnormally low size offlies (Kelling, 2001).

III. Pathogens Transmitted by House Fly

The most known way for house fly to transmit pathogens is mechanically (Fisher *et al*., 2017). Hence, some reports have shown that house fly is a disruptive pest and an important pathogenic micro-organism vector such as bacteria, viruses, fungi, and protozoa among human and animal (Sanchez-Arroyo and Capinera, 2014). Adults houseflies consume human foodstuff, various excretions, animal compost, moisture, meat potage, milk, trash, and damp or decomposing material of pet litter because of their strong odor. They usually suck up their food through their proboscis because they cannot grind or chew.

If a fly sucks up food from any infectious source, some of the germs attach to the fly's mouth/body part, and when the fly comes in contact with human food, pathogens move on it (Malik *et al.*,

2007). Szalanski *et al.* (2004) reported that flies breeding in feces and other organic waste could become inhabited with pathogenic bacteria such as *Escherichia coli* O157:H7, which affects humans with hemorrhagic colitis and *Campylobacter.* Moreover, Rosef and Kapperud (1983) separated 161 strains of *Campylobacter fetus subsp. Jejuni* from house flies. They noticed that their carrier rates were 50.7% and 43.2% in farms of chicken and pig, respectively. They assumed that flies play a connecting function in the epidemiology of *Campylobacter* contamination in humans by spreading these bacteria from animals to human nutrition. Sukontason *et al*. (2000), in North Thailand and urban areas of Chiang Mai province, evaluated the number of bacteria on house flies and found that about 60 percent of the *M. domestica* flies transported around 1 to 5 strains of bacteria and that *Staphylococci* were the most excessive. Various studies isolated highly infectious bacteria from house flies, comprising enteropathogenic strains such as enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enterohaemorrhagic *E. coli* (EHEC), and enteropathogenic E. coli (EPEC) (Fleming *et al.*, 2014; Solà-Ginés *et al.*, 2015; Songe *et al.*, 2017).

The areas of flies' collection are related to the micro-organisms transmitted by these insects. Places such as hospitals and animal farms where antibiotic and growth stimulators are applied extensively had flies carrying antimicrobial-resistant micro-organisms (Davari*et al.*, 2010; Nazari *et al.*, 2017). Previously, Rady *et al.* (1992) isolated 21 bacterial species of house flies collected from four general hospitals in Cairo (Egypt). Nine species of *Enterobacteriaceae,* two species of *Brucellaceae, one species of Acromobacteriaceae,* and *Pseudomonodaceae*. Boulesteix *et al.* (2005) also explored how the house fly is spreading multi-resistant microbes at the intensive care units of hospitals in sub-Saharan Africa. They revealed that 99 flies from 120 carried human pathogenic micro-organisms, and alarmingly, 17 flies carried antibiotic-resistant bacterial strains (including methicillin-resistant *Staphylococcus* and ticarcillin resistant *Pseudomonas*). Furthermore, Khamesipour *et al.* (2018), were able to isolate 130 pathogenic organisms from the house fly were bacteria was the most frequent.

In their study, Macovei and Zurek (2006) mentioned that houseflies in food-handling and supply amenities houseflies can transport and may be able to deliver antibiotic-resistant and potentially virulent bacteria. Moreover, several studies registered multiple antibiotic bacterial species isolated from house flies: *E. coli*; *Klebsiella pneumoniae* (Davari *et al.*, 2010; Fotedar *et al.*, 1992) and *Pseudomonas aeruginosa* (Davari *et al.*, 2010; Hemmatinezhad *et al.*, 2015). On the other hand, Olsen and Hammack (2000) isolated *Salmonella enteritidis, S. infantis,* and *S. Heidelberg* from house flies around poultry houses. Also, Nazni *et al.* (2005) isolated *Bacillus sp., Staphylococcus sp.* and *Micrococcus sp*. from feces and spews of houseflies extra than from their outer body. In India, during a craze, Fotedar (2001) showed the ability of house flies as a vector in transmitting *Vibrio cholerae*.

Reports indicated that antimicrobial-resistant strains responsible for 10% of in-hospital nosocomial infections such as *Klebsiella* species were transmitted by pests, including house flies and cockroaches (Fotedar *et al*., 1991; Davari *et al.,* 2010; Tajbakhsh *et al.*, 2015).In Japan, Sasaki *et al.* (2000) mentioned that house flies transmitted a toxic strain of *Escherichia coli*. Moreover, the World Health Organization (2004) reported that just trachoma transmitted by fly can cause six million cases of childhood blindness yearly. Because of their high activity, house flies are involved in transmitting many severe and widespread diseases. Flies come into contact with excreta, cadavers, garbage, and different infected matter, and at the same time, flies are closely associated with human's food and tools (Keiding 1986; Nichols, 2005). The kind and quantity of micro-organisms transported by flies are closely related to the presence of these organisms in the excreta and other wastes where flies grow and feed (Nichols, 2005). Usually, most antibiotic species have been secluded from insects collected from hospital and farms (Solà-Ginés*et al.*, 2015; Hemmatinezhad *et al.*, 2015; Nazari *et al.*, 2017), signifying that house fly shows a part in propagation of antibiotic-resistant species in the ecosystem (Zurek and Ghosh, 2014). A growing problem in hospitals and other health care facilities is house flies' involvement in transmitting life-frightening antibiotic-resistant bacteria (Boulesteix *et al.* 2005; Macovei and Zurek, 2006). A recent study, mentioned the contribution of house flyin the spread of avian influenza (Graham *et al.,* 2009).

Because *M. domestica* can bear a variety of bacteria, viruses, fungi, and parasites diseases over their appendages, several significant steps should be accomplished to combat these micro-organisms. One of these actions is to recognize pathogenic agents that enhance health civilization's status and monitor and reduce the population of house flies in human and animal activities (Service, 2000).

IV. Different Approaches used for House Fly Control

a) Mechanical control

Some self-protection behaviors prevent house flies by frequent cleanliness of indoor and the correct way of removing recycling rubbish (Urban and Broce, 2000). It is of importance to enhance ecological purification and hygiene to control house flies (Keiding, 1986).Effective control method for house flies producing in domestic and animal wastes is by removing properly compost or any other organic matter causing

propagation of house fly eggs. Around 50% of houseflies in metropolitan areas occur due to poor management in arranging waste materials from houses, hospitals, and markets.

b) Physical control

Numerous pests are susceptible to ultraviolet light with a frequency of roughly 350 nm. The adults of houseflies are phototactic positively and are captivated to light blue-green (450-550 nm) and ultraviolet (340-365 nm) (Bellingham and Anderson, 1993). Thus, electrocuting traps with fluorescent lamps emitting light in the ultraviolet range are usually used for indoor control of houseflies (Bellingham and Anderson, 1993; Sanchez-Arroyo and Capinera, 2014). It is challenging to preserve a hygienic ambiance and avoid house flies from transmitting diseases. As a substitute, through different physical methods such as light traps, adhesive tapes, fly swats, and electrocuting grids, monitoring the house fly population can be achieved. These techniques are used to precisely kill, repel, or capture the flies without creating any resistance in the flies' body, as observed in the case of chemical insecticides. Methods for physical control are simple and very secure to use. They often do not influence the surroundings but are not very effective in controlling a high density of house flies (Urban and Broce, 2000).

c) Chemical control

Numerous chemical compounds affect different insect systems, including the nervous system, energy production, cuticle production; endocrine system, or water stability that can also be used through various application modes such as topical application, baits, and fumigants to effectively manage house fly population (Shen and Plapp, 1990; Oi *et al*., 1992).For many years, house fly control has been performed by treating the surfaces where the flies usually rest with different chemical compounds such as chlorinated hydrocarbons Dichlorodiphenyltrichloroethane known as DDT and methoxychlor, as well as other (lindane, and chlordane), organophosphates (malathion, diazinon, and dimethoate), carbamates (methomyl), pyrethrins (usually with piperonyl butoxide), pyrethroids (permethrin, fenvalerate, and cyfluthrin), and most recently spinosad (limited use) and neonicotinoid baits (imidacloprid) (Noorman, 2001).

Although chemical insecticides were toxic against a large selection of pests, they also affected non-target organisms. These substances cannot be decayed by organisms and their residues sustained in the environment, get into the food chains, and stored in the body tissue of non-target organisms, as well as humans (Pimental & Perkins, 1980).In addition to the increase of tolerance and resistance of flies to insecticides, the high costs of using insecticides and their toxicity to other organisms make them less desirable for fly control.

Over the years, new pesticides were produced but flies reacted by producing resistance to organophosphate, carbamate, and pyrethroid pesticides (Kozaki *et al.*, 2009; Memmi, 2010). The continual introduction of flies to chemicals has encouraged the development of pesticide resistance (Sanchez-Arroyo and Capinera, 2014). Further pesticides that are safe for mammals were synthetic pyrethroids, although they could affect crustaceans and fish. But, at the same time some of these products are biologically broken down (Hill, 1985).

d) Botanical control

Basic oils insecticides, have been well-known for their fumigant properties, and their method of activity might include components that inhibit the acetylchol inesterase and octopaminergic impacts (Isman, 2000). More impacts could be found in the behavior variation (attraction/repellency) and contact harmfulness for several life stages (Koul *et al*., 2008). Normal oils are composed of numerous biological active constituents, including terpenes, acyclic monoterpene alcohols, monocyclic alcohols, aliphatic aldehydes, sweet-smelling phenols, monocyclic ketones, bicyclic monoterpene ketones, acids, and esters (Koul *et al.*, 2008). For this purpose, a massive effort was performed to investigate different components similar to the established essential oils effective as insecticides (Isman, 2000; Koul *et al*., 2008).

Terpenoids showed different effects on house flies. Some compounds had an attractant effect, others acted as a repellent of females, and both inhibited the larval development (Sharma and Saxena, 1974). Furthermore, Neem extracts and Azadirachtin had been somewhat effective against larvae of the horn fly (*Haematobiairritans*), however, doses required to kill house fly larvae were not useful because they were too high to be manipulated (Miller and Chamberlain, 1989).

The effect of essential oils as insecticide and repellent in flies' control has been reported in several research such as essential oils from orange peel and eucalyptus (Palacios *et al*., 2009 a, b); essential oils of pennyroyal mint (*Mentha pulegium*) and rosemary (*Rosemarinus officinalis*) Pavela (2008). Ezeonu *et al.* (2001), also reported that sweet orange peel extracts (Citrus sinensis) showed a positive effect on adult house flies when used as fumigants. Moreover, Kumar *et al*. (2011), reported that between 6 plant extracts that have been investigated against house fly (*Mentha piperita*) and blue gum (*Eucalyptus globulus*) were the most efficient as insecticidal and repellents. Also, Urzua*et al*. (2010), reported that essential oils from *Haplopappus foliosus* (Asteraceae) were effective on adult house flies.Hence, plant extracts can be used as larvicidal, pupicidal, and adulticidal. Others act as repellents, feeding inhibitors, oviposition reducing, and insect growth managers for house fly as well as for some other

pests (Tsao *et al.*, 1995). Botanical pesticides could be economically and ecologically beneficial as these are more specific than chemical pesticides and do not affect the non-target organism (Willikins and Metcalfe, 1993). Plant oils effect of on flies varies with the sex and the developmental stage of the house fly and the mode of application (Malik *et al.*, 2007).

e) Hormonal control

Searches for alternatives other than insecticides have increased in the last years. Insect growth regulators are called third-generation pesticides. They do not usually kill the target pest immediately, these substances show some selectivity and take a longer time to reduce insect populations than with nerve insecticides (Myamoto *et al.,* 1993). Lindquist *et al.* (1992), mentioned that discharging sterilized male flies could destroy flies population as it was effective against the screwworm fly *Cochliomyia homonivora*in Libya. Also, Howard and Wall (1996 b, c), used triflumuron in sugar -baited targets to sterilize house flies, and they reported that this chemical could decrease the population of house flies in combination with the discharge of predators and parasites. Anyhow, usage of sterile insect technique (SIT) has been constrained by its high cost and logistic complication. Otherwise, discharging a large number of sterilized males around human residency could increase the frustration problem at least for a brief time. Anyhow insect growth regulators IGRs, have no dangerous influences on humans, animals or the environment when applied as listed on the product labels (Oberlander *et al.* 1997). Though widespread resistance against IGRs, also has developed (Pap and Farkas, 1994).

f) Biological control

There are various substitutes to chemical insecticides for house fly control (Achiano and Giliomee, 2005). Entomopathogenic bacteria are additional alternatives to chemical compounds. In addition to their effectiveness, such as safety for humans and other non-target species, elimination of pesticides left in food, defense of natural enemies, and improved biodiversity in the environment, various benefits can be seen in using entomopathogens. Although there are several natural enemies of house flies such as entomopathogenic bacteria, fungi, nematodes, predatory beetles, parasitic wasps, mites, flies, and birds, few cases showed successful results of control by natural enemies, mainly when mixed with other control strategies (integrated fly control) (Urzua *et al.*, 2010).Because pathogenic fungi could be found on animal supplies, their activity varies on temperature and moisture. Besides, contamination of flies in summer is not very high, while it is most needed in summer (Hung and Gerry, 2013). Hence, natural enemies are thought to successfully suppressing the fly, if the right genus and strains are employed in the right region (Pawson & Petersen, 1988).

V. Parasites and Predators

King (1997), explored the efficacy of the parasitoid wasps *Spalangia cameroni* and *Muscidifurax raptor* in controlling fly populations and reported that *S. cameroni* alone seemed to be reliably more efficient in destroying flies' pupae than *M. raptor.* Greene *et al.* (1998), reported that the parasitoid *Spalangianigroaenea* induced mortality in pupae of *M. domestica* by 23 to 58 %, depending on the parasitoid to host ratio. Moreover, *Spalangia cameroni* Perkins and *Muscidifurax raptor* Girault and Sanders (Hymenoptera: Pteromalidae) are ectoparasites of filth fly and they are widely distributed (Taylor *et al*., 2006). These two pupal parasitoid species are commercially available to control house fly*Musca domestica* L. and stable flies *Stomoxys calcitrans* (L.), two pests of medical and veterinary importance. Some researchers pronounced that parasitoids wasps (Pteromalid) that attack pupae were used for fly management as they are the best biocontrol agents (Skovgaard and Nachman, 2004; Geden and Hogsette, 2006), Tsankova and Luvchiev (1993), mentioned that the second and third instar larvae of *Ophyra capensis* can execute as much as 17 housefly larvae varying on the larval instar and the population density. Some studies reported the effect of Histerid beetles and macrochelidae mites as predators on egg and larvae of house flies (Kaufman *et al.,* 2002; Achiano and Giliomee, 2005).

VI. Entomopathogenic Nematodes

Entomopathogenic Nematodes are small roundworms (much less than 1-3mm), parasites of soil-inhabiting insects. These parasites are stated as insecticidal nematodes, such as some species within the genus *Steinernema*(family: Steinernematidae) and *Heterorhabditis* (family: Heterorhabditidae) of the Phylum Nematoda (Mwamburi,2008). Steinernematid and heterrhabditid nematodes when used in the control of filth flies, the larval stage was very sensitive to these entomopathogenic nematodes (Mullens *et al*., 1987; Taylor *et al*., 1998). There is a mutualistic association between Nematodes and micro-organism inhabiting their digestive tracts, these bacteria execute the insect after the nematode conquers its body, some of these bacteria species are *Xenorhabdis nematophilisis* associated with *Steinernematid Steinernema carpocapsae* while *Photorhabdis luminescensis* associated with *Heterorhabditis bacteriophora* (Kaya and Gaugler, 1993; Mwamburi, 2008).Penetration of nematodes into the insect body depends on the host and nematode species, although there are many methods of penetrations such as the mouth or the anus the infection of house fly larvae and leaf miners is through the anus mainly (Renn, 1998), some studies mentioned that the mouth is the most successful way (Cui *et al.*, 1993). *Steinernema feltiae* can go into the

body insect via the cuticle or the inter segmental membranes, penetration through the integument was shown to be their main route of entry (Peters and Ehlers, 1994). An Additional way of entry to the adult insect is the genital openings (Samish and Glazer, 1992). After entering the hemocoel, nematodes feed on the blood, and at the same time, they evacuate the excretions, discharging the symbiotic bacteria (Martens *et al.*, 2004). Bacteria rapidly inhabits the insect and kill it for 1 to 3 days. The nematodes consume the bacteria and tissues of the larval body, it develops and undergo 2-3 generations in a period ranging from one to 2 weeks. The last generation leaves the cadaver searching for a new host (Ciche *et al.*, 2006). Bacteria from nematode destroy the insect as soon as it enters its body, so it cannot form a host-parasite relation. This allows the nematode to visit many hosts and cover most insects' orders (Grewal and Georgis, 1999). The tough behavioral barrier in some insect hosts could limit the efficacy of nematode (Gaugler, 1988).

VII. Entomopathogenic Fungi

Some studies have evaluated the effect of infective fungi for house fly management in the field such as *Entomophthora muscae* (Cohn) Fresenius, and they mentioned that sometimes the pathogenic *E. muscae* could destroy fly populations (Geden *et al.*, 1993; Steinkraus *et al.*, 1993; Watson and Petersen, 1993).Kuramoto and Shimazu (1997), used house flies infected with *Entomophthora muscae* in experimental poultry houses, these flies were able to kill 90% of the originally existing flies after 33 days of their introduction. Normally, the effect of *Beauveria bassiana* and *Metarhizium anisopliae* against house flies and stable flies are low in the field (Skovgaard and Steenberg, 2002). Nevertheless, they showed a high effect in the laboratory trials against larval and adult flies, their virulency depends on the strain and the formulations (Lecuona *et al*., 2005). It is important to use a mixture of pathogenic fungi with chemical insecticides to improve their effectiveness as biological control (Ericsson *et al.,* 2007). Fungi enter the body of the insect through the cuticle (Charnley, 1989) or the trachea (Feng *et al.*, 1994). The conidia attach to the cuticle (Boucias and Pendland, 1991), then germination begins and the insect becomes infected. The hyphae penetrate the cuticle and proliferate into the hemocoel, which causes the insect's death due to toxemia (Khachatourians, 1991).

VIII. Entomopathogenic Virus

One of the Hytrosaviridae family is the salivary gland hypertrophy virus that contaminates house flies, tsetse flies (*Glossina* spp.), and the narcissus bulb fly (*Merodon equestris* Fabr.) (Lietze *et al*., 2011). Contaminated flies do not show any external disease signs. The most visible infection characteristic is the incidence of significantly enlarged (hypertrophied) salivary glands with a blue-whitish presence that often dominates the abdominal cavity of the fly after dissection. Viral duplication and morphogenesis are confined to salivary gland cells, although complete virions are also found in asymptomatic tissues such as the midgut, ovaries, fat body, and brain (Lietze *et al*., 2010). The virus in both sexes of infected flies causes a decrease in mating achievement and shorten life periods. Sustainable virus particles pass by the digestive system of infested flies and are evacuated with feces, even if at low rates (Lietze *et al*., 2007; Lietze *et al*., 2009).

