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Therapeutic Potentials of *Mitracarpus scaber* and *Ocimum gratissimum* in the Treatment of Cutaneous Leishmaniasis in Imo State, Nigeria

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ABSTRACT

Background and Objective: Leishmaniasis is considered a neglected tropical disease (NTD) and hasbeenthe subject of concerted global efforts for its control. The study was conducted to determine the treatment of Cutaneous Leishmaniasis using *Mitracarpus scaber* and *Ocimum gratissimum* in Imo State. **Materials and Methods:** Preliminary qualitative and quantitative analyses were carried out on *Euphorbia helioscopia* and *Ocimum gratissimum* using established methods. Data obtained in this study were analyzed using analysis of variance at a 0.05% probability level. **Results:** The Percentage infection was highest at Umuaku Ntu 19 (13.77), while the least was observed from Umunam Atta 8 (5.79) subjects administered with 0.5% ivermectin in Umucheke and Umuaku Ntu communities had the highest rates (87.5%) of resolution of clinical signs after 0.5% ivermectin intake while those in Umunam Atta community had the lowest rates (70%). Out of the 275 (33.3%) number of subjects, 58 (47.2%) participants benefited from the consumption of extracts of *M. scarber* in all three communities. Subjects in the Umuaku Ntu community had the highest rates (75%) of resolution of clinical signs after consumption of the extract, while subjects in the Umucheke community had the lowest rates (30%). Out of the 275 (33.3%) number of subjects administered with 20% *O. gratissimum*, 112 (91.1%) of the participants in all the studied communities had resolution of their clinical signs. Subjects in Umuaku Ntu and Umucheke communities had the highest rates (88%) of disappearance of their clinical signs and symptoms while subjects in Umunam Atta communities had the lowest rates (50%) after the intake of Doxycycline. **Conclusion:** This study suggests that CL is a public health problem surrounding the study areas; urgent measures should be adopted to mitigate the incidence.

KEYWORDS

Transmission, cutaneous leishmaniasis, treatment, *Mitracarpus scaber*, *Ocimum gratissimum*, Imo State

INTRODUCTION

The treatment of leishmaniasis with available chemotherapy has been associated with systemic toxic effects, economic cost[1], and poor treatment compliance, necessitating most research in the past decade and even now to focus on the advancement of substitute dosage schedules, approaches of delivery, or management[2]. The establishment of the species composition of sand flies could reveal possible

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transmission of human leishmania parasites[3]. There is another documented work by Bongiorno *et al*.[4] on species composition of sandfly and occurrence of cutaneous leishmaniasis in Owerri North LGA of Imo State, showing the presence of two genus, Phlebotomus and Lutzomyia, in the study area but with no physical manifestation of cutaneous leishmaniasis[5,6]. There is therefore a need to ascertain if misdiagnosis of the infection has contributed to the absence of documented information of the disease, or if it is the visceral form of the disease that is present in Imo State. It is therefore pertinent to note that knowledge, attitude, and practices and determine the treatment of cutaneous leishmaniasis using *Mitracarpus scaber* and *Ocimum gratissimum* in Imo State.

Therefore, many patients seek alternative plant medicine which is cheaper and readily available, yet most of the herbs traditionally used in treatment have still not been evaluated scientifically. Natural products have long been providing important drug leads for infectious diseases, and while vaccination for leishmaniasis is not yet available, and conventional treatments are arguably unsatisfactory, the need for potential anti-leishmanial agents from natural products that have lower side effects is urgently needed. This research seeks to provide affordable, accessible, and quality but less toxic treatment for leishmaniasis.

The study aims to assess the therapeutic potential of *Mitracarpus scaber* and *Ocimum gratissimum* in the treatment of cutaneous leishmaniasis in Imo State, Nigeria, focusing on their antimicrobial, anti-inflammatory, and wound-healing effects.

