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Ectoparasiticidal Activity of Neem (*Azadirachta indica*), Fish Poison (*Tephrosia vogelii*), Lemon Grass (*Cymbopogon citratus*) and Orange Peel (*Citrus sinensis*) against Mosquitoes, Ticks and Mites

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Abstract

Introduction: *Inflammatory bowel disease (IBD) requires patient-centered care beyond pharmacological therapy. DOCLIVI, a holistic digital assistant integrating nutrition, physical activity, and stress–emotion regulation with personalized coaching, was developed to promote lifestyle modification in IBD. This study evaluated its impact on quality of life and behavioral indicators of health.*

Methods: *This prospective, single-arm, multicenter study enrolled patients with Crohn's disease or ulcerative colitis on stable biologic therapy. Participants used the DOCLIVI app, which included three programs (nutrition, physical activity/sedentary lifestyle, and stress–emotion regulation), and attended three personalized video coaching sessions over six months. Outcomes included the IBD Disk (quality of life and functional disability), PSS-10 (stress), and behavioral questionnaires assessing nutrition, physical activity (RICCI & GAGNON), sedentary lifestyle, sleep, circadian rhythm, connection to nature, and social-emotional well-being.*

Results: *Forty-one patients were included; 34 completed the program (mean age 35.5 years, 28 women). IBD Disk scores improved significantly (55.8 → 37.1; mean difference = 18.7; $p < 0.001$; Cohen's $d = 1.01$), with 40% achieving satisfactory quality of life (score < 40). Improvements were observed in 9 of 10 domains, including anxiety, fatigue and sleep. Healthy eating habits improved (+0.97; $p = 0.010$), physical activity increased by 23.6% ($p = 0.043$), and contact with nature rose from 58.8% to 76.5% ($p = 0.014$). Adherence and satisfaction were high, with 65% of participants achieving their goals.*

Conclusion: *Digital holistic care combining lifestyle modules and personalized coaching significantly improved quality of life and lifestyle behaviors in IBD patients. Sustained engagement may enhance long-term benefits.*

Introduction

Mosquitoes can transmit more life-threatening diseases such as malaria, dengue, chikungunya, zika, yellow fever, encephalitis and filariasis and make life at risk for millions of people particularly in tropical world [1,2]. Oribatid is one of the largest groups of mites, comprising more than 163 families, 1,300 genera and subgenera and 11,207 species and subspecies [3]. To prevent occurrence of mosquito-borne, ticks, mites diseases and promote public health, it is necessary to control their population. The use of organo-chlorine and organo-phosphorus pesticides in fighting against mosquitoes, ticks and mites pose more hardship on the farmers, due to high cost of these pesticides, their stability in environment, increase in resistance to pesticides, harmful effects on human health and other non-target populations, they have been used of them is limited [4]. These reasons have prompted researchers [3,5] to look for alternative methods, especially use of natural substances to fight and reduce the mosquitoes, ticks and mites population, which are compatible with environment. One of most effective alternative methods is use of materials of plant origin, which is a simple and sustainable method for mosquito and general pest control [6].

In addition, they are coordinated with behavioral and physiological processes and also create little resistance [7,8]. More than 2000 plant species have been identified that

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have valuable metabolites in pesticide application [9]. Members of plant family *Solanaceae, Asteraceae, Cladophoraceae, Labiatae, Miliaceae, Oocystaceae* and *Rutaceae* have been used against various mosquito, ticks and mites species.

Figure 1a. *Neem (Azadirachta indica).*

Neem (*Azadirachta indica*) is a highly versatile evergreen tree native to the Indian subcontinent. Revered in traditional medicine, its bark, leaves, flowers and oil are used globally for their powerful anti-bacterial, anti-fungal and anti-inflammatory properties to treat skin conditions, support oral hygiene, and act as natural pesticides (Figure 1a).