IX. Entomopathogenic Bacteria

To reduce the effect of chemicals on health and the ecosystem, other selected approaches have been applied for insect control. Many different genera of micro-organisms have been utilized as biological insecticides (Rodrigues *et al.*, 1988), and there is a tremendous review on the insecticidal impacts of *Bacillus thuringiensis(Bt)* beside the different isolates that are effective against house fly(Ruiu *et al.*, 2006). *B. thuringiensis,* has many advantages over conventional pesticides, itis specific to certain pest species, eco-friendly, and safe to non-target organisms, mosquitoes did not develop significant resistance to it in the field so far (Bravo *et al.,* 2007).Johnson *et al.* (1998), described the utilization of *Bacillus thuringiensis* as a protected and successful method for controlling rural pest and particularly houseflies. The active factor in the bacteria is a member of Cry IB class of protoxins, and it is created in some strains of *B. thuringiensis.*

Carramaschi *et al.* (2015), reported that *Brevibacillus laterosporus* (Laubach) is a biological control agent. It showed broad entomopathogenic activity against various insects such as blowflies (Pessanha *et al.,* 2015) and house flies (Ruiu *et al.*, 2006; Ruiu *et al.*, 2008; Ruiu *et al.*, 2011; Zimmer *et al.,* 2013). Innovative bacterial species with advanced methods of action have been found and prepared as new biological insecticide products (Ruiu *et al.,* 2013). *Bacillus thuringiensis* has proven an enormous potential factor in the control of livestock pests. Investigation and improvement of the toxins and their method of activity against pests are progressing in several countries (Pinnock, 1994).The impact of *Bacillus thuringiensis* against filth flies was encouraging, the control against larvae was achieved by feeding cattle and chickens with a spore formulation of *Bt* bacteria in that way animals can deliver these bacteria in the manure known as house flies rearing places (Miller *et al.*, 1971), also by blending *Bt* straight forwardly with fly reproducing substrates (Rupes *et al.*, 1987). Some studies used the exotoxin delivering *Bt* strains where flies showed higher

sensitivity than most other pests to the exotoxin (Carlberg, 1986). Indrasith *et al*. (1992), and Johnson *et al*. (1998), detected numerous strains to be effective against adult house flies, and they mentioned that all the *Musca domestica*-active strains had in them the endotoxin Cry1B which might be the key entry of these strains effect against house flies (Lysyk *et al.*, 2010). *Bacillus thuringiensis* was found to be more efficient against house fly when mixed with poultry food rather than added directly to the manure (Labib and Rady, 2001). Additional to the crystal-related poisonous proteins related to sporulation, some *Bt* isolates can produce proteins during their development such as vegetative insecticidal proteins. These vegetative proteins were effective against a big range of Lepidoptera (Estruch *et al.*, 1996; Schnepf *et al.*, 1998).

Most researches have focused on the validity of *Bt*on pest insects that are routes of human infections (Kellar and Langenfruch, 1993; Rajakulendran, 1993; Teakle, 1994). *B. thuringiensis israelensis* were applied as a pesticide compound to control medical dipteran pests such as mosquitoes and blackflies (MullaBecker and Margalit, 1993; Becker, 1997), Btisraelensis showed toxicity to the house fly (Zhong *et al.,* 2000).

The oral effect of bacterial toxins crystalogenic proteins (Cry) and cytolytic (Cyt) affect the larval stage by stimulating the formation of cell membrane lytic pore in the lining epithelia of the midgut, which causes an increase in the permeability of the membrane, paralysis of the intestine, stop digestion and finally kills the larva (Kongsuwan *et al.*, 2005). The recognition of insecticidal bacterial strains against the synanthropic housefly is of great importance. Zimmer *et al.*(2013), evaluated (in artificial medium) the entomopathogenic effects of *B. laterosporus* (Bl), *B. thuringiensis* var. *israelensis* (Bti), *B. thuringiensis* var. *kurstaki* (Btk), against immature and adult life stages of *M. domestica*. There is a convincing opportunity for using microbial control agents against flies as they are reasonably selective, active, and there are many options for implementations. *Bacillus thuringiensis* (Berliner) (Bt) is a naturally occurring bacterium creating proteins that are active as insecticides against many species.

House fly and antimicrobial resistance strains

Regrettably, restricting the human diseases transferred by house flies has not been successful due to the shortage of knowledge of this species' basic molecular process (Scott *et al.,* 2009). Adjustment to distinct ecological environments might result in the progression of specific immunity of house flies. Therefore, comparing the instinctive immune systems of *Musca domestica* with those of the species that face different ecological pressures and pathogens such as *Drosophila* and *Anopheles* can be very informative and thus offer clues on how house flies can flourish in close contact with many pathogens (Scott *et al.,* 2009).

There are some public health concerns regarding the global use of agricultural antibiotic and the increasing of drug-resistant bacteria(Levy & Marshall 2004; Erb *et al.*, 2007). A significant quantity of antibiotic-resistant bacteria with resistant genes have been found in poultry litter, where antibiotics are used to produce poultry (Nandi *et al.,* 2004).The house fly could take part in in disseminating these antibiotic-resistant bacteria from the poultry or hospital areas to the ecosystem (Winpisinger *et al.*, 2005; Akter *et al*., 2020). The antibiotic-resistant *enterococci* and *staphylococci* have been isolated from poultry litter (Hayes *et al.,* 2004; Simjee *et al.,* 2007).

X. Conclusion

Several studies confirmed the competence of house flies in dispersing numerous species of micro-organisms. Hence, the flies transport these micro-organisms, including bacterial species on their body surface or through their internal digestive tract and transmit them to human and animal food while their feeding mechanism. Previous studies indicated that among the bacteria transmitted by house fly, some antibiotic-resistant species worsen the problem. Recently, different species of bacteria proved their efficiency in reducing the population density of house fly. Therefore, it is of importance that researchers focus on biological pest control to avoid the damage inflicted by chemical insecticides.

Abbreviations

Bt: Bacillus thuringiensis

E. coli: Escherichia coli

M. domestica L.: *Musca domestica* Linnaeus

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Enhancing the Production of Carp Polyculture and Tilapia by Integrating with Duck Farming in Nepal"- Aquaculture for Small Scale Farmers and Sustainability

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Abstract- Integrated duck cum fish farming is suitable for developing countries like Nepal as it uses the locally available resources. This study was conducted for 120 days in an earthen pond of area 575 m^2. The fish stocked were *Labeorohita*(25%), *Cirrhinus Mrigala* (10%), *Cyrinuscarpio* (25%), *Aristichthys nobilis* (5%), *Ctenopharyngodonidella* (15%) and *Oreochromis niloticus*(20%) with the stocking density of 13000 fingerlings/ha. Fish were fed with dough formed with locally available ingredients like MOC and rice bran containing 20% CP at the rate of 2% of total body weight daily. The results showed the extrapolated GFY to be 4.0 t/ha/yr and extrapolated NFY was 2.9 t/ha/yr of total fish species. The total fish yield was 53.2 kg and the total feed supplied was 76.8 kg. The overall survival rate of fish was 66.0% whereas the AFCR was 1.4. Duck growth showed a normal trend from mean stock weight of 161±69.8 g/duck to mean harvest weight 1114.4±296.4 g/duck. Similarly, daily weight gain was 7.95 g/duck/day. The benefit: cost ratio for duck and fish production was 1.24and 1.65 respectively. This study concludes that carp- tilapia polyculture in integration with duck is reliable, economically viable, and effective for the small-scale fish farmers as well as the marginal groups.

Keywords: *integrated duck-fish, polyculture, tilapia, economic efficiency, small-scale farmers, nepal.*

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I. Introduction

Sustainable aquaculture, innovation of modern technologies, enhancing livelihoods, and global food security are the long-term goals of aquaculture development (Rai *et al*., 2008). Nepal is a landlocked country with an abundance of freshwater water bodies having a high possibility of aquaculture. Fish is considered auspicious and symbolizes a sign of fertility, power, and prosperity in Nepal (Gurung *et al*., 2003). In Nepal, fish culture is the prevailing type of aquaculture and is cultured in different systems. Aquaculture is at its blooming phase with an annual growth rate of about 8-9% in Nepal (Gurung, 2016).Aquaculture and fisheries contribute about 4.29% in agriculture domestic production (AGDP) and nearly 1.34% in GDP (DoFD, 2017). National production of fish was 77,000 mt of which about 72% contributed by aquaculture and 28% from capture fisheries (Kunwar and Adhikari, 2016).

Stocking the complementary species of fishes in a pond can increase the maximum standing crop by allowing a wider range of available foods and ecological niches (Da silva *et al*., 2006). Polyculture is also known as multi-trophic aquaculture, co-culture, or integrated aquaculture (Bunting, 2008).One of the most widely practiced pond aquaculture systems in Central Asia is carp polyculture (Woynarovich, 2010). Literally, polyculture fits the principles of sustainable aquaculture. With advanced ecological stability and optimizing the use of available resources, this system reduces the environmental impact of the activity and increases producer profitability (McKinnon *et al.,* 2002). In Nepal, pond fish culture contributes about 89.1% of total production from aquaculture which is mainly prevailed by the carp polyculture in earthen ponds (CFPCC, 2018).

Combination of mainly IMC (Indian Major Carps) and CMC (Chinese Major Carps) along with Common carp (*Cyprinus carpio*) is the most commonly used concept in carp polyculture Polyculture of carp species contributes about 70% of total aquaculture production in four countries (India, Myanmar, Nepal and Pakistan) of south Asia (FAO, 2016). Among different experiments, the addition of Nile tilapia (*Oreochromis niloticus*) and Sahar (*Tor putitora*) has been successfully proven to increase the total production and gross margin in pond aquaculture (Shrestha *et al.,* 2011).

Nile tilapia, in comparison to other species, has many aquaculture attributes such as excellent growth rates, low dietary protein requirement, and its prolific breeding nature. Tilapia can tolerate wide ranges of environmental conditions, less susceptibility to disease, and responsive to handling and captivity.

Integrated fish farming mainly focuses on production, integrated management, and comprehensive use of aquaculture, agriculture, and livestock, with an emphasis on aquaculture. The major features of this system include recycling of by-products

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in which the waste of one system becomes the input of other systems and efficient utilization of space. According to Latif (1993), integrating duck farming with aquaculture is an economically viable and productive system for both farmers and commercial entrepreneurs.

The duck droppings act as excellent organic fertilizer for the fish pond which accounts for 60% of the total input cost in fish culture (Shrestha& Pandit, 2012). Duck manure is considered one of the effective nutrients for enhancing the growth of natural food (Latif, 1993). Some fish species like common carp *(Cyprinus carpio)* intake duck dropping directly as their feed (Biswas, 2015).

Integrating duck with fish farming has many benefits like utilization of duck droppings by the fish as natural food, space utilization, and droppings can be used as manure. If the ducks are raised in ponds 2-3% of protein in duck feeds will be reduced. Ducks act as natural aerators by their swimming and dabbling activities. Integrating duck with fish culture ensures the farmers high profit with less investment (Majhi, 2018).

II. Materials and Methods

a) Study Site

The duck cum fish integrated farming was practiced in the Aquaculture farm of Fisheries Program, Agriculture and Forestry University located in Rampur, Chitwan. The study was conducted for 120 days from March 15 to mid-July. The area of the pond was 575 m^2 with a duck shed on the dyke previously constructed by the farm.

b) Materials and Methodology

Pond was prepared by draining, thorough cleaning, and removal of the existing fish and aquatic vegetation. Dry liming was done at the rate of 200 kg/ha with agriculture lime ($CaCO_3$). After 7 days of liming, the pond was fertilized with fresh cow dung at the rate of 3000 kg/ha. The ponds were filled with fresh water after the organic fertilization. Water depth was maintained at 1 meter deep. Inorganic fertilizers such as Urea and DAP were applied at the rate of 4.7 g/m^2/week and 3.5 g/m^2/week respectively.

The pond was stocked with Common carp (25%), Bighead carp (5%), Grass carp (15%), Rohu (25%), Mrigal (10%), and Nile tilapia (20%). The number and amount of fingerlings stocked is tabulated below:

Table 1: Stocking number and weight of the fish species in the pond

S.No.	Species	Stocked Number(No/Pond	Total Stocked Weight (g/pond)	Percentage (%)
1	*Cyprinus carpio*	145	2760	25
2	*Aristichthys nobilis*	30	1730	5
3	*Ctenopharyngodonidella*	82	890	15
4	*Labeorohita*	140	6000	25
5	*Cirrhinusmrigala*	55	530	10
6	*Oreochromis niloticus*	130	8200	20
	Total	582	20110	100

Feed containing 20% CP was given twice a day at 10 am and 3 pm at the rate of 2% of total body weight. Farm-made feed was fed to the fish for the reduction of the cost of production. The mixture of locally available rice bran and mustard oil cake in a 1:1 ratio was made in a dough form each day. The vitamin and mineral mixture was added at the rate of 1kg per 100 kg feed. For Grass carp, different types of vegetation like *Colocassia*, banana leaves, Para grass, and *Napier* were fed by chopping them into small pieces. Sampling was done every 2 weeks to check the growth performance and to estimate the amount of feed required. After 3 months of stocking the fish, partial harvesting was initiated. Complete harvesting was done by draining the pond water completely.

c) Rearing of Duck

Prior to stocking, the duck shed was cleaned with water thoroughly. Total 14 ducklings with an average weight of 161±69.8g were stocked. Mainly, rice husk was fed by mixing homogeneously with water in a feeding tray. Feed was given 4 times daily at 10 am, 12 pm, 3pm, and 5 pm. Sampling of duck was done every month to observe the growth rate. Each duckling was weighed individually on a weighing machine separately. After 4 months of rearing, ducks were harvested.

d) Analytical Method

i. Fish Growth Measurements

Growth and production was calculated using the following formulae:

$$Daily\ Weight\ Gain(g/fish/day) = \frac{Average\ Harvest\ Weight(g) - \ Average\ stocked\ Weight\ (g)}{Culture\ period\ (days)}$$

$$Total\ Weight\ Gain = \frac{Harvested\ Weight\ (g) - Stocked\ Weight(g)}{Pond\ Area(m^2)} * 100$$

$$Survival\ rate\ (\%) = \frac{Total\ number\ of\ fish\ harvested}{Total\ number\ of\ fish\ stocked} \times 100$$

$$Net\ fish\ yield\ (g/m^2/day) = \frac{Total\ harvested\ weight\ (g) - \ Total\ stocked\ weight\ (g)}{Culture\ peiod\ (days) \times Culture\ Units\ (m^2)}$$

$$Apparent\ food\ conversion\ ratio\ (AFCR) = \frac{Quantity\ of\ feed\ fed\ (kg)}{Net\ fish\ yield\ (kg)}$$

$$Extrapolated\ NFY\ (t/ha/yr) = \frac{Total\ harvest\ weight(g) - Total\ stocked\ weight(g)}{Culture\ period(days) * Culture\ Unit(m^2) * 1000 * 1000} * 10000 * 365$$

$$Extrapolated\ GFY\ (t/ha/yr) = \frac{Total\ harvest\ weight(g)}{Culture\ period(days) * Culture\ Unit(m^2) * 1000 * 1000} * 10000 * 365$$

e) Water quality analysis

Water quality parameters were monitored and recorded daily during the entire culture period. Physical parameters like dissolved oxygen (DO), temperature with DO meter (Lutran, DO 5519), and pH by using pH meter (Lutran, pH 222)were recorded daily.

f) Economic analysis

Simple gross margin analysis was done after the complete harvesting of the fish. Gross margin analysis was based on the farm prices for the harvested fish. The rate of fish per kg was estimated as NRs.300 per/day for all species of fish.

$$Gross\ margin\ (NRs) = Gross\ return\ (NRs) - \ Total\ variable\ cost\ (NRs)$$

$$Gross\ return\ (NRs) = Price\ of\ fish\ \left(\frac{NRs}{kg}\right) \times Total\ quantity\ produced(kg)$$

$$Total\ variable\ cost\ (NRs) = \sum cost\ incurred\ in\ all\ the\ variable\ items\ (NRs)$$

g) Statistical analysis

Statistical analysis of data was performed by using MS- Excel. Mean and standard deviation was calculated and differences were compared. Means were given with standard deviation (Mean± SD).

III. Results and Discussion

a) Water quality analysis

Water quality parameters like temperature, dissolved oxygen, and pH were recorded daily during the culture period of 117 days. The mean and range of water quality parameters recorded are presented in Table 2 and Figures 1-3. The range of all the water quality parameters is similar as reported by Jena *et al.,*(2002) and Jha *et al*., (2018).