MATERIALS AND METHODS

Study area: The study was conducted in Imo State, Southeast Nigeria, from July to November, 2024. Imo State has a total of twenty-seven Local Government Areas (LGAs). The people of Imo State are basically of Igbo extraction, having similar cultural activities like other Igbo communities, and they speak the Igbo language. The state is influenced by urban sprawl, whereby smaller communities merge. Imo State is divided into three political zones; the three zones are: Owerri Zone, Okigwe Zone, and the Orlu Zone. Based on the foregoing, a study sampling was doneto reflect the zones.

Ethical consideration: Ethical approval for the study was obtained from the Post Graduate Board of the Department of Biology, Federal University of Technology Owerri, Ethical Committee. Written/oral consent was sought and obtained from the health officers and local government health authorities, as well as the subjects.

Study population: The sample populations were drawn from three Local Government Areas (Umucheke Okwe in Orlu, Umunam Atta in Okigwe and Umuaku Ntu in Owerri) to cover the three Geopolitical Zones of the study area with a sample size of 600 subjects[3]. The choices of these Local Government Areas were to ensure good geographical spread, and subjects were selected based on obtaining informed consent. The study populations were cut across inhabitants who had lived for 10 years or above in the communities.

Study design: The three study sites, Orlu, Okigwe, and Owerri, were randomly sampled for sandfly species and subjects having leishmaniasis.

Procurement of materials for the study: Procuring the materials for the study involved the construction of trap nets for sandfly collection, hand lenses, petri dishes, a microscope, Soxhlet extractor, rotary evaporator, dissecting pins, slides, and cover slips from Final Nigeria Limited, Owerri, Imo State, Nigeria.

Familiarization and identification of endemic communities: Familiarization and identification of communities' endemic for cutaneous leishmaniasis prevalence and incidence in the three zones of Imo State to ascertain the level of morbidity and mortality, and obtaining other relevant demographic data from the Health and Environmental Protection Department will be undertaken.

Mobilization of selected communities and subjects: Preliminary survey and mapping out of the study into sites, interaction/discussion with community Heads of the designated study sites on the benefits of the research work. Mobilization and training of community volunteers as research assistants on the distribution of questionnaires and interviews, the census of the communities was taken, and the number of households was noted. Administered to individuals 18 years and above who have lived in the community for ten years or above and who gave their consent and agreed to participate in the research. This was to determine their knowledge about the presence of sand flies and cutaneous leishmaniasis. One hundred and fifty questionnaires were distributed to each of the communities, giving a total number of four hundred and fifty questionnaires to ascertain their knowledge, attitude, and practice concerning the prevention and control of leishmaniasis and sandfly vectors.

Clinical examination of cutaneous leishmaniasis: Those that gave their consent were clinically examined for the presence of cutaneous leishmaniasis and skin lesions, bumps, and ulcers, and potential breeding sites in and around the selected households, which include animal burrows, caves, tree holes, termite hills, rocks, etc., were noted. Hourly catches of flies were collected for four random days in a month per site from 6 am to 7 pm each day. Flies trapped were preserved in seventy percent ethanol in labeled sample bottles and transported to the Entomology unit, Department of Biology, FUTO, for proper identification. Climatic variables such as temperature and humidity on the days of the catch were recorded.

Screening techniques for cutaneous leishmaniasis: The screening technique for leishmaniasis was performed on randomly selected villagers who gave consent and presented themselves for examination. Two methods of screening were adopted in the diagnosis of cutaneous leishmaniasis. They were on-site clinical case detection in the field and the use of a cutaneous leishmaniasis rapid test kit. Concentration was given more to those with lesions.

Ivermectin administration: Ivermectin was administered by the trained health workers to subjects who volunteer themselves for the treatment.

Identification, collection, and authentication of plant materials: Samples of *Euphorbia helioscopia* and *Ocimum gratissimum* were collected and authenticated by Botanists in the Plant Taxonomy Unit, Department of Biology, FUTO. Voucher specimen numbers were assignedto each plant and deposited at the herbarium for future reference.

Preparation of plant extracts and application: The leaves of *Euphorbia helioscopia* and *Ocimum gratissimum* (Plates 1 and 2) were carefully separated from other morphological parts of the plants and washed clean with running water to remove dust particles and other water-soluble impurities settled on them[7]. The fresh leaves were squeezed and the liquid contents were used in the treatment of cutaneous leishmaniasis.