Fig 1b. *Fish-poison-bean (Tephrosia vogelii).*

Fish-poison-bean (*Tephrosia vogelii)* (Figure 1b) is a herb or small tree that is native to tropical Africa and has also been used in tropical America as well as South and Southeast Asia [11]. It is commonly used to deter pests and diseases, specifically fleas and ticks on animals. It is not suitable for livestock or human consumption because it is not highly nutritious and can be poisonous for fish and some other animals

Figure 1c. *Lemon grass (Cymbopogon citratus).*

Lemon grass *(Cymbopogon citratus)* (Figure 1c) belongs to the *Poaceae* family. *Cymbopogon*, also known as lemongrass, barbed wire grass, silky heads, oily heads, Cochin grass, Malabar grass, citronella grass or fever grass, is a genus of Asian, African, Australian and Tropical Island plants in the grass family. It is a tropical herb and one of the main medicinal and aromatic plants in Asia and Africa, as an anti-septic, anti-microbial, anti-inflammatory, anti-tussive, anti-rheumatic, to treat back pain, sprains and hemoptysis in some countries. In African it is used to treat diabetes [12,13].

Citrus sinensis (sweet orange) peels (Figure 1d) are highly potent, nutrient-dense by products that contain concentrated levels of essential oils, flavonoids and vitamin C. Instead of being discarded, they can be dried, extracted [14], or powdered for versatile use in cooking, cosmetics and eco-friendly

Figure 1d. *Orange peels (Citrus sinesis).*

household cleaning. Sweet orange peels (*Citrus sinensis)* and other citrus wastes cannot be used in landfills due to their low pH, high water and organic matter content; however, they have great potential as raw materials for fermentation and as a source of carbohydrate polymers and enzymes. There are various bioactive compounds with medicinal applications [15].

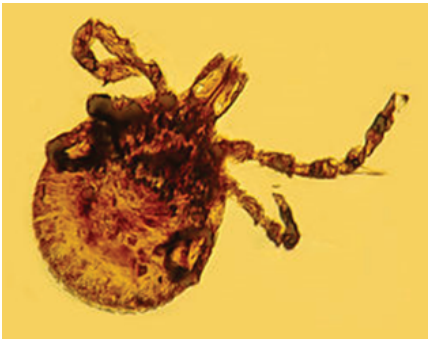

Figure 1e. *Ticks (Ixodida)*

Figure 1f. *Mosquito*

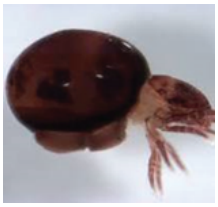

Figure 1g. *Mites*

In Nigeria inorganic mosquito repellent such as Raid, Mobil are not accepted in closed houses especially those having infants and young children due to their side effects and high cost, rather, people use smoke of some particular types of herbs such as leaves of Eucalyptus, Neem (*Azadirachta indica*) [16], Fish poison (*Tephrosia vogelii*), lemon grass and orange peel in community to keep mosquitoes, ticks and mites away [17]. Therefore, this study evaluated pesticidal efficacy of Neem (*Azadirachta indica*), Fish poison (*Tephrosia vogelii*), orange peel (*C. sinensis)* and lemon grass (*C. citratus*) on mosquitoes, ticks and mites.

Materials and Methods

The study was carried out at the biological garden of University of Agriculture and Environmental Sciences, Umuagwo, Imo State, Nigeria. It is geographically situated at coordinates 5°18'25" N latitude and 6°56'37" E longitude. The community lies along the Owerri-Port Harcourt Road, roughly 26 km south of Owerri, Imo State, Nigeria. The year has two seasons: the dry season and the rainy season. The rainy season extends from April to October, with average rainfall varying from 400 to 800 mm [18]. The dry season, which lasts 6 months, is marked by the harmattan, a hot, dry wind that sweeps across the region along a northeast to south-west axis. Temperatures oscillate between 20°C and 40°C. Vegetation was characterized by the shrub and tree savannah in the southern part, dominated by *Moringa oleifera, Gliricidia sepium, Citrus species and Tephrosia vogelii*, depending on soil type.

Sample collection

Live Mosquitoes, ticks and mites were collected from their natural breeding sites around the University campus and biological garden of University of Agriculture and Environmental Sciences, Umuagwo, Imo State, Nigeria using simple drag net for mosquitoes, ticks, mites and were transported to the laboratory for identification. From collected groups of mosquitoes, *Anopheles* mosquitoes were separated based on features like - having long palps with white-bands and 3 dark and 2 white spots on wing vein, the maxillary palps and a heavy footstep. *Culex* mosquitos were small, brown, with blunt abdomens and without band legs. Mosquito selected for this study were *Anopheles* spp.

