Epidemiological Study of *Falciparum* Malaria and Sensitivity of *Plasmodium* Species to Plant Extracts of *Aconitifolius cnidoscolus*.

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Outline

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Background

- **Complex poverty related infectious disease**
- ***3rd* most infectious disease globally**
- ***Plasmodium falciparum* most lethal spp.**
- **216 million cases of malaria in 91 countries annually.**
- **In Nigeria 207,000 die annually from malaria.**
- **Greater mortality rate in pregnant women, children below 5 years and neonates.**
- **Development of drug resistance to all available drugs**
- **Emergence and spread of resistance remains a global concern**

Aim and Objectives

Aim

- **The aim of this study was to carry out an epidemiological study on malaria and sensitivity pattern of *plasmodium* species to plant extracts of *C.aconitifolus.***

Objectives

- **To determine the prevalence of falciparum malaria in Ado-odo, Ota, Ogun State.**
- **In vitro detection of drug resistance genes in clinical isolates of *p. falciparum***
- **To determine the presence of *Plasmodium falciparium* markers in saliva sample in a bid to develop a non-invasive "point-of-care" diagnostic method for *p. falciparum*.**
- **To determine the antiplasmodial activity of *Aconitifolius cnidoscolus* against *Plasmodium berghei berghei* in mice**

Method

Study site and duration

- **2mls venous blood and corresponding saliva samples from subjects presenting with fever ≥37.5 from selected hospitals in Ado-odo/Ota LGA in Ogun state were over a period of two(2) years.**

Study group

- **The study group cuts across different age groups of patients, sex irrespective.**

- **Persons with parasitaemia of ≥ 2,000parasites/ul of blood.**

CONT'D

- **Parasite DNA from these samples were extracted using a DNA extraction kit.**

- **Point mutations in the *P. falciparum* chloroquine resistance transporter (*Pfcrt*) gene and in the *P. falciparum* multidrug resistance 1 (*Pfmdr1*) gene as well as nonsynonymous Pkelch (*pk*13) mutated genes were analysed by PCR/Nested PCR RFLP methods (Djimde *et al*, 2001*)*, and were further used to amplify and confirm the mutation of resistant genes.**

- **Afterwards, the amplicons were run electrophoretically on gel to view bands size of amplified resistance gene regions.**

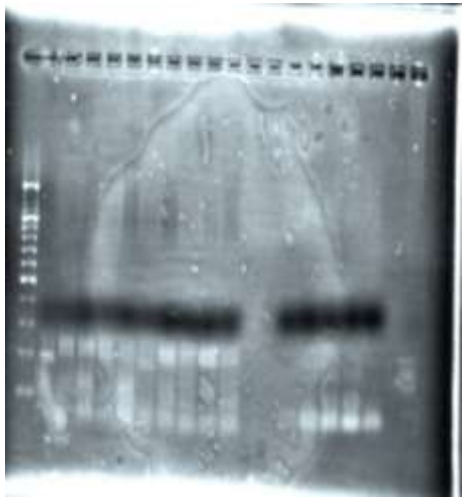
Result (Incidence)

Sex	No of samples collected	No of positive cases	Percentage incidence(%)
Male	590	295	50.00
Female	623	318	51.04
Total	1213	613	50.53

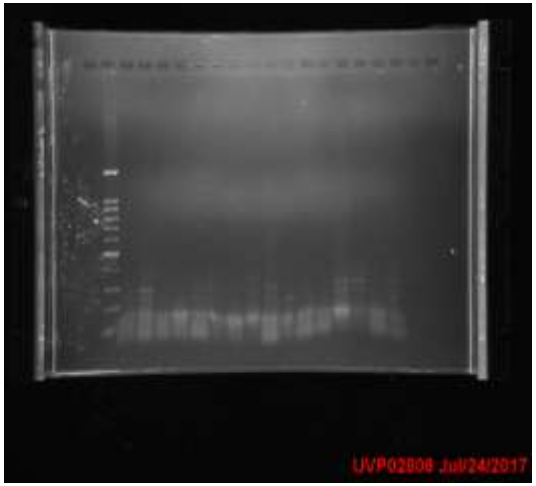
Result (PCR/Nested-RFLP)