Table 2: Mean and range of water quality parameters during the culture period (Mean±SD)

Parameter	Unit	Average	Range
Dissolved oxygen	mg/L	3.0±0.6	0.9-7.2
Temperature	°C	27.9±1.4	23.8-30.6
pH		6.7	6.09-9.7

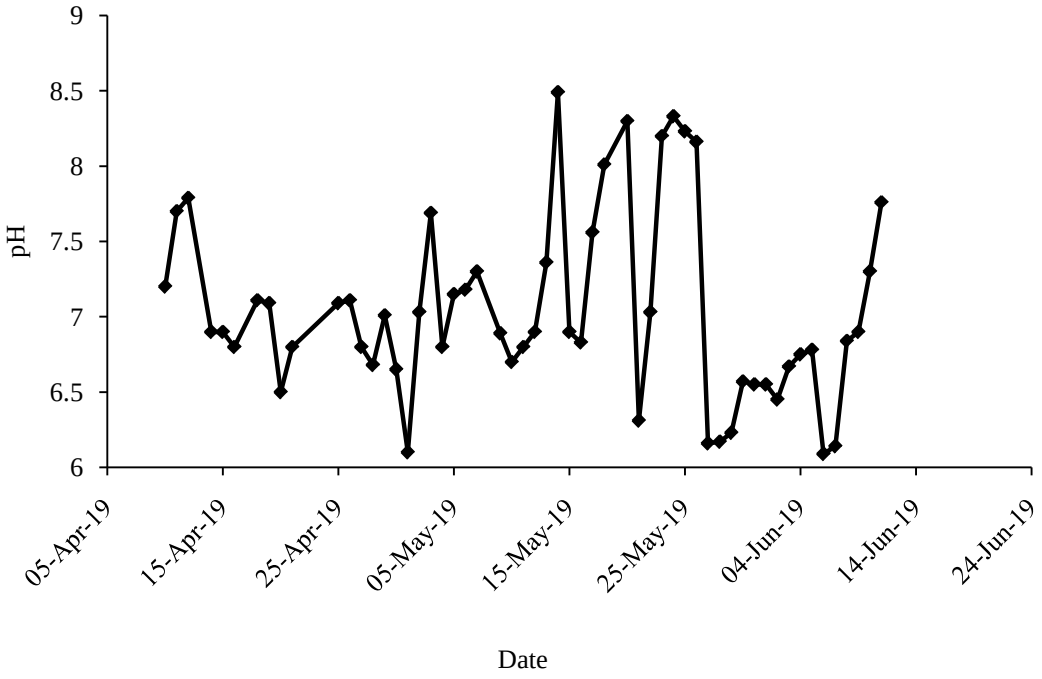

Figure 1: pH of pond water during the culture period

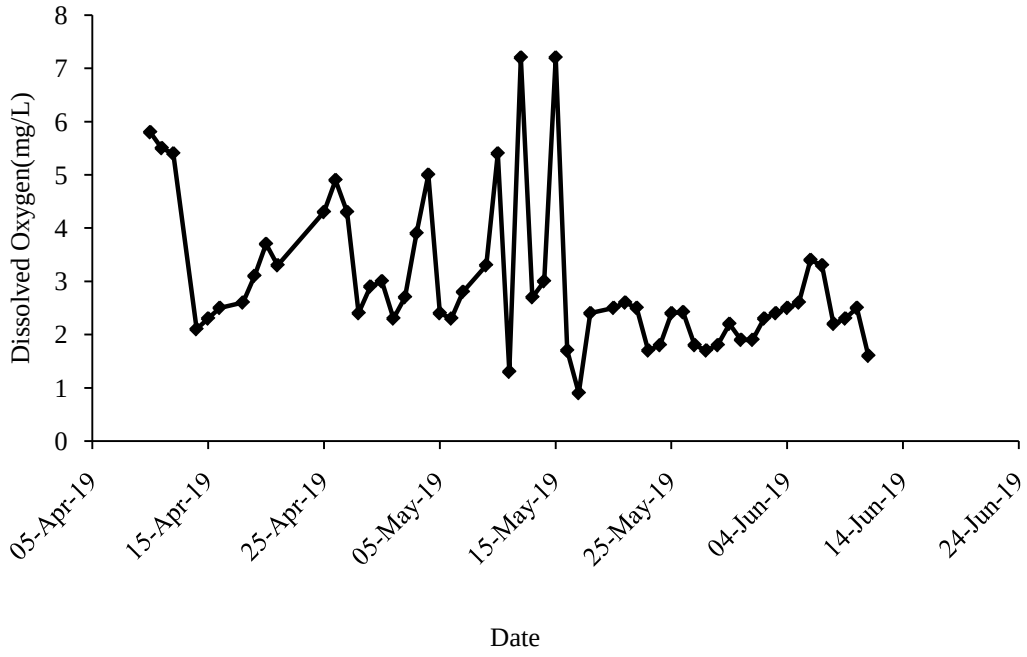

Figure 2: Dissolved oxygen (mg/L) during the culture period

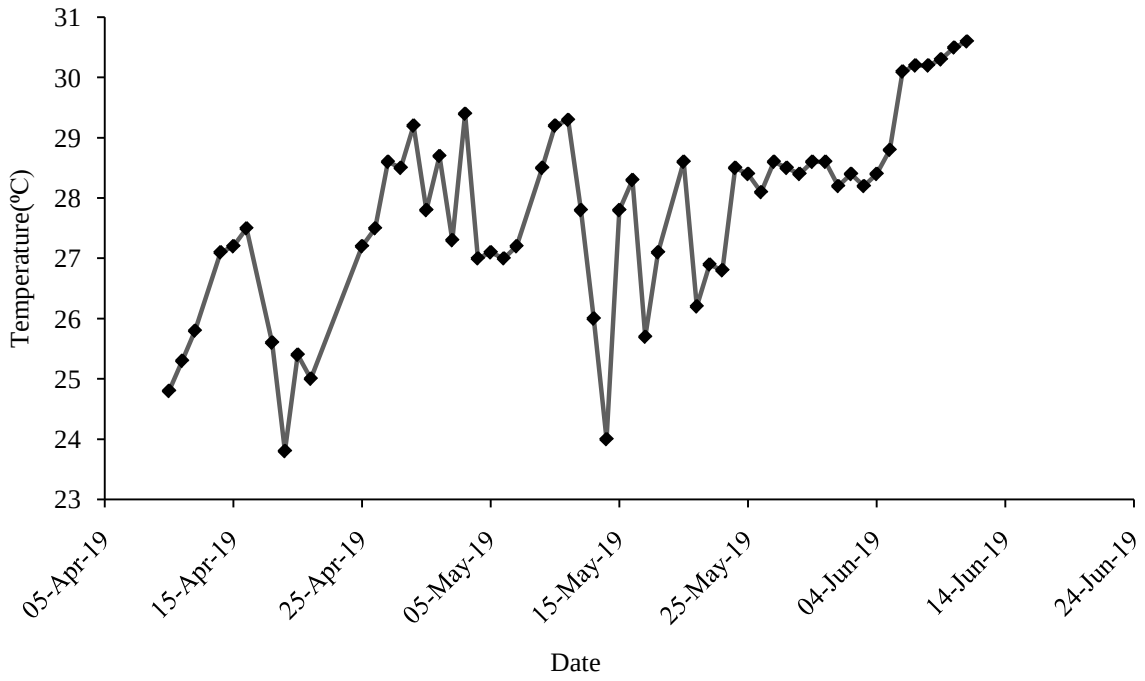

Figure 3: Temperature (°C) during the culture period

b) Fish growth and production

Table 3: Growth and production parameters

Growth and Production Parameters	*C. mrigala*	*C. carpio*	*A. nobilis*	*C. idella*	*O. niloticus*	*L. rohita*
Total weight gain (g/100m$^{2)}$	240.3	3742.0	1736.0	359.4	1110.1	2050.3
Daily weight gain (g/fish/day)	2.0	1.7	3.0	0.6	0.6	1.5
Survival rate (%)	14.5	80.7	93.3	47.6	81.5	61.4
Extrapolated GFY (t/ha/yr)	0.1	1.3	0.6	0.2	0.8	1.0
Extrapolated NFY (t/ha/yr)	0.07	1.17	0.54	0.11	0.35	0.64

Table 3 shows the growth and production parameters of all fish species during the culture period of 117 days. The daily weight gain and total weight gain of Rohu was found to be 1.5 g/fish/day and 2.05kg/100m^2 respectively. Similarly, the survival rate was 61.4%, and extrapolated GFY and NFY were1.0 t/ha/yr and 0.64 t/ha/yr respectively. The daily weight gain and total weight gain of Mrigal was found to be 2.0 g/fish/day and 240.3 g/100m^2 respectively. Similarly, the survival rate was 14.5% and extrapolated GFY and NFY 0.1 t/ha/yr and 0.07 t/ha/yr respectively. The daily weight gain and total weight gain of Common carp were found to be 1.7 g/fish/day and 3742.0 g/100m^{2}respectively. Similarly, the survival rate was 80.7% and extrapolated GFY and NFY 1.3 t/ha/yr and 1.17 t/ha/yr respectively. The daily weight gain and total weight gain of Bighead carp were found to be 3.0 g/fish/day and 1736.0 g/100m^2 respectively. Similarly, the survival rate was 93.3%, and extrapolated GFY and NFY was 0.6 t/ha/yr and 0.54 t/ha/yr respectively. The daily weight gain and total weight gain of Grass carp were found to be 0.6 g/fish/day and 359.4 g/100m^2 respectively. Similarly, the survival rate was 47.6%, and extrapolated GFY and NFY were0.2 t/ha/yr and 0.11 t/ha/yr respectively. The daily weight gain and total weight gain of Tilapia were found to be 0.6 g/fish/day and 1110.1 g/100m^2 respectively. Similarly, the survival rate was 81.5%, and extrapolated GFY and NFY were0.8 t/ha/yr and 0.35 t/ha/yr respectively.

c) *Growth Trend of Fish*

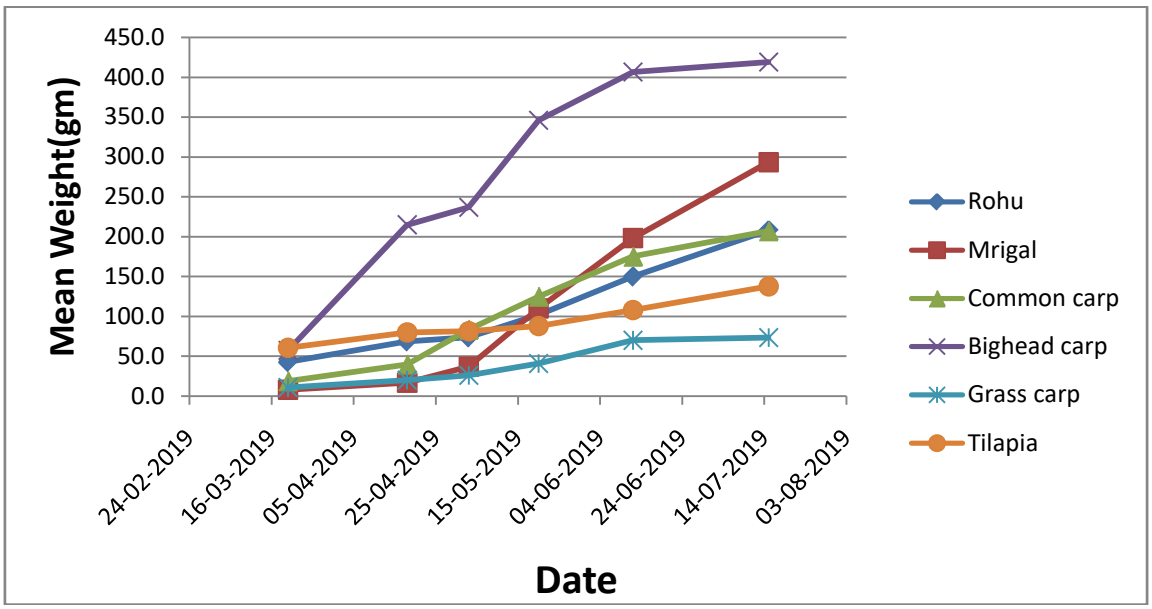

Figure 4: Growth trend of carps and nile tilapia during the culture period

i. *Rohu (Labeorohita)*

From the graph, it is clear that the growth of Rohu followed a normal trend from an average stocking weight of 42.1±4.6 g/fish to an average harvested weight of 212.2±6.8 g/fish. The daily weight gain of Rohu in the present study showed lower (1.5 g/fish/day) than as reported (2.5±0.1 g/fish/day) by Mandal *et al*., (2018) but higher than as reported by Jha *et al*. (2018). The higher weight gain might be due to the availability of natural food due to duck droppings incorporation. The survival rate of present work was 61.4% which is lower than as reported by Uddin *et al*., (2012), lower than that reported (91.0%) by Roy(2016), and also lower than as reported by Azim & Wahab (2003) which was 71%.

ii. *Mrigal(Cirrhinusmrigala)*

From the graph, it is clear that the growth of Mrigal followed a normal trend from an average stocking weight of 9.8±0.4 g/fish to an average harvesting weight of 242.8±32.4g/fish. In the present work, the daily weight gain was 2.0 g/fish/day which was slightly higher than as reported (1.8±0.1 g/fish/day) by Mandal *et al*., (2018) and also higher than as reported (0.7±0.1 g/fish/day) by Jha *et al.,*(2018). According to Uddin *et al*., (2012), the survival rate of Mrigal was 90.2±2.20 % which was 14.5% during the present work. Also, the survival rate of Mrigal as reported by Mandal *et al*., (2018) in previous work was 40.5±4.1%. and 66.0±5.3 % as reported by Jha *et al.,*(2018).

iii. *Common Carp (Cyprinus carpio)*

From the graph, it is clear that the growth of Common carp followed a normal trend from an average stocking weight of 15.1±3.7g/fish to an average harvesting weight of 208.6±84.0g/fish. DWG of Common carp during present work is 1.7 g/fish/day which is lower than 2.2±0.1 g/fish/day as reported by Mandal et al., (2018) and also lower than as reported (5.1±1.7 g/fish/day) by Jha *et al.,* (2018) but higher than as reported (1.11±0.07 g/fish/day) by Bhandari (2016). The survival rate of Common carp is higher in present work (80.7%) than as reported (22.7±3.8%) by Jha *et al.,* (2018) and (78±7%) by Bhandari(2016) but lower than as reported (84%) by Azim & Wahab(2003) in development of a duckweed fed carp polyculture system in Bangladesh.

iv. *Bighead Carp(Aristichthys nobilis)*

From the graph, it is clear that the growth of Bighead carp followed a normal trend from an average stocking weight of 57.2±2.5g/fish to an average harvesting weight of 411.1±42.7g/fish. Bighead Carp showed a daily weight gain of 3.0 g/fish/day in the present work which is higher than as reported (2.6±0.8 g/fish/day) by Mandal *et al.,*(2018) and also higher than 2.1±0.1 g/fish/day as reported by Jha et al. (2018). The survival rate of bighead carp was relatively similar as reported by Mandal et*al.,*(2018). It was remarkably higher than that as reported (45.4±2.5 %) by Jha et al.(2018) in the production of periphyton to enhance yield in polyculture ponds with carps and small indigenous species.

v. *Grass Carp(Ctenopharyngodon idella)*

From the graph, it is clear that the growth of Grass carp followed a normal trend from an average stocking weight of 15.1±3.7g/fish to average harvesting weight of 208.6±84.0g/fish. As reported in Jha *et al.,*(2018), the daily weight gain of Grass carp is 2.2±0.2 g/fish/day which is only 0.6 g/fish/day in the current

work. Pandit *et al*.,(2004) reported the daily weight gain of Grass carp to be 3.14±0.15 g/fish/day when stocked at 0.5 fish/m^2. The survival rate of Grass carp is 47.6% in the present work is similar to as reported (45.4±2.6%) by Jha *et al.,*(2018) but reported higher by Bhandari (2016) in carp and tilapia culture.

vi. *Nile Tilapia(Oreochromis niloticus)*

From the graph, it is clear that the growth of Tilapia followed a normal trend from an average stocking weight of 59.4±13.4g/fish to an average harvesting weight of 132.2±15.9g/fish. According to Guerrero III *et al*.,(1988), the survival rate of Nile tilapia was 100% in commercial diet and 93% in chicken manure but it is 81.5% in the present work where duck droppings were used. Also, the survival rate was reported lower as compared to the present work by Bhandari(2016) which was 69±5 %.The DWG of Nile tilapia was estimated to be 0.8-1.0 g/fish/day by Shrestha & Jaiswal (2011) is higher compared to the present work (0.6 g/fish/day).But the DWG in the present work is higher as compared to the result reported by Pandit *et al*., (2004) which was 0.40±0.02 in polyculture of grass carp and Nile tilapia with Napier grass as the sole nutrient input in the subtropical climate of Nepal where tilapia was stocked at 0.5 fish/m^2.

d) Combined Fish Production

Table 4: Production of fish species

Production parameters	Unit	Value
Stocked weight	kg/pond	19.92
Stocked number	Number/pond	582
Harvested fish	kg/pond	73.13
Harvest number	Number/pond	384
Fish yield	kg/pond	53.21
Feed supplied	kg	76.75
Pond area	m^2	576
Culture period	Days	117
Extrapolated GFY	t/ha/yr	4.0
Extrapolated NFY	t/ha/yr	2.9
Overall survival rate	%	66.0
AFCR		1.4

Table 4 presents the production and yield parameters of fish during the culture period of 120 days. Total fish yield of 53.21 kg of fish was gained from the pond of 575m^2.Extrapolated NFY and GFY were calculated to be 4.0 t/ha/yr and 2.9 t/ha/yr respectively. The overall survival rate was 66 %. The apparent food conversion ratio was found to be 1.4.

The overall survival rate of the present work was estimated to be 66% which is lower than that as reported by Bhandari (2016) in the value of Nile tilapia and Sahar in carp polyculture pond in improving pond productivity. Similarly, Mandal *et al*., (2018) reported a survival rate of 75.2±5.8 %, and Jena *et al.,*(2002) reported an 88.0±0.2% survival rate. Bhandari (2016) reported the FCR to be 2.62±0.17 which in present work is 1.4. Similarly, Mandal *et al.,*(2018) reported the FCR value of 1.5±0.2 which is nearly equal to that of the present work.

e) Duck growth and production

Figure 5 indicates the average weight of duck during the rearing period. The growth of duck showed a normal trend from mean stock weight of 161±69.8 g/duck to mean harvest weight 1114.4±296.4 g/duck.

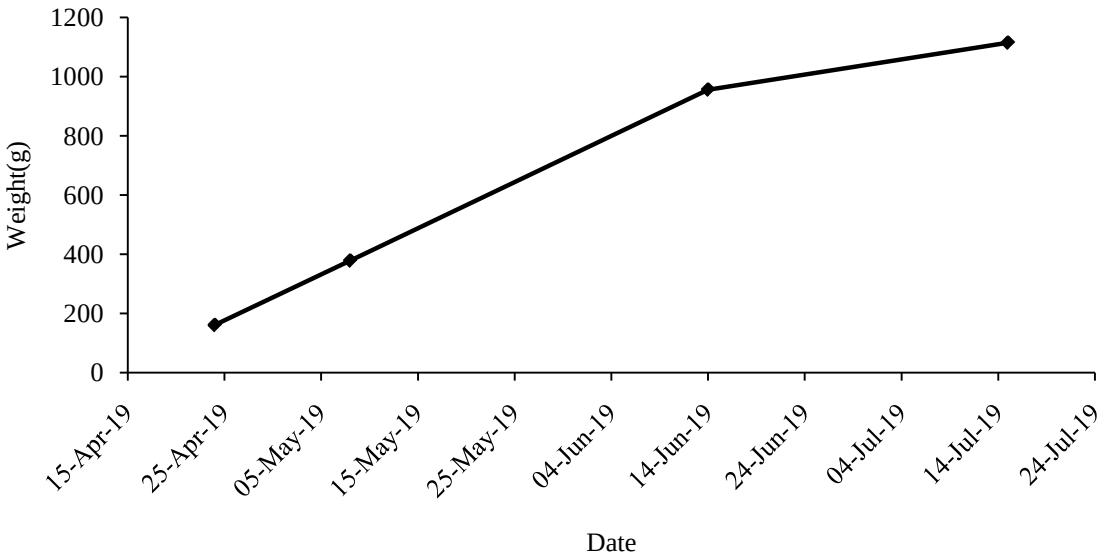

Figure 5: Average weight of duck during rearing period

Table 5 indicates the growth of duck during the rearing period. The rearing period of duck was about 120 days. The mean stock weight and mean harvest weight was 161±69.8 g/duck and 1114.4±296.4 g/duck respectively. Similarly, daily weight gain was 7.9 g/duck/day.