Collection and preparation of plant materials

Citrus sinensis were obtained from fruits of orange, *Tephrosia vogelii, Azadirachta indica* and *Cymbopogon citratus* also collected from the biological garden, University of Agriculture and Environmental Sciences, Umuagwo, Nigeria were cut into small pieces, spread on clean white cardboard papers and air-dried at room temperature for 7 days. The dried peel and other plant materials were pounded using a mortar and pestle into powdered form. One hundred gram (100g) of the powder (each) was transferred into beaker separately and soaked in 500ml of water steered occasionally and left overnight. The extracts were filtered using muslin cloth and served as stock solution. Three different concentrations (1.5%, 3.0% and 4.5%) were prepared using serial dilution procedure, from each extract. They were then spread on filter paper and the filter paper was placed inside the beaker. Ten mosquitoes, ticks and mites were introduced in each beaker using a funnel from the captured stock and covered by a fine wire net.

Control mosquitoes, ticks and mites were kept in similar condition but filter paper was treated with distilled water only. Each set of experiment was replicated three times. The observations were recorded visually from outside at 30 minutes, 1, 6, 12 and 24 hours' intervals. No movement in mosquitoes, ticks and mites bodies were taken as evidence of death.

Results and Discussion

The results of the study showed that 1.5% concentration of *C. sinensis* extracts had 20% mortality within 30 minutes; while same concentration caused 40% mortality within 24 hours exposure. The 4.5% concentration of the extract produced maximum mortality at all exposure periods. No mortality was recorded among control group. In all cases mortality was time and dose dependent (Table 1).

It was showed that 1.5% concentration of extract of *C. citrates* caused 5% mortality in treated mosquitoes, ticks and mites within 30 minutes of exposure and 25% at 24 hours exposure period; while 4.5% concentration of the extract produced 25% mortality within 30 minutes and 60 % mortality was recorded within 24 hours exposure. No mortality was recorded among control group (Table 2).

Table 1: *Mortality (%) among treated mosquitoes, ticks and mites as affected by aqueous extracts of Neem flowers, Tephrosia vogelii seeds and C. sinensis.*

	Exposure period				
Concentrations	**30 mins**	**1hr**	**6 hrs**	**12 hrs**	**24 hrs**
1.50%	20	25	25	30	40
3.00%	25	30	45	45	50
4.50%	40	45	50	60	65
Control	0		0	0	0
Key: mins= minutes, hr = hour, hrs = hours					

Table 2: *Mortality among treated mosquitoes as affected by aqueous extract of Neem flowers, Tephrosia vogelii seeds and C. citratus*

	Exposure period				
Concentrations	**30 mins**	**1hr**	**6 hrs**	**12 hrs**	**24 hrs**
1.50%	5	10	20	20	25
3.00%	10	25	30	45	45
4.50%	25	40	40	45	60
Control	0		0	0	0

Tephrosia can be used for various purposes besides human and livestock consumption which makes it a diverse and helpful plant. Its most common use is for organic tick control. First, leaves from the *Tephrosia* plant are ground up and a juice is extracted which is then used on the animal. The green liquid from the plant is mixed with water and is then applied to the animal's skin with a piece of cloth or a sponge. A little bit of soap can be added to the liquid to make it stick to the skin. Usually it is left on the animal for a week after which results appear. It is only effective against ticks, mites and mosquitoes that still have soft skin and are immature. This is a great practice for farmers that do not have access to veterinary medicine. *Tephrosia* can also be used as a fish poison, because chemicals in the plant react to chemicals in the fish and stupefy them so they can be easily caught.

Although *Tephrosia vogelii* is not consumed directly, it can be used to increase the yield of various crops. For example, leaf extracts of *Tephrosia vogelii* are used as chemical pesticides. Crops that have had this extract applied show significant decreases in insect and other pest activity. Therefore, crops can grow in areas that they could not before, if they are grown alongside *Tephrosia vogelii*. In fact, studies have shown a 46.2-52.2% decrease in grain damage. Grain damage was measured by the amount of kernels that were destroyed and also by the net weight loss of the crop. This number varies based on method of storage, type of insect measured and type of stored grain. In one particular study, the extract killed 40% of the attacking insects after a 21-day period. This decrease is about what would be expected from most chemical pesticides.