Genes	Total blood sample	Total saliva Sample	Positive Blood samples	Positive Saliva samples	Blood samples incidence (%)	Saliva samples incidence (%)
Pfcrt	55	35	26	11	47.3	31.4
Pfmdr1	30	30	8	8	26.7	26.7
*Pk*13	55	18	15	8	32.7	53.3
Total	140	83	49	27	35	32.5

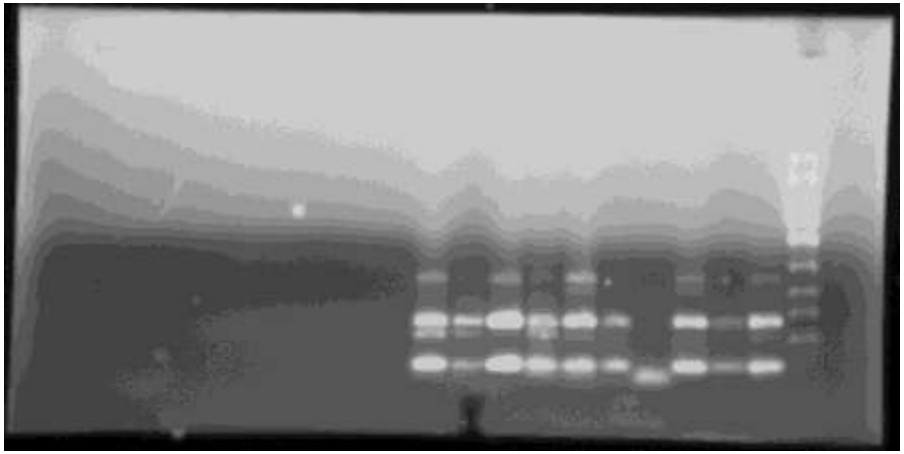
pfcrt (Blood)



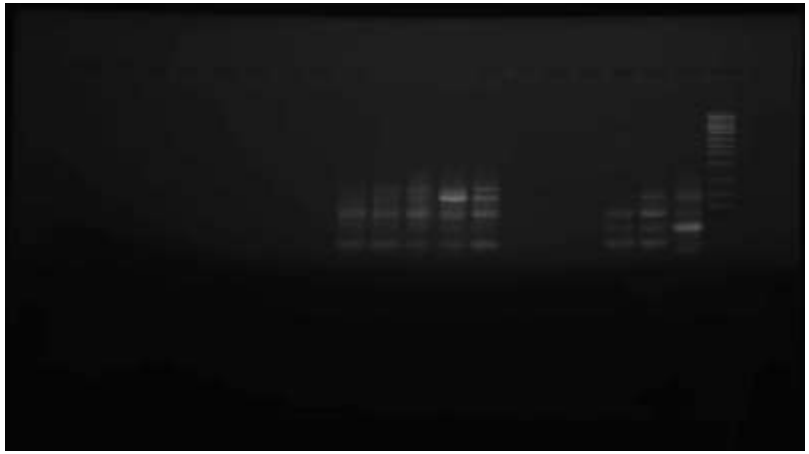
Pfcrt blood and saliva



Pfmdr blood



*Pf*K13 (blood)



*pf*K13 Nested PCR gel electrophoresis (Saliva)

Plant Sample collection

Plant collection

- Fresh leaves of plant were collected from the New Estate area of Covenant University. The leaves were left to dry at room temperature for a period of two weeks.

Plant Identification and authentication

- Fresh sample of the species were identified by certified botanist and authenticated at the Federal Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo state.