During the rearing period of a duck the mean weight harvest was 1114.1±296.4 g/duck in the present work which is similar as reported(1304g) by Kumar *et al*.,(2012) for the same work period. According to Latif *et al*.,(1993) the final mean weight ranged from 1200-1800g for a period of 4-6 months.

Table 5: Growth and production of Duck during rearing period

Growth Parameters	Unit	Value
Stocked no	Number	14
Stocked weight	kg	2.25
Mean stocked wt.	g/duck	161±69.8
Total harvest no	Number	14
Total harvest wt.	kg	15.6
Mean harvest wt.(g/duck)	g/duck	1114.4±296.4
Daily weight gain (g/duck/day)	g/duck/day	7.94

f) Gross margin Analysis

i. *Gross margin analysis of duck cum fish integration*

The total variable cost involved in the fish production was NRs.18,286.54. Similarly, the production cost was NRs. 150.7 per kg and benefit: cost ratio was 1.69. All the variables and costs are tabulated below in Table 6.

Table 6: Gross margin analysis of Carp-Tilapia-Duck integrated farming

Variable cost	Unit	Quantity	Rate (Rs/kg)	Amount
Variables				
Urea	kg	5.41	20	108.2
DAP	kg	4.0	55	221.76
Cow dung	kg	57.6	2	115.2
Lime	kg	11.52	12	138.24
Feed				

MOC	kg	38.378	30	1151.34
Ricebran	kg	38.378	35	1343.23
Vitamin	kg	0.76	300	228
Fish seed	kg	19.92	300	5976
Diesel	L	9.25	100	925
Electricity	kWh	81	10	810
Ducklings	Number	14	300	4200
Duck feed	kg	65.31	47	3069.57
Total variable cost(NRs.)				18286.54
Return				
Rohu	kg	17.8	300	5340
Mrigal	kg	1.9	300	570
Common	kg	24.2	300	7260
Bighead	kg	11.7	300	3510
Grass	kg	2.9	300	870
Tilapia	kg	14.6	300	4380
Duck	kg	15.6	576	8985.6
Gross return (NRs.)				30915.6
Net return (NRs.)				12629.06
Production cost (NRs./kg)				150.71
B:C ratio				1.69

g) Cost analysis of duck farming

Table 7 indicates the economic analysis of duck farming integrated with the fish culture. The ducklings were reared for 120 days. The production cost was estimated as NRs.465.99 per kg and the benefit: cost ratio was 1.24.

Table 7: Economic analysis of duck farming

Variable cost	Quantity(kg)	Rate (Rs/kg)	Amount
Ducklings	14	300	4200
Duck feed	65.31	47	3069.57
Total variable cost(NRs.)			7269.57
Return			
Duck	15.6	576	8985.6
Production cost (NRs./kg)			465.99
B:C ratio			1.24

IV. Conclusion

Integrated fish farming is a sustainable and effective tool for improving the livelihood of rural people. It offers the effective and efficient utilization of the locally available resources and diversification of the income of the small-scale farmers. This research concluded that integrated duck-fish farming can resolve the issues of sustainability effectively.

Acknowledgment

We would like to thanks the Department of aquaculture and Fisheries, Agriculture and Forestry University for providing research facilities to carry out this work. We would also like to thanks Mr. Rahul Ranjan, Prof. Dr. Madav Kumar Shrestha, Prof. Dr. Sunila Rai, Prof. Dr. Dillip Kumar Jha, and Asst. Prof. Dr. Narayan Prasad Pandit for their invaluable suggestion, persistent co-operation, continuous supervision, and guidance throughout the period of this research.

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Nitrogen Sorption Isotherms of Biologically Synthesized Non-Stoichiometric Hydroxyapatite Nanoparticles (HAp NPs) Extracted from Fish Bones

By Mohamad Rais Hasan, Mohd Sabri Mohd Ghazali & Nor Fazliyana Mohtar
University Malaysia Terengganu

Abstract- Hydroxyapatite (HAp) with the chemical formula of $Ca_{10}(PO_4)_6(OH)_2$ is a mineral component found in bone structure, and has broad application in many fields. Several sources can be used for the extraction of HAp either synthetic or natural. However, sources such as porcine and bovine have drawbacks that decreased the demand of HAp.This study aimed toextract and determine particle size distribution and pore characteristics ofhydroxyapatite nanoparticles (HAp NPs) derived from spotted sardinella (*Amblygaster sirm*). Further characterization of extracted HAp NPswas carried out by Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Spectroscopy (EDS)and Brunauer–Emmett–Teller(BET).SEM analysis has shown that the extracted HApNPs has an irregular sphere-like shape with particle size distribution ranged from 95nm to 100nm.

Keywords: *HAp NPs, nitrogen sorption, fish bone, characterization, non-stoichiometric.*

GJSFR-C Classification: *FOR Code: 279999*

Strictly as per the compliance and regulations of:

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Mohamad Rais Hasan [α], Mohd Sabri Mohd Ghazali [σ] & Nor Fazliyana Mohtar [ρ]

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Keywords: HAp NPs, nitrogen sorption, fish bone, characterization, non-stoichiometric.

I. Introduction

Bone is a combination of organic and inorganic components that provides mechanical strength and stability to the structure (Dorozhkin, 2013). The organic components include fat, collagenous, and non-collagenous proteinthat are packed together with inorganic nano-hydroxyapatite (nano-HAp) to form a bone structure(Poinern et al., 2016). Nano-Hap with achemical formula of Ca_{10} $(PO_4)_6(OH)_2$ is an organic mineral component found in bone and teeth which possesses excellent biological properties (Barakat et al., 2009; Jahan et al., 2017). It has exceptional biocompatibility due to their similar structure and composition with boneand enamel of teeth (Dorozhkin, 2013; Tsai et al., 2008; Zhou and Lee, 2011). Nano-Hap constitutes in bone and enamel structure of teeth usually in rod-like shaped nano-crystal with an average size of 20 to 50nm(Ajami et al., 2016).

Nano-HAp has many applications in medical, pharmaceutical, and dentistry due to its enhanced biocompatibility, bioactivity, non-inflammatory behavior, non-immunogenicity and high osteoconductive properties (Giraldo-Betancur et al., 2013; Huang and Chu, 2013). Nano-HAp has the capability to assist the growth of new bone and enhanced the bone structure due to its osteoconductivity without any inflammatory effects (Sobczak-kupiec et al., 2018). The application of nano-HAp as a scaffold in generating a new bone depending on the optimum condition of its porosity and pore size (Ofudje et al., 2018). Nano-Hap can be used in the medical field as an alternative coating material to increase theattachment strength of bone to metalimplants(Coatchup et al., 1999). It also has the potential to be applied in dentistry field for regenerating the enamel layers of damaged teeth(Zhou and Lee, 2011). Nano-Hap fulfilled the required criteria to be applied in most medical and dentistry purposes related to living things due to its excellent biocompatibility.

However, the morphological structure and size of nano-HAp influence the mechanical properties and biological behavior of the particles. Different size of nano-HAp has different application due to its different properties. The composition, crystallinity, morphology, and particle size of nano-HAp are the essential characteristics which affect its performance and utilization (Jahan et al., 2017, Sun et al., 2017).

The properties of nano-HAp are also influenced by the source and methods of extraction process (Sun et al., 2017). The extraction process of nano-HAp consists of natural and synthetic methods. Nano-HAp

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can be produced syntheticallyfrom chemical such as wet chemical precipitation Fernando et al., 2015; Gentile et al., 2015; Kamieniak et al., 2016; Nazari et al., 2014; Zanotto et al., 2012), sol-gel (Agrawal et al., 2011), spray drying (Chow and Hockey, 2004; Ruphuy et al., 2016), hydrothermal transformation (Hu et al., 2001; Sivaperumal and Mani, 2017). Meanwhile, natural approach involve heat treatment of bone such as bovine bone (Barakat et al., 2009, Bahrololoom et al., 2009; H|Khoo et al., 2015; Gentile et al., 2015), fishbone (Boutinguiza et al., 2012; Ozawa et al., 2007; Venkatesan et al., 2015; Pal et al., 2017), fish scale (Huang and Chu, 2013; Prasad et al., 2017), eggs shell (Gergely et al., 2010; Khandelwal and Prakash, 2016; Rivera et al., 1999), and snail shell (Adak and Purohit, 2011).This method is preferred over the synthetic method due to its low-cost, time-saving and simple procedure (Sun et al., 2017).

There are a few studies have been reported on the extraction of nano-HAp from natural sources for different field of applications. In this paper, the preparation of natural nano-HAp has been extracted from spotted sardinella (*Amblygastersirm*) bone using heat treatment. The fish bone was obtained from fish processing industries which in return will help to reduce the environmental pollution by promoting the potential use of the fish by-products. Therefore, this study aimed to extract and characterize the elemental composition and pore characteristics from nitrogen (N_2) isotherms of nano-HAp from *A.sirm* bone and compare with standard. The extraction was carried out at different calcinations temperatures of 600°C, 700°C, 800°C, 900°C and 1000°C. The nano-Hap particles were characterized using Scanning Electron Microscope (SEM) equipped with EDAX and Micromeritics devices.

II. Materials and Methods

a) Preparation of raw material

A total amount of 50kg of spotted sardinella (*Amblygastersirm*) bone was collected from local fish processing industry and stored in a freezer at -20°C. Standard hydroxyapatite(HAp) was purchased from Sigma and used as control. The frozen raw material proceeded to bone separation process and boiled before it was then rinsed with tap water to remove adherent fish meat. The raw material was dried in an oven.

b) Extraction of nano-hydroxyapatite

The extraction of nano-hydroxyapatite (nano-HAp) was carried out according to the method of Boutinguiza et al., (2012).The dried bone was heated in a furnace(Carbolite, UK) at different temperature and it was cooled isothermally in dessicator. The calcined bone was milled for using the ball-mill(Retsch PM 100, Germany).

c) Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) analysis was carried out according to the method of Boutinguiza et al., (2012). The morphology of nano-Hap was determined by a Scanning Electron Microscope(JEOL JSM-6360LA, Japan) and (JEOL JSM-6610LV, Japan). The nano-HAp were coated with a thin gold layer before observed under a microscope.

d) Energy Disperse Spectroscopy

Energy Disperse Spectroscopy (EDS)analysis was carried out according to the method of Boutinguiza et al., (2012).The composition of nano-Hap was determined by a Scanning Electron Microscope (JEOL JSM-6360LA, Japan)equipped with an EDAX detector for energy dispersive microanalysis (EDX) to analyze local chemical composition.

e) Nitrogen sorption analysis

Pore parameters analysis was carried out based on Bruaauer–Emmett–Teller (BET) theorem followed to the method from Ciobanu et al., (2011)using Micromeritics (ASAP 2020, USA).Thepore parameters such as pore size (μm),pore volume (cm^3/g) and BET surface area (m^2/g)were calculated from the adsorption branch of isotherms based onBarrett–Joyner–Halenda (BJH).

f) Statistical analysis

Analysis of Variance (one way ANOVA) and multiple comparisons (Posthoc test) was applied in this study. Comparisons of changes in Ca/P and pore parameters were performed to determine the significant. The level of significance was set at $\alpha = 0.05$. Statistical analyses were performed using SPSS for Windows (version 23.0).

III. Results and Discussion

a) Morphological structure and particle size distribution

SEM analysis was performed to observe the morphological composition and particle size distribution of raw bone (RB), extracted hydroxyapatite nanoparticles (HAp-1) compared to the standard hydroxyapatite (HAp-2). The SEM images and particle size distribution of RB, HAp-1 and HAp-2 are presented in Figure 1. All of the SEM images show the particle structure on the surface of the powders. The morphological structure of raw bone (RB) showed an amorphous shape and size consisting of decomposed inorganic materials and the small size of the hydroxyapatite (HAp) particle. This phenomenon could be due to the composition of inorganic materials contained in the RB, including HAp particle that has a particle size distribution ranging from 90 to 100 μm. The particle size of RB seems to have larger particle structure compared to the HAp-1 and HAp-2 due to the packed structure of particles with a combination of inorganic materials such as collagen proteins and HAp

crystals. This finding is further supported by Sutapaet al., (2016) who stated that the raw bone consists of inorganic materials including collagens, fats, proteins and mineral compounds which decomposed after the calcination process except for HAp.

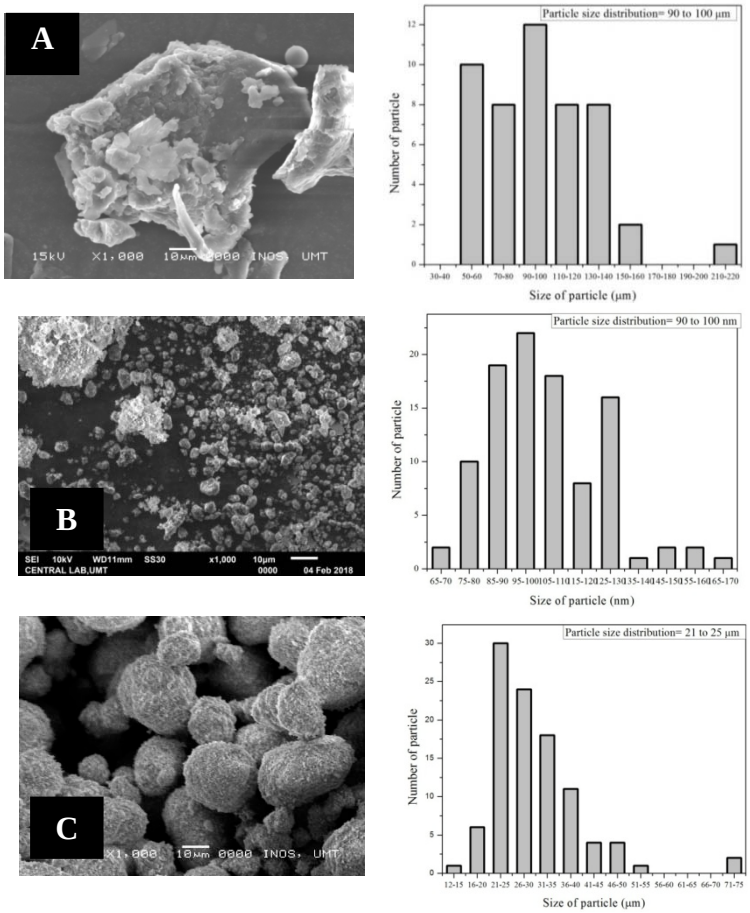

Figure 1: SEM images and particle size distribution of RB (A), HAp-1 (B) and HAp-2 (C). Magnification: X1000, Volt : 15 kv, 10 kv.

*RB represents: Raw bone

*HAp-1 represents: Extracted nano-hydroxyapatite

*HAp-2 represents: Standard hydroxyapatite

There were slight differences in the morphological structure of HAp-1. It exhibited smaller particles compared to the ones observed for RB and HAp-2. These HAp particles consisted of irregular and agglomerated sphere-like shapes with particle size distribution ranging from 95 to 100 nm. The morphological structure of HAp-1 was found to be agglomerated, consisting of tiny crystal particles. The spherical morphology and agglomerated structure of HAp-1 are similar to the particle morphology reported by Poinern et al., (2016). This phenomenon may be due to the effect of the milling process and also was influenced by the calcination temperature. The calcination temperature used in the present study was 700°C and this has changed the particle size of HAp-1, leading to the formation of the agglomerated nano-sized particles after the milling process (Sofronia et al., 2014). The calcination temperature has altered the size and shape of the HAp-1 particles with high agglomeration due to the low temperature used (Guo et al., 2013). The finding of the present study is supported by Sun et al., (2017) who found a similar finding on the structure of calcined-HAp which has an irregular shape with a particle size ranging from 20 to 100 µm. This finding is further supported by Sofronia et al., (2014). who found similar findings on the morphological structure of HAp-1 which had pseudo-spherical shape particles consisting of a large agglomerated structure with sizes less than 955 nm. The nano-sized HAp was previously reported to have an elongated shape with a particle size of less than 100 nm (Jahan et al., 2017).

The HAp-2 exhibited a larger particle size with fixed shape when compared to those The HAp-2 exhibited a larger particle size with fixed shape when

compared to those observed for RB and HAp-1. The Hap-2 consisted of the spherical shape of the HAp particle with a particle size distribution ranging from 21 to 25 μm. The particle size of HAp-2 seems to have larger particle structure compared to the HAp-1 sample due to the high crystallinity and growth of the HAp crystal particles. The increasing of calcination temperature influenced the grain growth and crystallization of HAp particles due to the absorption of heat energy during synthesis process (Guo et al., 2013; Khoo et al., 2015). The present finding is further supported by Sun et al., (2017) who found similar conclusions on the morphological structure formed by the standard HAp particle which has a spherical shape with a size range of 5 to 15 μm. A summary of findings on the morphological structure and particle size distribution of HAp from various studies is shown in Table 1. It can be seen that the morphological structures of HAp consist of various shapes, depending on the source of extraction. It also influences the particle size distribution of HAp.