Neem (*Azadirachta indica*) is celebrated in traditional medicine for its anti-microbial and anti-inflammatory properties. It is frequently used as a complementary or home remedy to manage symptoms of various ailments, particularly skin conditions (acne, eczema, psoriasis), fungal infections (ringworm, athlete's foot), and gum diseases (gingivitis, plaque). Extracts from its leaves, bark, and seeds are utilized to help manage and treat a variety of ailments: Skin Diseases: Neem oil and leaf paste are widely used in remedies to soothe and treat inflammatory and chronic conditions like psoriasis and eczema. Fungal Infections: The anti-fungal properties of neem leaves help treat common fungal infections such as candidiasis, athlete's foot, and ringworm. Due to its natural anti-bacterial compounds, neem is used in toothpastes and mouthwashes to help cure gingivitis and reduce gum bleeding. Malaria: Neem bark and leaf extracts have a long history of use in curbing the symptoms and progression of malaria, though clinical research is still evaluating its parasiticidal efficacy. Stomach Ulcers and Gastro-intestinal Issues: Pre-clinical trials suggest that compounds in Neem can reduce stomach ulcers and regulate issues like diarrhea and constipation. Type 2 Diabetes: Bioactive ingredients in Neem extracts are studied for their potential to help regulate blood sugar levels, which is relevant for the management of type 2 diabetes.

Ticks are parasitic arachnids of the order Ixodida (Figure 1e). They are part of the mite superorder *Parasitiformes*. Adult ticks are approximately 3 to 5 mm in length depending on age, sex and species, but can become larger when engorged. Ticks are external parasites, living by feeding on the blood of mammals, birds and sometimes reptiles and amphibians. The timing of the origin of ticks is uncertain, though the oldest known tick fossils are around 100 million years old and come from the *Cretaceous* period. Ticks are widely distributed around the world, especially in warm and humid climates.

Mosquito-borne (Figure 1f) diseases are considered major threats to global health in developing and developed countries. Their tendency to spread outside their geographic range and cause large epidemics is clearly demonstrated [19,20].

Sarcoptiformes includes other mites (Figure 1g) of veterinary importance such as *Psoroptes, Knemicodoptes, and Notoedres*. In humans, *Sarcoptiformes scabiei* causes disease known as scabies and in animals the disease is referred to as sarcoptic mange. The use of chemical insecticides is very effective against mosquitoes, ticks and mites but due to development of resistance, it leads to a rebounding vectorial capacity [21]. In recent years, the use of pesticides containing natural substances, especially of plant origin, which are compatible with the environment, has been considered for vector control, because they are rich in bioactive chemicals, active against a limited number of species, including specific target pests and were biodegradable [22]. The mosquito detects the odor when the volatile odor of the plant is repelled.

The olfactory receptor protein, which is expressed on the ciliated dendrites of olfactory receptor neurons exposed to the external environment, is often displayed on the antennae and mandibular floor of mosquitoes, ticks and mites causing mosquito, ticks and mites mortality. In each of the plants, the higher the escape rate, the higher the mortality and the lower the escape, the lower the mortality.

The results of this study showed that both plants *C. citrates* and *C. citratus* possess insecticidal efficacy against tested mosquitoes, ticks and mites but *C. sinensis* caused more mortality than *C. citratus* extract. In both cases observed effect was time and dose dependent which can be due to the fact that active moieties present in these extract get accumulated with time over prolonged period cause a cumulative effect in mosquito's body. The extract *C. citrates* was more potent at short exposure period which revealed that active moieties present in it are more volatile and thus absorbed rapidly while *C. citratus* showed persistent increase in mortality of mosquitoes, ticks and mites which probably shows that the active moieties present in this extract had slow but continuous release.