Phytochemical tests: The phytochemical analysis for the various plant parts was performed according to Vivero *et al.*[8].

Test for saponin: Half a gram of the crude extracts was diluted in 2 mL of distilled water and shaken rigorously for three minutes in a graduated cylinder. The formation of a stable layer of foam that is about 1 cm, confirmed the presence of saponins.

Test for alkaloids (Wagner test): Half a gram of the crude extracts was dissolved in 5 mL of distilled water, followed by filtration. The filtrate was then treated with Wagner's reagent, and the formation of a red brown precipitate was looked for to indicate the presence of alkaloids.

Plate 1: *Euphorbia helioscopia*

Plate 2: *Ocimum gratissimum*

Test for flavonoids: An amount of 5 mL of dilute ammonia solution was added to a portion of the aqueous filtrate of the extract, followed by the addition of concentrated sulfuric acid. The appearance of yellow coloration indicated the presence of flavonoids.

Test for tannins: To half a gram of the extracts, 1 mL of 5% ferric chloride was added. The formationof a greenish black color indicated the presence of tannins.

Test for glycosides: To 1 mL of extracts, 1.5 mL of chloroform and 10% ammonia solution were added. Pink colour formation indicated the presence of glycosides.

Test for triterpenoids: To 1.5 mL of the extracts, 1 mL of Libemann-Buchard Reagent (acetic anhydride+concentrated sulphuric acid) was added. The formationof blue-green color indicated the presence of triterpenoids.

Test for anthraquinones: About 0.5 g of each extract was boiled with 10 mL of sulphuric acid and filtered while it was still hot. The filtrate was shaken with 5 mL of chloroform. The chloroform layer was then pipetted into another test tube and 1 mL of dilute ammonia was added. A colour change was observed in the resulting solution.

Test for phytosterols: To 0.5 mL of the extracts, 2 mL of cold acetic acid and a few drops of 5% ferric chloride were added. This was overlaid with 1 mL of concentrated sulphuric acid. Brown ring formation at the interface indicated the presence of steroids.

Data analysis: Data obtained in this study were analyzed using Analysis of Variance (ANOVA) subjected to SAS Package Version 20.0 software, and means were separated using Duncan's Multiple Range Test (DMRT) at a $p<0.05$ significant level. Prevalence was calculated as simple percentages and standard deviation.

RESULTS AND DISCUSSION

Demographic characteristics of the study participants: The level of education was higher among those with secondary education (45.82%), while the lowest level of education was recorded among those with no formal education (9.09%). Results further revealed that most of the participants are married (61.82%) while about 3.27% are separated/divorced. Farming is the main economic activity (30.91%) followed by Civil Service (24.00%), Trading (20.36%), and studying (9.46%), respectively. The majority of the participants (53.09%) earned less than ₦30,000, while 14.55% earn above ₦41,000 (Table 1).

Phytochemical properties of *M. scarber* and *O. gratissimum*: Mean concentrations of the quantitative phytochemical constituents of the plant extracts (*M. scarber* and *O. gratissimum*) used for the study are presented in Table 2. Results obtained showed the presence or presence of bioactive compounds such as Alkaloids, Flavonoids, Saponins, Tannins, Steroids, Cardiac glucosides (%), and Anthraquinone (mg/100 g) in the plant extracts. *Mitracarpus scarber* extracted with absolute ethanol yielded the highest Alkaloids with a mean value of 17.94, while the lowest value of Alkaloids was obtained from *O. gratissimum* (10.16). Similarly, the Flavonoids (%) content of *O. gratissimum* yielded the highest phytoconstituents with a mean value of 35.71 (%), while the lowest value was obtained from *M. scarber* with a mean value of (13.50%), *M. scarber* (%) quantitatively yielded the highest value of Cardiac glucosides (%) with 28.72, while *O. gratissimum* recorded the least concentration with a mean value of 23.06. Results from the quantitative analysis of the plant extracts further showed that Anthraquinone (mg/100 g) yielded the highest value of phytochemicals, with a mean value of 3.51, while *O. gratissimum* had no value for Anthraquinone (mg/100 g).