Table 1: Various findings of morphological structures and particle size distributions of HAp extracted from different sources

Sample	Morphological structure	Particle size distribution (μm)	Reference
Tuna bone	Irregular crystal-like structure	5-10	Sutapa et al., (20160
Bovine bone	Nano rod-like structure	0.3	Barakat et al., (2009)
Seabass bone	Crystalline aggregates	0.2	Ozawa et al., (2007)
Sea bass bone	Irregular structure	0.005-0.055	Pal et al., (2017)
Carp fish scale	Crystal-like structure	0.1	Muhammad et al., (2016)
Bovine bone	Irregular	20-100	Sun et al., (2017)
Bovine	Irregular	45	Khoo et al., (2015)

The size of HAp-1 was in the range of nanoparticles size and with an inconsistent shape. The production of HAp from biological sources commonly exhibited slight inconsistencies in shape and size. This phenomenon is related to the composition, properties of the bone and effect of the milling process that contributed to the shape and size of the HAp particles. Venkatesan et al., (2015) stated that the morphological characteristic of HAp extracted from salmon fish bone was in agglomerated and irregular particles. The finding of the present study is further supported by Sunil and Jagannatham (2016). who found that the SEM images of HAp extracted from roholabio fish bone exhibited large agglomerated particles. Other researchers (Coelho et al., (2007); Corrêa and Holanda, (2019); Mondal et al., (2012); also reported that the SEM images of HAp nanostructure extracted from fish bone demonstrated agglomerated and irregular particles. A comparison of SEM images of this study of the structure of fish bone HAp with previous studies demonstrated similar agglomerated and inconsistent HAp particles with variations in particle size distribution. This finding is also in a good agreement with the result in Section 3.5, which demonstrated that the specific surface area ofHAp-1 was higher than the other samples. The correlation of the size of the particles with the surface area was inversely proportional. Generally, the surface area of nanoparticles (NPs) isrelatively large due to presence of many reactive areas on the particle structure Christian et al., (2008). The shape and size of HAp particle is important to this study due to the primary characteristics of an abrasive material in toothpaste formulation.

b) Transition phase

Energy Dispersive X-ray Spectroscopy (EDS) analysis was conducted mainly to confirm the calcium-to-phosphorus (Ca/P) ratio of raw bone (RB), extracted hydroxyapatite nanoparticles (HAp-1) and standard hydroxyapatite (HAp-2) and its elemental composition. The Ca/P ratio determination is compulsory data for supporting the transition phase for the formation of hydroxyapatite (HAp). There are several types of calcium phosphate-based materials such as HAp, beta-tricalcium phosphate (ß-TCP), tetratricalcium phosphate (TTCP) and alpha-tricalcium phosphate (α-TCP). These materials can be generally distinguished by the Ca/P molar ratio through EDS analysis.The transition phase of the materials is inconsistent depending on the source of extraction and temperature.

c) Elemental composition

The elemental composition of hydroxyapatite (HAp) is an important analysis to determine the major and other possible minor elements. Figure 2 shows the elemental contents of RB, extracted HAp-1 and HAp-2. The elemental composition of RB and HAp-1 contained abundant of elements compared to HAp-2.

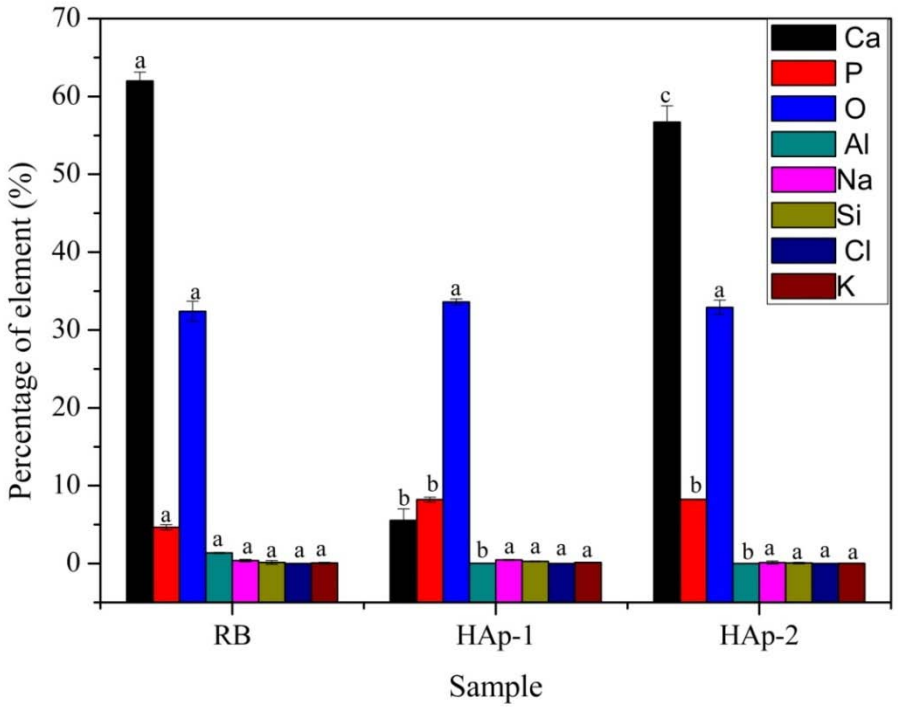

Figure 2: Comparison of percentage of elements of RB, HAp-1 and HAp-2

*Values are given as mean ± SD for triplicate determinations.

*Values with the same superscript letters were not significantly different ($p<0.05$).

RB: Raw bone; HAp-1: Extracted hydroxyapatite nanoparticles;

HAp-2: Standard hydroxyapatite

This phenomenon could be owing to the inheritance of mineral partly from the bone, which contains high amount of calcium and other trace elements such as aluminium (Al), sodium (Na), silicon (Si), chlorine (Cl) and potassium (K). HAp-2 contained fewer trace elements compared to RB and HAp-1 due to the chemically synthesized process which lacked of trace elements. This finding is further supported by another similar finding by Giraldo-Betancur et al., (2013) who demonstrated that the HAp possessed a high amount of other trace element and minerals that originated from the bone compared to the chemically synthesized HAp.

d) Calcium to phosphorus ratio

Based on the chemical formula of the hydroxyapatite (HAp), the theoretical stoichiometric calcium-to-phosphorus (Ca/P) ratio was 1.67 (Michael, et al., 2016). Figure 3 shows the Ca/P ratio of raw bone (RB), extracted hydroxyapatite nanoparticles (HAp-1) and standard hydroxyapatite (HAp-2). The Ca/P ratio of HAp is a vital characteristic which confirms the purity of the extracted HAp. It is related to the ionic interchange of HAp structure and other minor calcium phases, including calcium oxide, calcium carbonate and calcium hydroxide Giraldo-Betancur et al., (2013). The result showed that the Ca/P ratio of RB, HAp-1 and HAp-2 were 3.18, 1.64 and 1.64, respectively. The Ca/P ratio of RB had a slightly higher value compared to HAp-1 and HAp-2. This phenomenon could be due to the non-stoichiometric ratio and variation of the element in the raw bone which influenced the ratio. The Ca/P ratio of HAp-1 and HAp-2 exhibited a lower value than the theoretical stoichiometric ratio of HAp.

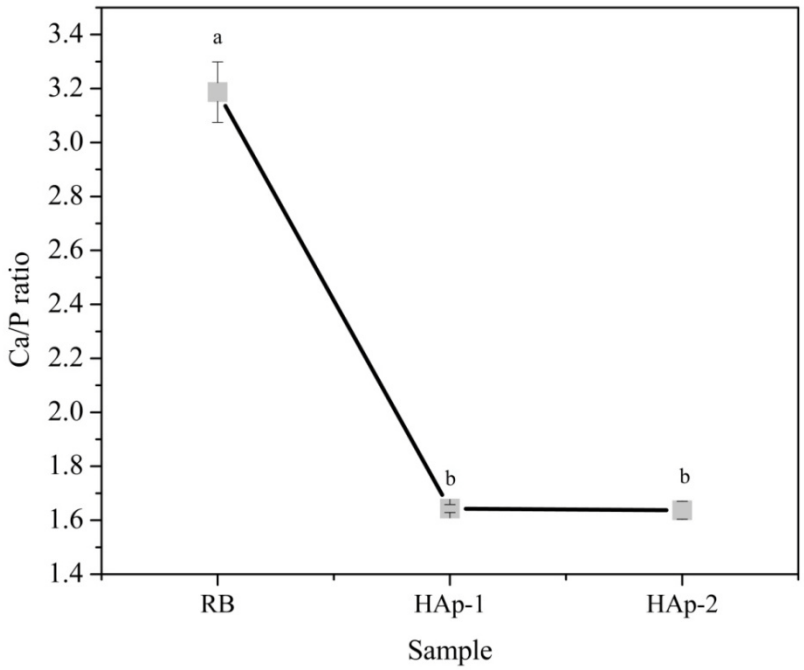

Figure 3: Comparison of Ca/P ratio of RB, HAp-1 and HAp-2 for the confirmation of HAp.

*Values are given as mean ± SD for triplicate determinations.

*Values with the same superscript letters were not significantly different ($p<0.05$).

RB: Raw bone; HAp-1: Extracted hydroxyapatite nanoparticles;

HAp-2: Standard hydroxyapatite; HAp: Hydroxyapatite

However, it is an acceptable range for the HAp stoichiometric molar ratio, which ranged from 1.56 to 1.86 (Barakat et al., 2009). This finding is further supported by Michael, et al., (2016). who stated that the extracted HAp at different conditions showed high Ca/P ratios which were 1.74, 1.81 and 1.87. The Ca/P ratio of HAp-1 in the present study is in a good agreement with those previously stated in Section 4.6.1 which demonstrated a high percentage composition of calcium (Ca) and phosphorus (P). The percentage composition of these elements varied depending on the source of extraction and temperature, thus determining the transition phase of the calcium phosphate-based material. The finding on Ca/P ratio (1.64) of HAp-1 indicated that the ratio was in the range of non-stoichiometric ratio for the formation of HAp. Therefore, the result indicated that the extracted HAp-1 was in the transition phase for non-stoichiometric HAp through the Ca/P molar ratio determination.

e) Pore parameters and specific surface area

Nitrogen (N_2) adsorption and desorption analysis were carried out to analyze the pore parameter and specific surface area of raw bone (RB), extracted hydroxyapatite nanoparticles (HAp-1) and standard hydroxyapatite (HAp-2) based on the Brunauer-Emmett-Teller (BET) theorem. The pore parameters and the specific surface area of the samples were evaluated from the adsorption-desorption branch of N_2 isotherm (Figure 4) according to the Barrett–Joyner–Halenda (BJH) model.

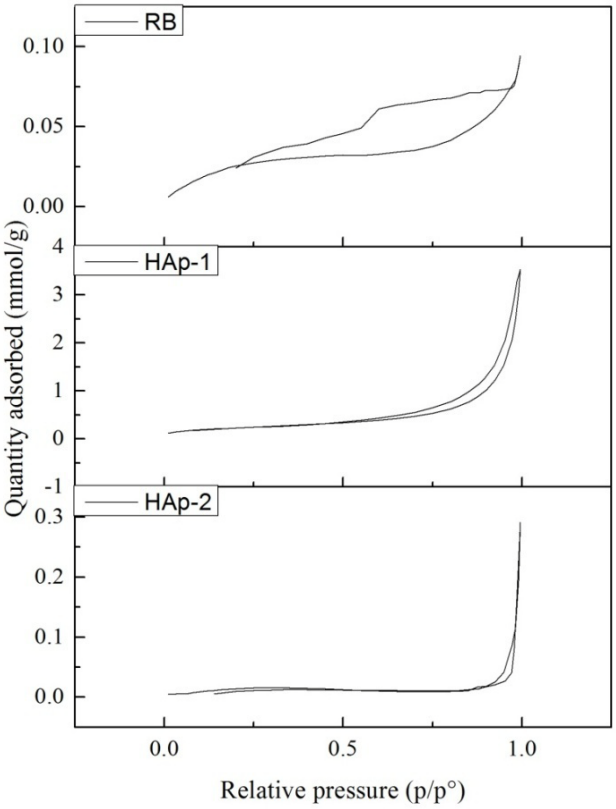

Figure 4: Comparison of N_2 adsorption-desorption isotherms of RB (a), HAp-1 (b) and HAp-2 (c) for the determination of pore structure of HAp NPs.

RB: Raw bone; HAp-1: Extracted hydroxyapatite nanoparticles;
HAp-2: Standard hydroxyapatite

The result demonstrated that all samples were identified as type I N_2 isotherms based on the International Union of Pure and Applied Chemistry (IUPAC) classification(IUPAC). However, extracted hydroxyapatite nanoparticles (HAp-1) demonstrated higher hysteresis loops at P/Po > 0.8 due to their higher surface area compared to standard hydroxyapatite (HAp-2) and raw bone (RB). This phenomenon is closely related to the adsorption of N_2in the pore structure of the particles which determined the pore characteristics. The differences in adsorption isotherms might due to the different pore volume, specific surface area and pore size of the particles.

The shape of the RB, HAp-1 and HAp-2 corresponded to the type I heterosis loop which appeared to be a long hexagonal pore structure. This finding is in alignment with the basic hexagonal shape of hydroxyapatite (HAp) crystal which was made up of calcium (Ca), phosphorus (P), oxygen (O) and hydrogen (H) atoms as shown by Figure 6. The arrangement of HAp crystal is usually in polycrystalline form which is slightly spaced between the crystals. The HAp obtained from biological sources commonly possesses polycrystalline crystals which differ depending on the source of HAp. The shape of HAp particles obtained from biological sources is usually irregularly shaped due to the simple form of extraction. In contrast, HAp obtained from synthetic sources has a fixed shape and the arrangement of the crystal is commonly crystalline. However, these synthetic sources usually require a higher cost, time-consuming and more complicated compared to biological sources. The differences in adsorption isotherms might due to the different specific surface area, pore volume and pore size of the particles.

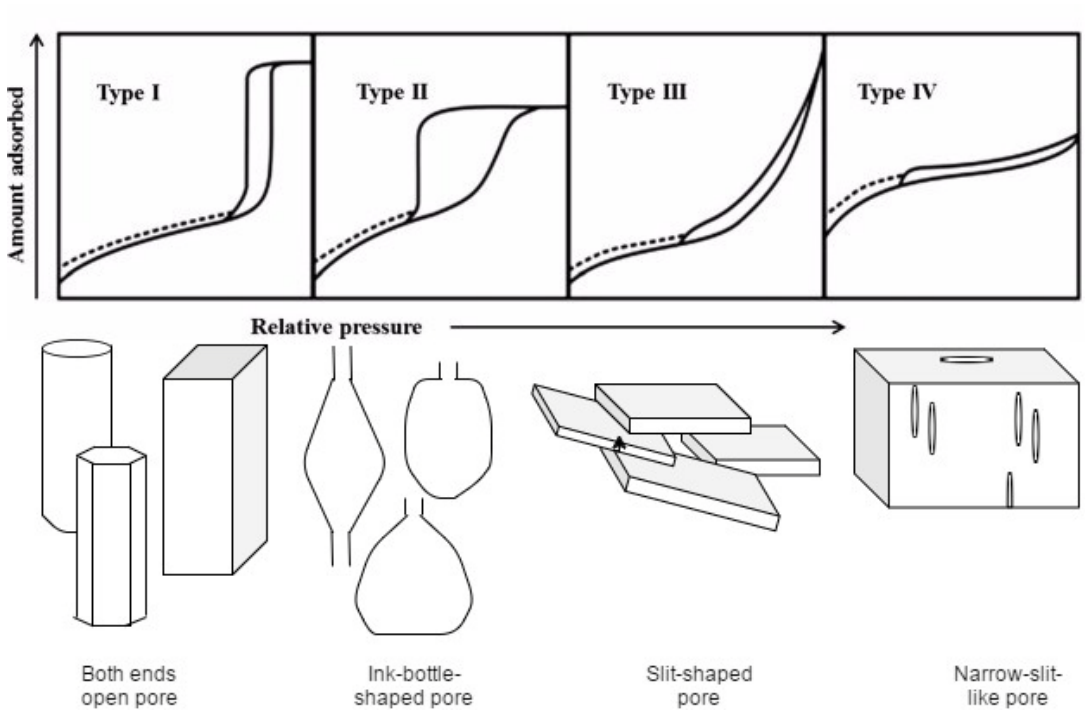

Figure 5: Pore structure characteristics based on N_2 sorption

The shape of the RB, HAp-1 and HAp-2 corresponded to the type 1 heterosis loop which appear to be a long hexagonal pore structure. This finding was in alignment with the basic hexagonal shape of HAp crystal which made up of calcium (Ca), phosphorus (P), Oxygen (O) and Hydrogen (H) atoms as shown by Figure 6.

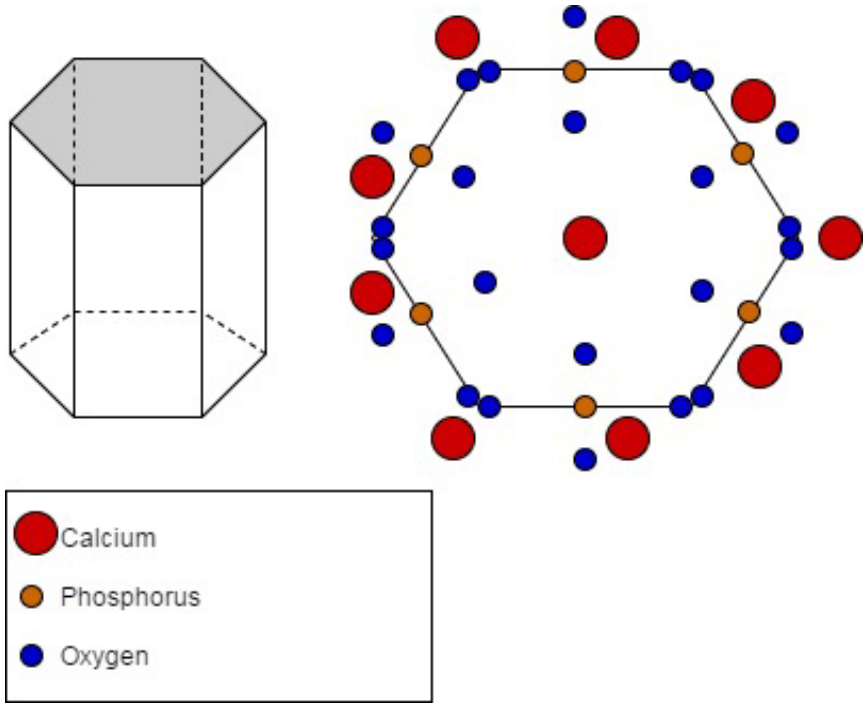

Figure 6: Crystal structure of HAp that composed of calcium (C), phosphorus (P) and Oxygen (O) atoms

The crystal shaped structure of the sample played a crucial role that influenced the N_2 adsorption which then was reflected on the pore characteristics. The crystal pore structure of the HAp demonstrated a long hexagonal rod-like shape.