Castillo *et al*. [23] examined the pupicidal, adulticidal and repellent activities of essential oils from 7 other plants included *Cymbopogon citratus* and *Citrus sinensis* against *Aedes aegypti* under laboratory conditions. The greatest pupicidal activity was exhibited at 390 ppm with a 48-h exposure by *C. sinensis* of exposure [23]. In the study the greatest pesticidal activity (65 and 60%) was exhibited at 4.5 % concentration with a 24-hour exposure by *C. sinensis* and *C. citratus* respectively. Of course, because the units of measurement and the type of extract are different in the fields, it is not possible to make a complete comparison.

Ezeonu *et al*. [24] investigated the effect of extracts of two types of orange peel *Citrus sinensis* (sweet orange) and *Citrus aurantifolia* (lime) on mosquitoes and concluded that these two extracts have very low biological performance, but this fact should be considered that a large amount of these peels are produced by orange juice industries. Extraction of skin oils is a suitable option to recover resources from waste. These facts show enough optimism for investment [24].

Dua *et al*. [25] showed the effect of *Valeriana jatamansi* root extract on larvicidal and adulticidal activity against different mosquito species. The results of their investigation showed that the average lethal concentration of the extract against the larvae of *Anopheles stephensi*, *Anopheles culicifacies*, *Aedes aegypti*,

Aedes albopictus and *Culex quinquefasciatus* was 68.1, 42.8, 51.2, 53.8 and 80 mg/liter respectively.

Harve and Kamath [26] tested the larvicidal activity of acetone and petroleum ether extracts of four plants *Murraya koenigii*, *Coriandrum sativum*, *Ferula asafoetida*, *Trigonella foenum graceum* in combination against *Aedes aegypti* larvae in laboratory conditions. All plants showed potential synergistic activity, although they showed relatively weak larvicidal activity when tested individually [26]. Thekkevilayil *et al*. [27] studied the in vitro bioassay of the essential oil extracted from *Ipomoea cairica* against the larvae of four mosquito vector species. The biometric test showed that the essential oil of the plant can cause 100% death in *Culex trita eniorhynchus* larvae. It was found that the essential oil is very toxic to the larvae of *Culex trita eniorhynchus* following mosquitoes *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinque fasciatus* [27]. Cavalcanti *et al.* [28] showed that *Ocimum americanum* and *Ocimum gratissimum* have LC50 ppm of 67 and 60 ppm, respectively, and the essential oils of these two plants have potential to control *A. aegypti* [28].

Vivekanandhan *et al*. [29] investigated the ethyl acetate extract of *Metarhizium anisopliae* (mycelia) on larvae, pupae and adults of *Anopheles Stephensi*, *Aedes aegypti* and *Culex quinque fasciatus* and concluded that in 24 hours after treatment *M. anisopliae* extract had significant toxicity on allIt had mosquito cheeks. The chemical compounds derived from *M. anisopliae* are effective on the target pests, are non-polluting, target specific and are alternative chemical pesticides [29,30]. Inocente *et al*. [31] tested the hypothesis that the extract of *Cinnamosma* spp. enriched with drimane sesquiterpenes, it is toxic to the *Aedes aegypti* mosquito. It showed that bark and root extracts, which contain higher abundances of drimane sesquiterpenes compared to leaves, were the most effective and drimane sesquiterpenes provide valuable chemical substrates for the development of pesticides and repellents to control mosquito vectors [31].

The biological effectiveness of *Solanum xanthocarpum* leaf extract on the larvae and pupae of *Culex quinque fasciatus* was investigated in laboratory conditions. Therefore, the present research clearly showed that *B. thuringiensis* can act as a high potential in the mortality of mosquito larvae. It is an eco-friendly biopesticide for the control of mosquito vector management program [32].

Of course, the units of measurement, the type of extract, the type of plants and the test stage were different in the various other studies.

Conclusion

It was concluded that *C. sinensis* and *C. citratus* possess pesticidal efficacy against tested mosquitoes, ticks and mites but *C. sinensis* caused more mortality than *C. citratus* extract. In both cases observed effect was time and dose dependent as waste of these plants is abundant in the industry, the pesticidal properties of the essential oils of these two plants should be evaluated by identifying the active components causing mortality and then using them in field experiments to evaluate their potential as an alternative to chemical larvicides.

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