Phytochemical properties of *M. scarber* and *O. gratissimum*: Results of the qualitative analysis of plant extracts of *M. scarber* and *O. gratissimum* used for the study are shown in Table 3. Results obtained showed the presence or presence of bioactive compounds such as Alkaloids, Flavonoids, Saponins, Tannins, Steroids, Cardiac glucosides (%), and Anthraquinone (mg/100 g) in the plant extracts. Terpenoids and steroids were present in *M. scarber* in large quantities, while alkaloid were present in all the plant extracts assayed; flavonoids were present in large quantities in *O. gratissimum*, but no Anthraquinone was found.

Disappearance of clinical signs with ivermectin: Figure 1 illustrates the effectiveness of 0.5% ivermectin in treating cutaneous leishmaniasis (CL) across the study area. Among the 275 participants (33.3% of the total surveyed), 102 individuals (82.9%) showed a complete resolution of clinical signs and symptoms following ivermectin treatment. The highest response rates were observed in the Umucheke and Umuaku Ntu communities, where 87.5% of treated subjects experienced recovery. In contrast, the lowest response rate was recorded in Umunam Atta, at 70%.

Figure 2 depicts the therapeutic outcome of *Mitracarpus scaber* extract administration. Of the 275 individuals involved, 58 (47.2%) demonstrated noticeable clinical improvement. The Umuaku Ntu community reported the highest recovery rate (75%), while Umucheke showed the lowest (30%).

Table 1: Socio-demographic characteristics of respondents in the study area

	Yes (%)			
Variable	Umucheke Okwe (n = 97)	Umunam Atta (n = 76)	Umuaku Ntu (n = 102)	Total (%) (n = 275)
Age (Years)				
≤20	7 (7.22)	5 (6.58)	9 (8.82)	21 (7.64)
21-30	13 (13.40)	11 (14.47)	15 (14.71)	39 (14.18)
31-40	20 (20.62)	17 (22.37)	23 (22.25)	60 (21.82)
41-50	24 (24.74)	21 (27.63)	27 (26.47)	72 (26.18)
51-60	19 (19.59)	16 (21.05)	21 (20.59)	56 (20.36)
≥61	14 (14.43)	6 (7.90)	7 (6.86)	27 (9.82)
Sex				
Male	55 (56.70)	40 (52.63)	59 (57.84)	154 (56.0)
Female	42 (43.30)	36 (47.37)	43 (42.16)	121 (44.0)
Educational level				
Primary	22 (22.68)	18 (23.69)	20 (19.61)	60 (21.82)
Secondary	43 (44.33)	37 (48.68)	46 (45.09)	126 (45.82)
Tertiary	26 (26.80)	17 (22.37)	21 (20.59)	64 (23.27)
None	13 (6.19)	4 (5.26)	15 (14.71)	25 (9.09)
Marital status				
Single	26 (26.80)	20 (26.32)	31 (30.39)	77 (28.0)
Married	56 (57.74)	52 (68.42)	62 (60.78)	170 (61.82)
Widow	10 (10.31)	3 (3.95)	6 (5.88)	19 (6.91)
Separated/divorce	5 (5.22)	1 (1.31)	3 (2.95)	9 (3.27)
Religion				
Christian	91 (93.81)	73 (96.05)	94 (93.16)	258 (93.82)
Islam	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Traditional	6 (6.19)	3 (3.95)	8 (7.84)	17 (6.18)
Occupation				
Trader	19 (7.22)	16 (21.05)	21 (20.59)	56 (20.36)
Farmer	38 (13.40)	7 (9.21)	40 (39.22)	85 (30.91)
Carpenter	9 (20.62)	6 (7.90)	8 (7.84)	23 (8.36)
Welder	4 (24.74)	9 (11.84)	6 (5.88)	19 (6.91)
Civil servant	16 (19.95)	31 (40.79)	19 (18.63)	66 (24.0)
Student	11 (11.31)	7 (9.21)	8 (7.84)	26 (9.46)

Table 2: Quantitative phytochemical properties of *M. scarber* and *O. gratissimum*