The pore size and pore volume of the samples were acquired from the Barrett-Joyner-Helenda (BJH) method while the surface area was determined by the Brunauer-Emmett-Teller (BET) method. Figure 7 presents the pore parameters and BET surface area of the samples. The pore volume of raw bone (RB), extracted hydroxyapatite nanoparticles (HAp-1) and standard hydroxyapatite (HAp-2) were 0.0017, 0.1179 and 0.0101cm^3/g, respectively.HAp-1 demonstrated higher pore volume compared to the RB and HAp. The specific surface area of RB, HAp-1 and HAp-2 were 2.27, 22.07 and 1.90m^2/g, respectively.HAp-1 exhibited a higher surface area compared to RB and HAp-2.This phenomenon might be due to the formation of the nanostructure of HAp that contributed to the greater size of the specific surface area.This phenomenon correlated with the different size of particles which influences the properties of the HAp particle. The formation of HAp-1 nanostructure has dramatically altered the performance and features of the particle. The pore size of RB, HAp-1 and HAp-2 were 0.0129, 0.0235 and 0.0489 μm, respectively.

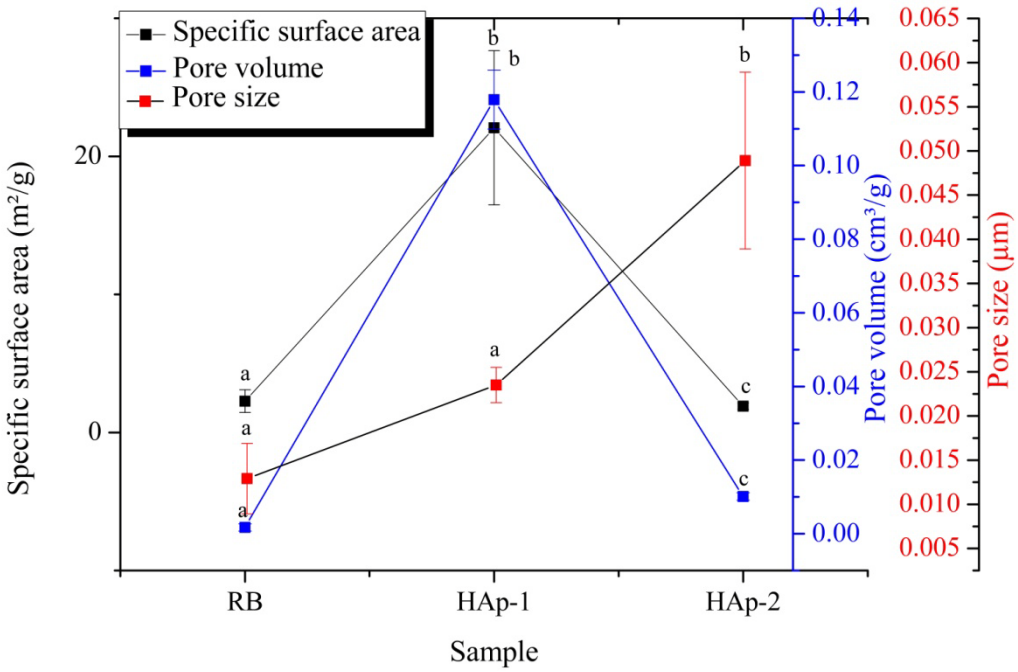

Figure 7: Comparison of pore parameters and BET surface area of RB, HAp-1 and HAp-2

RB: Raw bone; HAp-1: Extracted hydroxyapatite nanoparticles;
HAp-2: Standard hydroxyapatite

All of the samples showed a predominantly mesoporous pore structure.The pore structure of a sample can be classified into micropore (0.1 to 1 nm), mesopore (1 to 10 nm) and macropore (10 to 1000 μm).The specific surface area and pore volume of HAp-1 were increased with the decreasing of the particle size.This phenomenon may be due to the smaller particle size that contributed to the greater exposure of the atoms which led to the abundant formation of reactive sites (Cui et al., 2016). The present finding is further supported by Zanotto et al., (2012) who found that the specific surface area of HAp is inversely proportional with the temperature and particle size. This finding is also in good alignment with those previously stated in Section 3.1 which demonstrated the nanoparticle size of HAp-1. Therefore, it is indicated that the size of the particle was inversely proportional to the specific surface area. Figure 8 shows a possible mechanism of the nitrogen (N_2) adsorption through the pore structure of the HAp crystal.

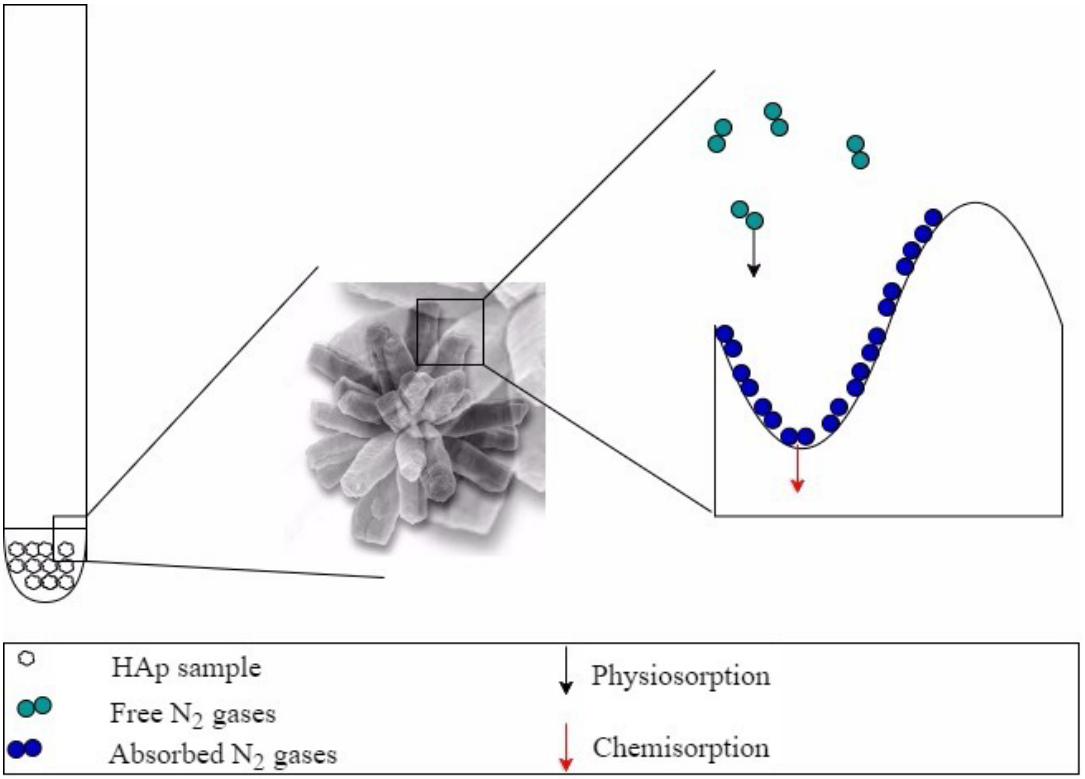

Figure 8: Mechanism of nitrogen (N_2) gases sorption into the pore of HAp crystal through physiosorption and chemisorption

HAp: Hydroxyapatite; N_2: Nitrogen

The adsorption of gas molecules into the pore structure of HAp can be illustrated by the binding of the free N_2 gas molecules into the pore surface of the HAp through physiosorption and chemisorption mechanisms. The absorbed gas molecules formed a monolayer at the first phase before forming a multilayer of the molecule. The physiosorption occurred at this stage due to the van der Waals attraction which is usually used for the pore parameters determination. The adsorption of the gas is generally dependent on the time, pressure, surface energy distribution and surface area. The gas may undergo chemisorption at a later phase due to the chemical bonding attraction which may be contributed by the activation energy.

IV. Conclusion

The extracted nano-hydroxyapatite (nano-HAp) extracted from spotted sardinella (*Amblygaster sirm*) bone can be an alternative to the synthetic hydroxyapatite (HAp). This is because theHAp extracted from *Amblygaster sirm* bonedemonstrated high yield (55.06%) at optimum calcination temperature (700°C).Morphological structure of nano-HAp was observed to exhibit a spherical-like shape with particle size distribution range from 95 to 100nm. The size of nano-HAp is an important factor that can influence its properties, performance and compatibility to be used in certain application. The particle size of nano-HAp can also be adjusted by the calcination temperature and effects of milling. Calcium to phosphorus (Ca/P) ratio of nano-HAp was close to theoretical stoichiometricand standard HAp ratio. It also demonstrated higher specific surface area and pore volume which were 22.07 m^2/g and 0.1179 cm^3/g, respectively. The increased on the specific surface area of nano-HAp has potential for efficient absorption mechanism and interchange of ion. The properties of nano-HAp are the important features to be used for removal of unwanted ions, bacteria and acidic medium in the teeth. This biological source of HAp is more preferable due to less toxic, low-cost, incomplicated and time-saving for the prodction process. These findings suggested that the extracted nano-HAp can be potentially commerciallized andused for various purposes ofapplications.

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Abbreviations

Al: Aluminium; ANOVA: Analysis of Variance; BET: Bruaauer–Emmett–Teller; BJH: Barrett–Joyner – Halenda; Ca: Calcium; Cl:Chlorine; EDS; Energy Disperse Spectroscopy; HAp: Hydroxyapatite; HAp-1: Extracted nano-hydroxyapatite; HAp-2: Standard hydroxyapatite; K: Potassium; Na: Sodium; N_2: Nitrogen; O: Oxygen; P: Phosphorus; RB: Raw bone; SEM: Scanning ElectronMicroscopy; Si: Silicon; TGA: Thermogravimetric Analysis.

Authors' contributions

MRH, NFM and MSMG designed the experiments. MRH performed theexperiments. MRH analyzed the data and drafted the manuscript. MRH, NFM and MSMG helped to draft the manuscript. All authors read and approved the finalmanuscript.

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Herbal Recipes, Drug Indications and Sustainability Potential of Traditional Oral Liquid Formulations in Ogbomoso, Nigeria

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Abstract- Confirmation of identity, along with determination of the quality and purity of herbal drug is an important step towards ensuring its safety and efficacy. This study therefore sought to document the botanical constituents and drug indications of traditional oral liquid herbal formulations (TOLHFs) manufactured in Ogbomoso, Nigeria. It also examined the conservation status of the medicinal plants so used alongside the cultivation efforts being made by the drug manufacturers in order to provide information on whether continual exploitation of the plants for TOLHFs is sustainable. Through a questionnaire, 14 traditional herbal medical practitioners (THMPs) provided information on the recipes of their products, the sources of their raw material herbs, and types of health conditions treated or managed with the drugs. Sustainability potential of the drugs was quantified as relative percentage of the three choices of sources of raw material herbs available to the manufacturers in conjunction with the conservation status of the plant species as recorded by the International Union for Conservation of Nature (IUCN).Fifty- seven medicinal plant species (in 34 angiosperm families) were used to formulate 71 herbal recipes that are indicated for treating 14 different health conditions.

Keywords: medicinal plants; traditional oral liquid herbal formulations, sustainable exploitation of medicinal herbs; ethno-medicine; forest conservation; ethno-botany.

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Abstract- Confirmation of identity, along with determination of the quality and purity of herbal drug is an important step towards ensuring its safety and efficacy. This study therefore sought to document the botanical constituents and drug indications of traditional oral liquid herbal formulations (TOLHFs) manufactured in Ogbomoso, Nigeria. It also examined the conservation status of the medicinal plants so used alongside the cultivation efforts being made by the drug manufacturers in order to provide information on whether continual exploitation of the plants for TOLHFs is sustainable. Through a questionnaire, 14 traditional herbal medical practitioners (THMPs) provided information on the recipes of their products, the sources of their raw material herbs, and types of health conditions treated or managed with the drugs. Sustainability potential of the drugs was quantified as relative percentage of the three choices of sources of raw material herbs available to the manufacturers in conjunction with the conservation status of the plant species as recorded by the International Union for Conservation of Nature (IUCN).Fifty-seven medicinal plant species (in 34 angiosperm families) were used to formulate 71 herbal recipes that are indicated for treating 14 different health conditions. The sources of raw material herbs, in relative terms, were purchase from herbal markets/suppliers(38.7%), collection from the wild vegetation (35.5%) and cultivation of some of the herbs for use (25.8%). The herbal material used included fruits, seeds, leaves, stem barks, flowers, roots and rhizomes, most of which can be sustainably harvested. Based on the IUCN records, only six (i.e. 10.5%) of the 57 plant species have been evaluated and placedin the categories of 'Least Concern' (*Ceiba pantandra*, *Cola acuminata*, *Ficus exasperata*), 'Vulnerable' (*Garcinia kola*, *Khaya senegalensis)* and Endangered' (*Jatropha curcas*). Production of TOLHFs in Ogbomoso was therefore, adjudged to be sustainable with minimal potential injury on the natural flora. Medicinal plant cultivation by THMPs and herb vendors should however, be further encouraged or enforced.

Keywords: medicinal plants; traditional oral liquid herbal formulations, sustainable exploitation of medicinal herbs; ethno-medicine; forest conservation; ethno-botany.

I. Introduction

The traditional herbal medical practitioners (THMPs) in Ogbomoso, Nigeria produce and market various herbal preparations used for different types of ill health conditions. Among these are the traditional oral liquid herbal formulations (TOLHFs) used by the residents belonging to different socio-economic classes in the city (Ogunkunle and Ashiru, 2011). The efforts of the THMPs are commendable against the backdrop of rising cost of imported medication in Nigeria(High Commission of India in Nigeria, 2020), along with scarcity and cost of the commodities used in manufacturing drugs locally (Fatokun, 2020), and the fact that there are some areas in which orthodox medicine is known to be weak (Isola, 2013). However, herbal products from Africa have been called to question on account of adulteration, substitution, contamination, misidentification of ingredients, lack of standardisation, incorrect preparation and/or dosage, inappropriate labelling and/or advertisement (Lau et al., 2003; World Health Organization, 2003). For these reasons, herbal products from Africa have not enjoyed worldwide acceptability compared to those from other countries such as India and China (Patwardhan *et al.* 2005).

Quality of herbal medicines is defined by World Health Organisation (2002) on the basis of their reproducible efficacy and safety, while Bauer (1998) identifies quality criteria in terms of the scientific definition of the raw materials. Based on these definitions, standardisation and quality control of herbal formulations can be said to recline on their identity and purity. So, correct identification and quality assurance of the starting materials are essential prerequisites to ensuring reproducible quality of herbal medicine, which will in turn contribute to its safety and efficacy (Kadam *et al*., 2012). The belief in many quarters is that it is difficult to establish comprehensive quality for herbal formulations because of professional secrecy of THMPs. However, recent developments have shown that this challenge is surmountable (Obu, 2015). Considering the raw medicinal herbs for TOLHFs in Ogbomoso as 'active ingredients' for these drugs (World Health Organization, 2000), their enumeration formed the main purpose of this study.

There is substantial evidence to show increasing human dependence of herbal medicine for primary health care (World Health Organisation, 1998). It is however regrettable that users of herbal medicine seldom seek to know where the herbs they use come from. We should be mindful of the source of our

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medicinal herbs because these items are inextricably connected to the processes that produce them; and we cannot be healthy unless our environment is healthy. To these extent, if we choose to use plants as our medicine, we become responsible for ensuring that the vegetation or environment that produced the plants are safe and sustainable. These are the thoughts of the professionals advocating for sustainable herbal medicine (Pastogi and Kaphle, 2011; Pesic, 2015; Chen et al., 2016).

According to World Health Organisation (2003), and Chen et al. (2016), the strategies for ensuring sustainability of medicinal plants production include *in situ* and *ex situ* conservation efforts, controlled cultivation and sustainable harvesting, among others. For these strategies to produce the desired effects in a country, the political will is a requirement. The government of Nigeria on 30 September, 1992 promulgated the Medical and Dental Practitioners' (Amendment) Decree number 78, which placed traditional and alternative medicine side by side with orthodox medicine (ABFR & Co., 1996). This step is commendable, but not enough, until it is backed with pragmatic policies and programmes. Presently, there is no government policy in place to ensure sustainability of herbal medicine and the protection of environment in the country with particular reference to medicinal plants (Osunderu, 2009). Therefore, there is no information on whether continual exploitation of medicinal plants for TOLHFs in Ogbomoso is sustainable or not. Filling this gap was another area of focus in this study.

The objectives of this study were to botanically characterise the TOLHFs from Ogbomoso; to ethno-medicinally document their health indications; and to evaluate the sustainability status of the drugs in the study area based on the IUCN's conservation status of the medicinal plants alongside efforts on their cultivation made by the THMPs.

II. Materials and Methods

This study, which was conducted in 2014 covered the five local government areas (LGAs) of Ogbomoso land, Oyo State, Nigeria. The target population consisted of the THMPs in the study location who produced, marketed and provided healing services with TOLHFs. Initial selection of the participants was doneusing stratified sampling technique, with each of the LGAs taken as a stratum. Fourteen of the THMPs were eventually found suitable for inclusion, to whom a questionnaire was administered or used as an interview schedule (Figure 1).

Question items were developed for four different sections of the questionnaire to seek information on the various areas of focus of the study: socio-demographic and professional profiles of the THMPs, types and drug indications of the TOLHFs they produced, recipes and formulations of the drugs, and sources of the medicinal herbs used as raw materials (Figure1). In seeking information on the sources of the herbs, the THMPs were provided a list of possible sources namely purchase, collection from the wild, cultivation, etc. and each of them was asked to tick as many of the options as applicable to him or her. In analysing the data supplied, each of the multiple choices was considered as a separate variable across the 14 participants and counted. A summation of the three alternatives selected was obtained and equated to 100%, from which the relative percentage of each choice was computed. The magnitude of the relative percentage obtained for cultivation of the required herbal materials was taken as sustainability index for the herbal liquid drugs in the study area.

The name of each of the 57 plant species used by TOLHF manufacturers was cross-checked against the red list of threatened plant species compiled by the International Union for Conservation of Nature (IUCN, 2017) to obtain information on its conservation status or population dynamics. The proportion of the plant species in the threatened category, taken alongside conservation efforts by the THMPs, was used to suggest whether manufacturing of TOLHFs in Ogbomoso was sustainable.