Method

Plant preparation

- **Extraction of plant *Cnidoscolus aconitifolus* (Iyana Ipaja) material were performed according to methods described by Peter, (1990) and modified by Olasehinde, (2014).**

- **Swiss albino mice, *Mus musculus* of either sex weighing between 18-25g were obtained from the Nigerian Institute of Medical Research (NIMR) and fed on a standard diet and water according to the NRC Guide for the care of laboratory animals.**

Acute toxicity test LD_{50}

- **For the ethanolic extract of *C. aconitifolius* leaves, oral acute toxicity testing was also carried out at concentrations of 25, 50 and 100ml/kg.**

- **No lethality was recorded across all concentrations and at 24, 48 and 72 hours. Upon physical examination, no convulsions, salivation, diarrhoea, changes on skin, changes in eyes and mucus membranes, behavioural patterns, trembling, diarrhoea, falling of the fur, sleep or coma were observed during the first 4h until the end of 72h of observation.**

Result

Drug	Dose	Chemo-suppression (%)
C.aconitifolus	25mg/kg	65.21
	50mg/kg	79.43
	100mg/kg	84.09
Chloroquine	10mg/kg	95.40

Discussion

- ***Plasmodium falciparum*** **was found prevalent in the samples collected.**
- ***Pfcrt*****,** ***pfmdr1*** **and** ***pk*****13 genes were found in blood and saliva.**
- **Saliva could be used for non-invasive "point-of-care" diagnosis.**
- ***C. aconitifolius*** **possesses effective antimalarial effect.**

Conclusion

- ***Falciparum*** **malaria remains endemic in Ado-odo/Ota, Ogun State.**
- **Resistance genes are present in the study are.**

- **Alternative non-synthetic drugs need to be developed;** ***C. aconitifolius*** **possesses effective antimalarial potentials and could serve as an alternative treatment for malaria.**

Contribution to knowledge

- **Establish the prevalence of malaria in the study site. May help suggest new/existing drugs of choice.**
- **Non- invasive method will be deployable to rural endemic areas.**
- **Determine the efficacy of ethnomedicinal *plant* on *plasmodium* spp.**
- **Enhance monitoring and surveillance of *P.falciparium* resistance genes in the study area which is in line with the WHO's recommendation on routine monitoring of antimalarial drug resistance.**

References

Elizabeth A. W. (2008). Malaria research in the post-genomic era. *Nature* volume; 455: 751–756.

Ofentse J. P., Addmore S. and Sungano M. (2011). A PCR screen for malaria carrier infections using human saliva samples. *African Journal of Microbiology Research*; 5(28): 5120-5126

Kenji O. M., Samuel T. Y., Livo F. E., Obase N. B., Jessica Y., Jean C. D., Calixt D. M., Diane W. T., Vivek R. N. and Rose F. L. (2017). Detection of Plasmodium falciparum DNA in saliva samples stored at room temperature: potential for a non-invasive saliva-based diagnostic test for malaria. *Malaria Journal*; 16:434

Rie I., Haruki U., Isao K., Yoshio I., John L., Ahmeddin H. O. and Akira K. (2015). *Novel Mutations in K13 Propeller Gene of Artemisinin-Resistant* Plasmodium falciparum. *Emerging infectious diseases*; 21:3

References

Salwa D., Hesham M. A., Init I., Jamaiah I., Wahib M. A., Awatif M. A., Hany S., Fatin N. E., Ado U. A., Saadatu I. Y., Abdulhamid A., Mona A. A., Lahvanya R. S., Nabil A. N., and Yee-Ling L. (2016). Is Nigeria winning the battle against malaria? Prevalence, risk factors and KAP assessment among Hausa communities in Kano State. *Malar J*; 15: 351.

WHO factsheet. World malaria report. Available at http://apps.who.int/iris/bitstream/10665/252038/1/9789241511711-eng.pdf

***Xavier C. D., Hans-Peter B., Giovanna R.*. (2011). *Plasmodium sensitivity to artemisinins: magic bullets hit elusive targets*. *Trends in Parasitology* Volume 27 (2):73-81**


Thank you