S/N	Parameters	*M. scarber*	*O. gratissimum*
1	Alkaloids (µg/mL)	17.94	10.16
2	Saponins (µg/mL)	3.43	2.23
3	Tannins (mg/100 g)	2.04	1.13
4	Steroids	2.32	2.02
5	Cardiac glucosides (%)	28.72	23.06
6	Flavonoids	13.50	35.71
7	Anthraquinone (mg/100 g)	3.51	-

Table 3: Quantitative phytochemical properties of *M. scarber* and *O. gratissimum*

S/N	Parameters	*M. scarber*	*O. gratissimum*
1	Alkaloids (µg/mL)	++	+
2	Saponins (µg/mL)	+	+
3	Tannins (mg/100 g)	+	+
4	Steroids	+	+
5	Cardiac glucosides (%)	+	+
6	Flavonoids	+	++
7	Anthraquinone (mg/100 g)	+	-

+: Present, -: Absent and ++: Present in large quantities

Figure 3 presents the effect of 20% *Ocimum gratissimum* on CL symptoms. Among the same group of 275 subjects, 112 (91.1%) benefited from the treatment. Umuaku Ntu and Umucheke communities both exhibited high resolution rates (88%), whereas Umunam Atta reported the lowest at 50%.

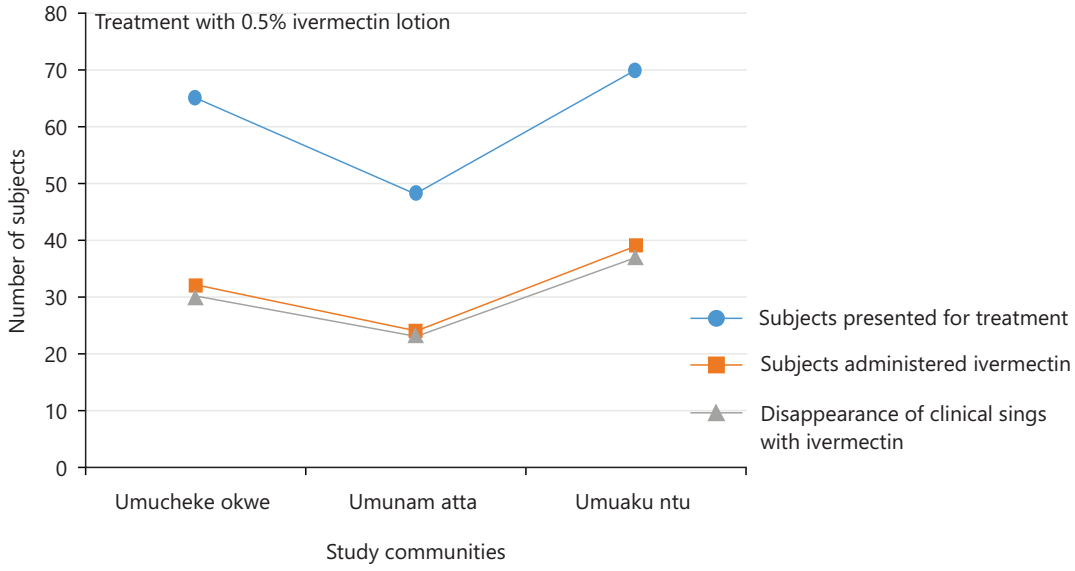

Fig. 1: Treatment with ivermectin in the study area

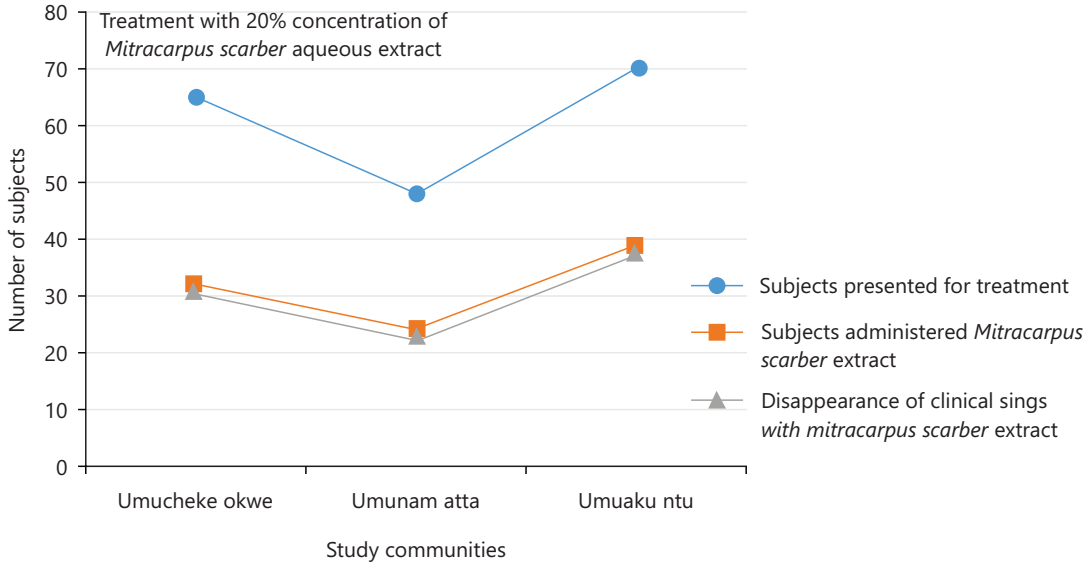

Fig. 2: Treatment with *Mitracarpus scarber* aqueous extract in the study area

Figure 4 shows the effect of Doxycycline treatment, which was less effective in comparison, with only 50% of subjects in Umunam Atta achieving symptom resolution.

DISCUSSION

The study was conducted to determine the therapeutic potential of *Mitracarpus scaber* and *Ocimum gratissimum* in the Treatment of Cutaneous Leishmaniasis in Imo State, Nigeria. Evidence based scientific reports in this study lend credence to the value of Medicinal plants[9,10] and documented evidence suggested that *M. scarber* and *O. gratissimum* remains a very rich source of extract and chemical compounds with diverse bioactivities that are of therapeutic benefit to man including the treatment of CL as researched by Honegger *et al.*[11].

In this research work, Doxycycline had a greater effect than *M. scarber* and *O. gratissimum* extract, which was observed to have minimal therapeutic effect on subjects. This is in line with the works done by Du *et al.*[12], where their results showed sustained effects of 6 weeks of Doxycycline treatment of