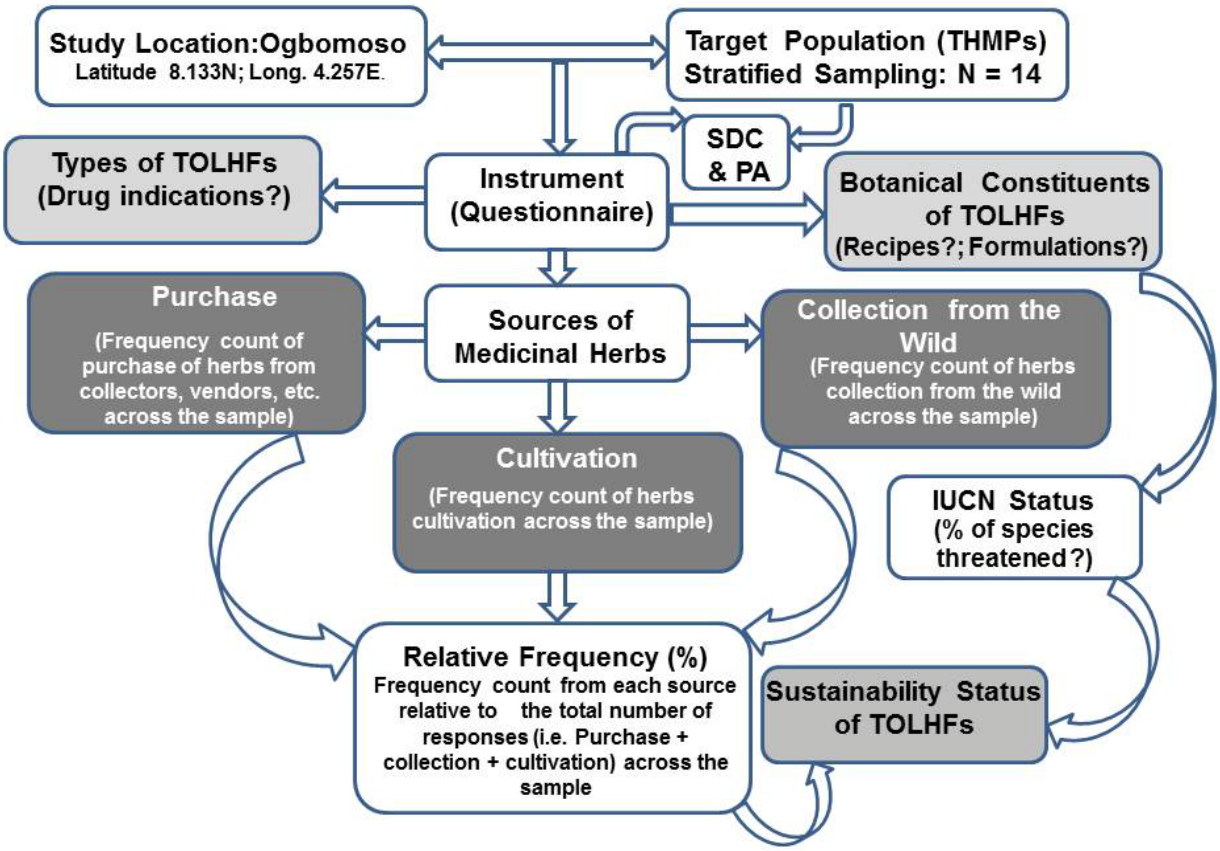

Figure 1: Summary of the methodology adopted towards achieving the three main objectives of the study (i.e. light-shaded boxes), and documenting the relative sources of medicinal herbs for TOLHFs in Ogbomoso, Nigeria (i.e. dark-shaded boxes). *THMPs*, traditional herbal medical practitioners; *TOLHFs,* traditional oral liquid herbal formulations; *SDC,* socio-demographic characteristics of *THMPS; PA,* professional activities; *IUCN,* International Union for conservation of Nature.

The 14 THMPs were assigned anonymity codes A, B, C, D, etc to N, while their products were labeled, each according to its indications (i.e. the health condition that the drug treats). To do this, each health condition was first given a short code of five alphabets, which was then used as a hyphenated prefix to the anonymity code of its manufacturer, such as YELLO-D (i.e. drug for yellow fever produced by healer D), PILES-C (i.e. drug for piles from healer C) and HIGBP-B (i.e. remedy for high blood pressure from healer B) (see Table 2).

III. Results

a) *Socio-demographic characteristics and professional activities of the traditional herbal medical practitioners*

As recorded in Table1, thirteen (i.e. 76.5%) of the 17 THMPs initially recruited to participate in the study were men and 4 (i.e.23.4%) were women; 10 of them (about 59%) were over 50 years of age. The majority of the healers (70.6%) had only primary and/ or secondary education, but up to 65% of them had practiced in the profession for more than 30 years. Historically, majority (88%) of these people came into the profession by descent, being their family trade, while few others either combined some form of training with this option or depended on their natural gifts or talents to become traditional healers. The THMPs ventured into updating their knowledge of medical practice through a wide range of choices, such as by intuition (35.3%), attendance at health talks or meetings (11.8%) and electronic media (about 6%), while 47% of them adopted various forms of a combination of these and other choices (Table 1).Fourteen of the initially recruited 17 THMPs were involved in the production, sale and application of TOLHFs against 14 different types of health conditions including maintenance of general body homeostasis and the management of some dreaded diseases such as diabetes, high blood pressure, typhoid and yellow fever. All of the 14 THMPs produced oral liquid herbal formulations for malaria therapy; about 93% for piles; 86% for typhoid; 64% for blood enricher and 57% for blood purifying drugs (Table 2).

b) *Medicinal herbs enumerated by the traditional herbal medical practitioners in Ogbomoso as recipes for oral liquid herbal formulations*

A total of 57 medicinal plant species from 34 angiosperm families were listed by the THMPs as constituents of TOLHFs in Ogbomoso, Nigeria. Plants of the families Fabaceae, Euphorbiaceae, Amaryllidaceae and Meliaceae were most widely used, followed by those of Anacardiaceae,Annonaceae, Apocynaceae, Combretaceae, Cucurbitaceae, Poaceae, Rutaceae, Sapotaceae, Solanaceae, Sterculiaceae and Zingiberaceae. Members of the other 19 families were seldom used by the THMPs (Tables 3-13). The plant parts used as herbs include fruits, seeds, leaves, stem barks, flowers, roots and rhizomes, and six categories of TOLHFs were being produced, namely: decoctions, infusions, syrups, juices, tinctures and cold infuse drugs. A total of 71 recipes were being formulated, with details of the procedure, processes and products as presented in Figure 2.

c) *Sources and conservation status of the raw material herbs for producing traditional oral liquid herbal formulations in Ogbomoso*

Information obtained from the THMPs indicated the sources of raw material herbs available to them in relative terms as purchased from herbal markets and suppliers (38.7%),

Table 1: Information about the traditional herbal healers who participated in the study

Variable	Number of participants		
	Male	Female	Total (N = 17)
Age (years)			
31-40	4	0	4
41-50	2	1	3
>50	7	3	10
Formal Education			
None	0	2	2
Primary	7	1	8
Secondary	3	1	4
OND/NCE	1	1	2
HND/Degree	1	0	1
Experience (years)			
<10	2	0	2
10-20	1	1	2
21-30	1	1	2
>30	9	2	11
Professional history			
By descent	11	4	15
By training	0	0	0
Both by descent and training	1	0	1
Others*	1	0	1
Manufacturer of Liquid herbal formulations (TOLHFs)			
Yes**	11	3	14
No	2	1	3
Update of knowledge in medical practice			
By intuition (A)	5	1	6
Attendance of meetings or health talks (B)	2	0	2
Electronic media (C)	1	0	1
Internet (D)	0	0	0
A and B	2	3	5
A, B and C	1	0	1
A, B, C and D	2	0	2

OND, Ordinary National Diploma; NCE, Nigeria Certificate in Education; HND, Higher National Diploma.

*Talent from God; ** of the 17 herbal healers 14 produced liquid herbal formulations for oral use and their residential homes doubled as factories, with none of them having evidence of registration of their products with National Food and Drug Administration and Control.

Table 2: List of health conditions indicated for traditional oral liquid herbal formulations manufactured and marketed in Ogbomoso, Nigeria

Number	Health Condition or type of liquid herbal formulation	Local name of health condition	Number of manufacturers (N = 14)	Types and Number of TOLHFs with recipe information
1	Arthritis and rheumatism (ARTRH)	*Làkúègbé*	1	*IRNA*
2	Back and/ waist pain (BAWAP)	*Èyìn dídùn*	1	Decoction (1)
3	Blood enricher/enhancer (BLENH)	*Atún èjè se*	9	Decoction (8); *IRNA* (1)
4	Blood purifier/thinner (BLPUR)	*Apa kòkòrò inú èjè*	8	Syrup (2); decoction (3); infusion (1); *IRNA* (2)
5	Body fatigue (BOFAT)	*Ara wíwó*	2	*IRNA*
6	Convulsion (CONVU)	*Gìrì omodé*	3	Decoction (3)
7	Diabetes (DIABE)	*Ìtò-súgà*	1	*IRNA*
8	Gonorrhea (GONOR)	*Àtòsí*	5	Decoction (3); *IRNA* (2)
9	High blood pressure (HIGBP)	*Èjè ríru*	8	Decoction (5); infusion (1); infusion or tincture (1); *IRNA* (1)
10	Jaundice/anaemia (JAUND)	*Afa èjè s'ára*	5	Decoction (3); decoction or tincture (1); *IRNA* (1)
11	Malaria fever (MALAR)	*Ibà*	14	Decoction (9); infusion (5)
12	Piles (PILES)	*Jèdíjèdí*	13	Decoction (5); decoction or tincture (2); infusion (1); infusion or tincture (3); juice (1); IRNA (1)
13	Typhoid (TYPHO)	*Ibà jèdòjèdò*	12	Decoction (10); infusion or tincture (1); juice (1)
14	Yellow fever (YELLO)	*Ibà pónjú-póntò*	1	Decoction (1)

IRNA, information on recipe not available

collection from the wild vegetation (35.5%), and cultivation of some of the herbs for use (25.8%). Furthermore, a scrutiny of the list of the plants against the IUCN red list of threatened plants revealed that only six of the 57 plant species have been evaluated for their conservation status and population dynamics (IUCN, 2017). From these six, three species are categorised as threatened, namely *Garcinia kola*(Vulnerable), *Khaya senegalensis* (Vulnerable) and *Jatropha curcas* (Endangered), constituting only 5.3% of all the plant species involved in the manufacturing of TOLHFs in the study area. The status of the other three species (i.e. *Ceiba pentadra, Ficus exasperta and Khaya senegalensis*) is of 'Least Concern' category.

Table 3: Names of plants and their parts used for the formulation of traditional oral liquid herbal drugs for the treatment of back and waist pain in Ogbomoso, Nigeria

	Plant Species	Family name	Local name	Part(s) used
1	*Parkia biglobosa* (Jacq.) R. Br. Ex G. Don.	Fabaceae	*Ìgbá/igi ìgbá*	Stem bark
2	*Bridelia ferruginea* Benth.	Euphorbiaceae	*Ìrà*	Stem bark
3	*Boerhaavia diffusa* L.	Nyctaginaceae	*Ètìponlá*	Leaves

Herbal recipe for one liquid decoction: BAWAP-A (1,2,3); suffix alphabet indicates the manufacturer's anonymity code. BAWAP, back and waist pain.

Table 4: Names of plants and their parts for the formulation of traditional oral liquid herbal drugs used as blood enricher or enhancer in Ogbomoso, Nigeria

	Plant species name	Family	Local name	Part(s) used
1	*Parkia biglobosa* (Jacq.) R. Br. Ex G. Don.	Fabaceae	*Ìgbá*	Stem bark
2	*Paullinia pinnata* L.	Sapindaceae	*Kàkànselà*	Leaves
3	*Theobroma cacao*	Sterculiaceae	*Kòkó*	Stem bark
4	*Harungana madagascariensis*	Hypericaceae	*Amùjè*	Stem bark
5	*Maranthes polyandra* Benth.	Chrysobalanaceae	*Ara/igi ara*	Stem bark
6	*Allium sativum* L.	Amaryllidaceae	*Aáyù*	Bulb
7	*Piper guinense* Schumach.	Piperaceae	*Ìyèré*	Fruits
8	*Anogeissus leiocarpus* (DC) Guill & Perr.	Combretaceae	*Àyin*	Stem bark
9	*Xylopia aethiopica* (Dunel) A. Rich.	Annonaceae	*Èrù*	Fruits

Herbal Recipes for eight oral decoctions: BLENH-A (1 only); BLENH-C (2 + potash); BLENH-D (3,4,5,6); BLENH-E (3,4, 5+ potash + cube sugar).; BLENCH-F (5,7 + potash); BLENCH-M (8,9 + potash); BLENCH-O (3,4,5+ potash); and BLENCH-P (3,4,6); suffix alphabets indicate the manufacturers' anonymity codes. BLENH, blood enricher.

Table 5: Names of plants and their parts for the formulation of traditional oral liquid herbal drugs used as blood purifying or thinning drug in Ogbomoso, Nigeria

	Plant species name	Family	Local name	Part(s) used
1	*Tetrapleura tetraptera* (Schumm. & Thonn.) Taub.	Fabaceae	*Àìndan*	Fruits
2	*Garcinia kola* Heckel	Clusiaceae	*Orógbó*	Seeds
3	*Cola acuminata* (P. Beauv.) Schott & Endl.	Sterculiaceae	*Obì àbàtà*	Seed
4	*Paullinia pinnata* L.	Sapindaceae	*Kàkànselà*	Leaves
5	*Parkia biglobosa* (Jacq.) R.Br ex G.Don	Fabaceae	*Ìgbá*	Stem bark
6	*Alstonia boonei* De Wild.	Apocynaceae	*Ahùn/wáwòn*	Stem bark
7	*Anacardium occidentale* L.	Anacardiaceae	*kajú*	Stem bark
8	*Ceiba pentadra* (L.) Gaertn.	Malvaceae	*Àràbà*	Stem bark
9	*Daniellia oliveri* (Rolfe) Hutch. & Dalziel	Fabaceae	*Iyá*	Stem bark
10	*Azadirachta indica* A. Juss.	Meliaceae	*Dógóyárò*	Stem bark
11	*Anogeissus leiocarpus* (DC) Guill & Perr.	Combretaceae	*Àyin*	Stem bark
12	*A. leiocarpus* (DC) Guill & Perr.	Combretaceae	*Àyin*	Root
13	*Xylopia aethiopica* (Dunel) A. Rich.	Annonaceae	*Èrù*	Fruits
14	*Eugenia aromaticum* (L.) Merr. & L.M. Perry	Myrtaceae	*Kànnáfùrù*	Fruits or flowers
15	*Khaya senegalensis* A. Juss.	Meliaceae	*Àgànó*	Stem bark

Herbal recipes for six oral liquid drugs;syrup: BLPUR-B (1, 2, 3+ honey); BLPUR-Q (1,2,3,6 + honey); decoctions: BLPUR-C (4 + potash); BLPUR-E (5,6,7,8,9,10); BLPUR-O (5,6,8,15); infusion: BLPUR-M (11,12,13,14);suffix alphabets indicate the manufacturers' anonymity codes.BLPUR, blood purifying or thinning drug.

Table 6: Names of plants and their parts used for the formulation of traditional oral liquid herbal drugs for the treatment of convulsion in Ogbomoso, Nigeria

	Plant Species name	Family	Local name	Part(s) used
1	*Parkia biglobosa* (Jacq.) R.Br ex G.Don	Fabaceae	*Ìgbá*	Stem bark
2	*Daniellia oliveri* (Rolfe) Hutch. & Dalziel	Fabaceae	*Iyá*	Stem bark
3	*Nicotiana tabacum* L.	Solanaceae	*Tábà*	Fresh leaves
4	*Jatropha curcas* L.	Euphorbiaceae	*Làpálàpá*	Leaves
5	*Crinum jagus* (J. Thomps.)	Amaryllidaceae	*Ògèdè-odò*	Corm

Herbal recipes for three decoctions: CONVU-A (1,2); CONVU-B (3, 4 + local table salt substitute 'obu-otoyo'); and CONVU-C (5 only); suffix alphabets indicate the manufacturers' anonymity codes. CONVU, convulsion.

Table 7: Names of plants and their parts used for the formulation of traditional oral liquid herbal drugs for the treatment of gonorrhoea in Ogbomoso, Nigeria

	Plant species name	Family	Local name	Part(s) used
1	*Citrullus colocynthis* (L.) Schrad.	Cucurbitaceae	*Bàrà/Ègúsí*	Fresh fruit
2	*Adenopus breviflorus* Benth.	Cucurbitaceae	*Tàgîrì*	Fruit
3	*Capsicum frutescens* L.	Solanaceae	*Ata wéwé*	Fruits
4	*Gladiolus psittacinus* Hook	Iridaceae	*Bákà*	Bulb or leaf base
5	*Anthocleista djalonensis* A. Chev.	Gentianaceae	*Sápó*	Stem bark
6	*Alstonia boonei* De Wild.	Apocynaceae	*Ahùn (wáwòn)*	Stem bark
7	*Securidaca longepedunculata* Fresen	Polygalaceae	*Ìpèta*	Root

Herbal recipes for three decoctions: GONOR-A (1 only); GONOR-E (1,2,3, 4); and GONOR-M (1,5,6,7); suffix alphabets indicate the manufacturers' anonymity codes. GONOR, gonorrhea.

Table 8: Names of plants and their parts used for the formulation of traditional oral liquid herbal drugs for the treatment or management of high blood pressure in Ogbomoso, Nigeria

Number	Plant Species name	Family	Local name	Part(s) used
1	*Parkia biglobosa* (Jacq.) R.Br ex G.Don	Fabaceae	*Ìgbá*	Leaves
2	*Vernonia amygdalina* Del.	Asteraceae	*Ewúro*	Leaves
3	*Ficus exasperata* Vahl.	Moraceae	*Ipín*	Leaf buds or juvenile leaves
4	*Ceiba pentadra* (L.) Gaertn.	Malvaceae	*Àràbà*	Root
5	*Anogeissus leiocarpus* (DC) Guill & Perr.	Combretaceae	*Àyin*	Root
6	*Daniellia oliveri* (Rolfe) Hutch. & Dalziel	Fabaceae	*Iyá*	Root
7	*Tetrapleura tetraptera* (Schumm. & Thonn.) Taub.	Fabaceae	*Àìndan*	Root
8	*Citrus aurantifolia* (Christ.) Swingle	Rutaceae	*Òrombó*	Root
9	*Anthocleista djalonensis* A. Chev.	Gentianaceae	*Sápó*	Root
10	*Dioscorea bulbifera* L.	Dioscoreaceae	*Isu-erin*	Peels of stem tuber
11	*Citrullus colocynthis* (L.) Schrad.	Cucurbitaceae	*Bàrà or Ègúsí*	Fresh fruit
12	*Sorghum bicolor* (L.) Moench	Poaceae	*Okàa- bàbà*	Leaf sheath
13	*Allium cepa* L.	Amaryllidaceae	*Àlùbósà*	Leaves
14	*Xylopia aethopica* (Dunel) A. Rich.	Annonaceae	*Èrù*	Fruits
15	*Capsicum frutescens* L.	Solanaceae	*Ata wéwé/ata ìjòsì*	Fruits

Herbal recipes for seven liquid drugs : Decoctions: HIGBP-B (1 only); HIGBP-D(3 only); HIGBP-E (4,5,6,7,8); HIGBP-N (12,13,14+ table salt); HIGBP-Q (1,15); infusion: HIGBP-C (2 only); infusion or tincture: HIGBP-M (9,10,11); suffix alphabets indicate the manufacturers' anonymity codes. HIGBP, high blood pressure.