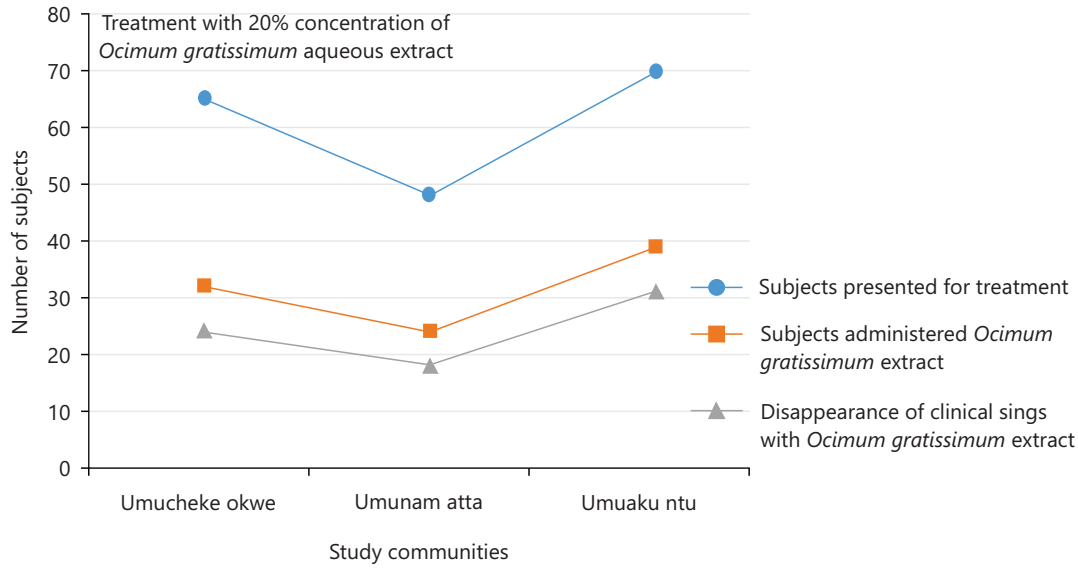

Fig. 3: Treatment with *Ocimum gratissimum* aqueous extract in the study area

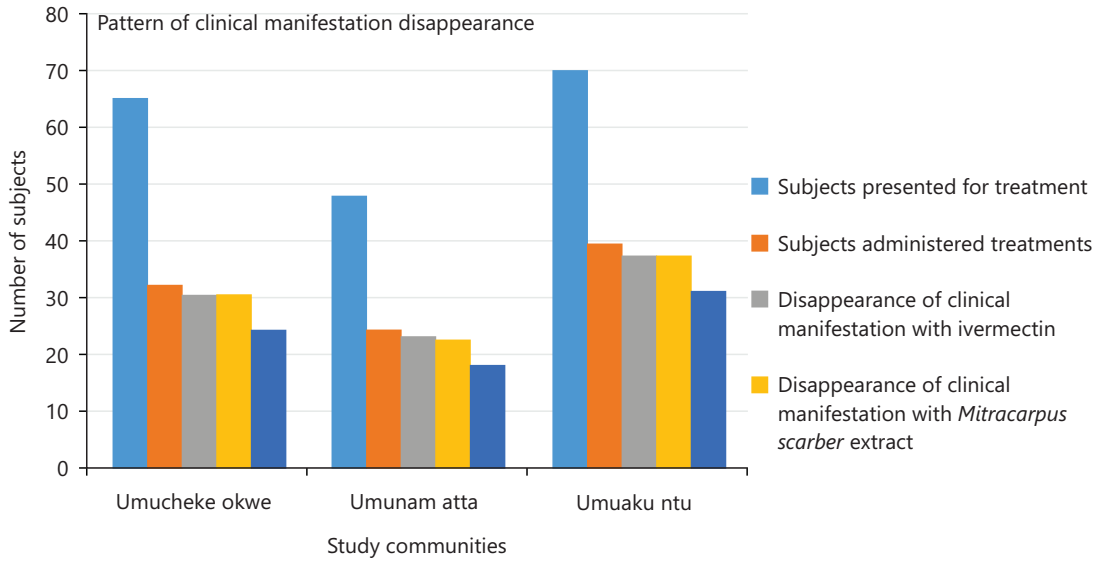

Fig. 4: Pattern of clinical disappearance with treatments in the study area

CL depletion when compared to those who received treatment with Ivermectin alone. In a randomized clinical study conducted by de Souza *et al.*[10] results showed that when Doxycycline was orally taken by subjects for three weeks, it significantly reduced the microfilaria load and was more effective in inducing microfilaremia for up to two years when compared to monotherapy with Albendazole or Ivermectin.

CONCLUSION

Although CL was not found to be endemic in the study area, its presence in a returning migrant highlights the risk of disease introduction and spread. This study affirms the therapeutic potential of *Mitracarpus scaber* and *Ocimum gratissimum* as effective alternatives to conventional drugs. Immediate public health interventions and awareness campaigns are recommended to prevent possible outbreaks and promote early detection and treatment of imported CL cases.

SIGNIFICANCE STATEMENT

Cutaneous leishmaniasis (CL) remains a persistent public health concern in many parts of Nigeria, with limited access to effective and affordable treatment options. This study investigated the therapeutic

potentials of two medicinal plants, *Mitracarpus scaber* and *Ocimum gratissimum*, in the management of CL in Imo State. The findings demonstrated that both plants exhibited promising anti-leishmanial properties, supporting their traditional use by local health practitioners. These results suggest that *M. scaber* and *O. gratissimum* could serve as cost-effective, plant-based alternatives to synthetic drugs for treating CL. This study contributes to the growing body of ethnopharmacological knowledge and encourages the integration of validated traditional remedies into modern healthcare systems, especially in resource-limited settings.

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