Table 9: Names of plants and their parts used for the formulation of traditional oral liquid herbal drugs for the treatment jaundice or anaemia in Ogbomoso, Nigeria

Number	Plant Species name	Family	Local name	Part(s) used
1	*Parkia biglobosa* (Jacq.) R.Br ex G.Don	Fabaceae	*Ìgbá*	Stem bark
2	*Paullinia pinnata* L.	Sapindaceae	*Kàkànselà*	Leaves
3	*Senna alata* (L.) Roxb.	Fabaceae	*Àsùnwòn òyìbó*	Root, flower and leaves
4	*Xylopia aethiopica* (Dunel) A. Rich.	Annonaceae	*Èrù*	Fruits
5	*Olax subscorpioidea* Oliv.	Olacaceae	*Ifon*	Root
6	*Alstonia boonei* De Wild.	Apocynaceae	*Ahùn/wáwòn*	Stem bark
7	*Khaya senegalensis* A. Juss.	Meliaceae	*Àgànó*	Stem bark
8	*Enantia chlorantha* Oliv.	Annomaceae	*Awopa/Dókítà igbó*	Stem bark

Herbal recipes for four liquid drugs;Decoctions: JAUND-A (1 only); JAUND-C (2 + potash) ; JAUND-M (3 and 4); and decoction or tincture: JAUND-N (5,6,7,8); suffix alphabets indicate the manufacturers' anonymity codes.JAUND, jaundice.

Table 10: Names of plants and their parts used for the formulation of traditional oral liquid herbal drugs for the treatment of malaria fever in Ogbomoso, Nigeria

Number	Plant Species name	Family	Local name	Part(s) used
1	*Bridelia ferruginea* Benth.	Euphorbiaceae	*Ìrà*	Stem bark
2	*Citrus aurantifolia* (Christ.) Swingle	Rutaceae	*Òrombó*	Fruits (sliced)
3	*Mangifera indica* L.	Anacardiaceae	*Móngòrò*	Stem bark
4	*Parkia biglobosa* (Jacq.) R.Br ex G.Don	Fabaceae	*Ìgbá*	Stem bark
5	*Allium cepa* L.	Amaryllidaceae	*Àlùbósà*	Bulb or leaf base
6	*Ananas comosus* (L.) Merr.	Bromeliaceae	*Òpe òyìbó*	Fruit (crushed)
7	*Alstonia boonei* De Wild.	Apocynaceae	*Ahùn/wáwòn*	Stem bark
8	*Capsicum frutescens* L.	Solanaceae	*Ata wéwé/ata ìjòsì*	Fruits
9	*Zingiber officinale* Roscoe	Zingiberaceae	*Atalè*	Rhizome
10	*Khaya senegalensis* A. Juss.	Meliaceae	*Àgànó*	Stem bark
11	*Anogeissus leiocarpus* (DC) Guill & Perr.	Combretaceae	*Àyin*	Stem bark
12	*Blighia sapida* K. D. Koenig	Sapindaceae	*Isin*	Stem bark
13	*Enantia chlorantha* Oliv.	Annomaceae	*Awopa/Dókítà igbó*	Stem bark
14	*Piper guinense* Schumach.	Piperaceae	*Ìyèré*	Fruits
15	*Terminalia glaucescens* Planch.	Combretaceae	*Idí-òdàn*	Stem bark
16	*Sarcocephalus latifolius* (Smith) Bruce	Rubiaceae	*Ègbèsì*	Stem bark
17	*Eugenia aromaticum* (L.) Merr. & L.M. Perry	Myrtaceae	*Kànnáfùrù*	Fruits/flower buds
18	*Aframomom melegueta* K.Schum.	Zingiberaceae	*Ataare*	Seeds/fruit
19	*Parkia biglobosa* (Jacq.) R.Br ex G.Don	Fabaceae	*Ìgbá*	Root bark
20	*Mangifera indica* L.	Anacardiaceae	*Móngòrò*	Root bark

Herbal recipes for 14 liquid drugs; Decoctions: MALAR-A (1,2); MALAR-B (3,4,5); MALAR-C (6 + fermented maize water); MALAR-E (7, 10, 11, 12); MALAR-F (2, 13, 14 + fermented maize water); MALAR-I (7, 13, 15); MALAR-L (13 +fermented maize water); MALAR-M (7, 17 + table salt); MALAR-O (7, 9, 18); infusions: MALAR-D (7, 8, 9); MALAR-J (3, 5, 16); MALAR-N (7, 13, with cold water or 7-Up beverage drink); MALAR-P (7, 8, 9); MALAR-Q (5, 19, 20); suffix alphabets indicate the manufacturers' anonymity codes. MALAR, malaria fever.

Table 11: Names of plants and their parts used for the formulation of traditional oral liquid herbal drugs for the treatment of piles in Ogbomoso, Nigeria

Number	Plant Species name	Family	Local name	Part(s) used
1	*Senna accidentalis* L.	Fabaceae	*Réré abo*	Root
2	*Senna tora* (L.) Roxb.	Fabaceae	*Réré ako*	Root
3	*Allium cepa* L.	Amaryllidaceae	*Àlùbósà eléwé*	Leaves
4	*Alcohorneae laxiflora* (Benth.) Pax & K. Hoffm.	Euphorbiaceae	*Ijàn*	Leaves
5	*Euphorbia hirta* L.	Euphorbiaceae	*Emilè*	leaves
6	Anacardium occidentale L.	Anacardiaceae	*Kajú*	Stem bark
7	*Bridelia ferruginea* Benth.	Euphorbiaceae	*Ìrà*	Stem bark
8	*Sarcocephalus latifolius* (Smith) Bruce	Rubiaceae	*Ègbèsì*	Stem bark
9	*Maranthes polyandra* Benth.	Chrysobalanaceae	*Ara/igi ara*	Stem bark
10	*Anogeissus leiocarpus* (DC) Guill & Perr.	Combretaceae	*Àyin*	Stem bark
11	*Allium sativum* L.	Amaryllidaceae	*Aáyù*	Bulb
12	*Ananas comosus* (L.) Merr.	Bromeliaceae	*Òpe òyìbó*	Fruit
13	*Citrullus colocynthis* (L.) Schrad.	Cucurbitaceae	*Bàrà/Ègúsí*	Fresh fruit
14	*Pseudocedrela kotchyii* Harms	Meliaceae	*Emi-gbègì*	Stem bark
15	*Ancistrophylum secundiflorum* L.	Areceae	*Òkùùku*	Stem bark
16	*Eugenia aromaticum* (L.)Merr. & L.M. Perry	Myrtaceae	*Kànnáfùrù*	Fruits
17	*Zingiber officinale* Roscoe	Zingiberaceae	*Atalè*	Rhizome
18	*Khaya senegalensis* A. Juss.	Meliaceae	*Àgànó*	Root bark
19	*Sorghum bicolor* (L.) Moench	Poaceae	*Okàa- bàbà*	Leaf sheath
20	*Aristolochia ringens* Vahl	Aristolochiaceae	*Akogùn*	Root
21	*Huntaria umbellata* K. Schum.	Apocynaceae	*Àbèrè*	Fruit

Herbal recipes for 12 oral liquid drugs; Decoctions: PILES-A (1, 2 + potash); PILES-B (3, 4, 5 + fermented maize water); PILES-F (14, 15, 16, 17 + few tablets of edible camphor); PILES-L (18, 19, 20); PILES-O (7, 11, 16, 17); decoction or tincture: PILES-C (6, 7); PILES-D (7, 8, 9, 10, 11, 16, 17 + few tablets of edible camphor); infusion: PILES-N (3, 21); infusion or tincture: PILES-M (3, 16, 18, 20); PILES-P (7, 10, 11, 16, 17); PILES-Q (3, 4 + fermented maize water); juice (crush, squeeze and strain): PILES-E (12, 13 + potash); suffix alphabets indicate the manufacturers' anonymity codes. PILES, piles.

Table 12: Names of plants and their parts used for the formulation of traditional oral liquid herbal drugs for the treatment of typhoid in Ogbomoso, Nigeria

Number	Plant Species name	Family	Local name	Part(s) used
1	*Bridelia ferruginea* Benth.	Euphorbiaceae	*Ìrà*	Stem bark
2	*Citrus aurantifolia* (Christ.) Swingle	Rutaceae	*Òrombó*	Whole fruit
3	*Anogeissus leiocarpus* (DC) Guill & Perr.	Combretaceae	*Àyin*	Leaves
4	*Vernonia amygdalina* Del.	Asteraceae	*Ewúro*	Root
5	*Xylopia aethiopica* (Dunel) A. Rich.	Annonaceae	*Èrù-alamo*	Empty pods of fruits
6	*Aframomum melegueta* K. Schum.	Zingiberaceae	*Ataare*	Seeds (7 or 9 pieces)*
7	*Capsicum frutescens* L.	Solanaceae	*Ata wéwé/ata ìjòsì*	Fruits (7 or 9 pieces)*
8	*Ananas comosus* (L.) Merr.	Bromeliaceae	*Òpe òyìbó*	Fruit
9	*Saccharum officinarum* L.	Poaceae	*Ìrèké*	Stem juice
10	*Gladiolus psittacinus* Hook	Iridaceae	*Bákà*	Bulb
11	*Citrus aurantifolia* (Christ.) Swingle	Rutaceae	*Òrombó*	Fruit juice

12	*Enantia chlorantha* Oliv.	Annomaceae	*Awopa/Dókítà igbó*	Stem bark
13	*Alstonia boonei* De Wild.	Apocynaceae	*Ahùn/wáwòn*	Stem bark
14	*Terminalia glaucescens* planch.	Combretaceae	*Ídi-òdàn*	Stem bark
15	*Khaya senegalensis* A. Juss.	Meliaceae	*Àgànó*	Stem bark
16	*Mangifera indica* L.	Anacardiaceae	*Móngòrò*	Stem bark
17	*Zingiber officinale* Roscoe	Zingiberaceae	*Atalè*	Rhizome
18	*Zanthoxylum zanthoxyloides* (Lam.) Zepern. & Timler	Rutaceae	*Àta*	Root
19	*Allium cepa* L.	Amaryllidaceae	*Àlùbósà eléwé*	Leaves
20	*Xylopia aethiopica* (Dunel) A. Rich.	Annonaceae	*Èrù-alamo*	Whole fruits
21	*Anogeissus leiocarpus* (DC) Guill & Perr.	Combretaceae	*Àyin*	Root
22	*Daniellia oliveri* (Rolfe) hutch. & Dalziel	Fabaceae	*Iyá*	Root bark
23	*Tetrapleura tetraptera* (Schumm. & Thonn.) Taub.	Fabaceae	*Àìndan*	Root

*Herbal recipes for 12 oral liquid drugs; Decoctions: TYPHO-A (1, 2); TYPHO-C (3, 4, 5); TYPHO-D (3, 5, 6, 7); TYPHO-I (12, 13, 14); TYPHO-J (15 only); TYPHO-L (16 only); TYPHO-N (14, 18, 19, 20); TYPHO-O (11, 21, 22, 23); TYPHO-P (12, 13, 14); TYPHO-Q (15 + potash); infusion or tincture: TYPHO-M (17 + 7-Up beverage drink + potash); juice (crush, squeeze and strain): TYPHO-E (5, 8, 9, 10, and 11); suffix alphabets indicate the manufacturers' anonymity codes. TYPHO, typhoid. *7 or 9 pieces for female or male user of the remedies respectively.*

Table 13: Names of plants and their parts used for the formulation of traditional oral liquid herbal drugfor the treatment of yellow fever in Ogbomoso, Nigeria

Number	Plant Species name	Family	Local name	Part(s) used
1	*Alstonia boonei* De Wild.	Apocynaceae	*Ahùn/wáwòn*	Stem bark
2	*Elais guinensis* Jacq.	Arecaceae	*Òpe*	Stem bark
3	*Sorghum bicolor* (L.) Moench	Poaceae	*Okàa- bàbà*	Leaf sheath

Herbal recipe for one oral herbal decoction: YELLO-D (1, 2, 3 +potash); suffix alphabet indicates the manufacturer's anonymity code. YELLO, yellow fever.

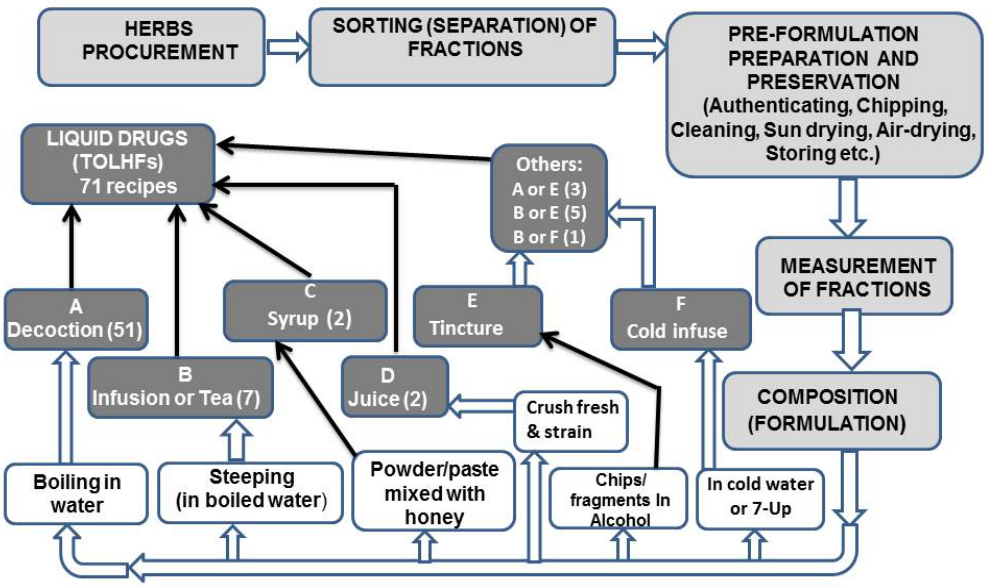

Figure 2: Documentation of the procedure (i.e. light-shaded boxes), process (i.e. un-shaded boxes), and products (i.e. dark-shaded boxes) of TOHLFs in Ogbomoso, Nigeria. *TOHLFs,* traditional oral liquid herbal formulations

IV. Disussion

The presentation in Table 2 is indicative of prevalence of malaria, piles, typhoid and high blood pressure; and awareness of the necessity for blood forming and blood thinning drugs among the residents of Ogbomoso. However, ill health conditions such as arthritis, back or waist pain, diabetes and yellow fever appear to be relatively uncommon, or else, people suffering from these ailments did not seek healing or management from THMPs. There are reasons to believe that malaria is a major public health problem in Nigeria, including accounting for more cases and deaths than any other country in the world (United States Embassy in Nigeria, 2011; World Health Organisation, 2013). Predominance of antimalarial liquid drugs among the products from the traditional healers in the study area is a confirmation that the disease is prevalent in the southwestern parts of the country(Okunade, 2001).

Going by the principle of sustainable herbal medicine (Pesic, 2015), it is necessary to examine whether production of TOLHFs in Ogbomoso is sustainable, and if the exercise will not result in irreparable loss to the local vegetation. No doubt, the number of plant species being exploited for TOLHFsin the study area is on the high side (Tables 3-13), but our chief concern should be the sources of these raw materials. The results of this study revealed that about 39% of the medicinal herbs were sourced through purchase from herb vendors or suppliers. Even as a few of the purchased herbs were collections from outside Ogbomoso and its environs, a scrutiny of the lists in Tables 3-13 revealed that a substantial number of them were obtainable in the savanna woodland to which Ogbomoso ecologically belongs (Keay, 1989). For this reason, there is the probability that most of these plants were readily available in the past for free collection in the neighbourhoods, but now become articles for purchase due to urbanization and other related factors that have made then less accessible (Hsuch, 2009; Liu *et al*., 2015). It is therefore reasonable to believe that the natural flora of Ogbomoso has been largely impacted to the extent that only about 36% of the herbs are available for collection in the wild.

In order to truly ascertain the sustainability status of TOLHFs production in Ogbomoso, empirical data on the quantities of raw material herbs extracted annually for this purpose are required, and this information was not available to this study. But then, if it is assumed that the 51 of the plant species used for TOLHFs yet to be evaluated by the IUCN are not threatened, it is logical to infer from available data from this study, and information in the red list of IUCN that production of TOLHFs in Ogbomoso is sustainable. This position is strengthened by the facts that exploitation of plant seeds as in *G. kola* is a sustainable practice, and in fact, healthy to the plant (Rokaya *et al*., 2017); exploitation of *J. curcas* (i.e. the leaves) is also sustainable, and in addition, its cultivation is now being widely advocated for biodiesel production and other purposes in Nigeria (Fakayode *et al*., 2012; Raufu *et al*., 2014; Akogwu *et al*., 2018; Yahuza *et al.,* 2020); and lastly, *K. senegalensis*, whose sustainable exploitation may be in doubt (i.e. the stem bark), is seldom employed (7.1%) in manufacturing TOLHFs in the study area, and in such circumstance, a rotation system of collection can be adopted to reduce plants' sensitivity to harvesting and enhance their resilience (WHO, IUCN & WWF, 1993). In addition, the efforts put up by the manufacturers of TOLHFs in cultivating about 26% of their raw material herbs are commendable, but there is room for improvement(Schippmannet al., 2002).

V. Conclusion and Recommendation

Seventy-one TOLHFs manufactured in Ogbomoso, Nigeria fell into five categories of remedies namely decoction (51), infusion or tea (7), syrup (2), juice (2), and others (9), which are indicated for the treatment of 14 different kinds of health conditions including malaria, piles and typhoid, and in the management of such dreaded diseases as high blood pressure, yellow fever, and diabetes. Herbs extracted from 57 plant species in 34 angiosperm families are used to produce these drugs, but the practice is adjudged sustainable with minimal injury on the neighbouring flora if sustainable harvesting can be encouraged or enforced alongside medicinal plants cultivation.

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Competing interests

The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this article.

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Memberships

Fellows/Associates of Science Frontier Research Council

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The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

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20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

Criterion for Grading a Research Paper (Compilation) by Global Journals

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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