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In vitro antimalarial activity of the ethanol extracts of *Afzelia africana* and *Cassia alata* commonly used as herbal remedies for malaria in Ghana

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Abstract: **In this study, two commonly used plants were investigated for their antimalarial activities against the 3D7 strain of the *Plasmodium falciparum* parasite.Ethnobotanical survey in the Sekyere central district revealed the usage of the aqueous decoction of the stem bark of *Afzelia africana* and the leaves of *Cassia alata* in combination therapy for the treatment of malaria; however, its efficacy has not been scientifically proven. In this study, the stem bark of *A. africana* and the leaves of *Cassia alata* were investigated for their antimalarial activity against the 3D7 strain of the *Plasmodium falciparum* parasite. This was done by employing the WHO microtest assay (Mark III). These were compared with artesunate-amodiaquine. The results indicated that, the standard drug, Artesunate-amodiaquine was the most active with an Inhibitory Concentration (IC_{50}) of 0.313µg/ml this was followed by *Afzelia africana*, and *Cassia alata* with IC_{50} values of 2.954µg/ml, and 17.270µg/ml respectively. This study has provided scientific evidence for traditional usage of *Afzelia africana* and *Cassia alata* as effective remedy for the treatment of malaria in Ghana.**

Keywords: Afzelia africana, Plasmodium falciparum, Cassia alata.

1. INTRODUCTION

Ethnobotanical survey in the Sekyere central district assembly reveals the combined aqueous decoction of the leaves of *Cassia alata* and the stem bark of *Afzelia africana* for the treatment of malaria. It happens that the two selected plants *Cassia alata* and *afzelia Africana* have the same family name which is *Caesalpiniaceae.*

Cassia alata is a large handsome shrub with thick downy branches, found wild almost throughout India. Leaflets are 8-12 pairs. It is known as ringworm shrub and winged senna in English and *sempe* in Ghana. The leaves of the plant are used as purgative, expectorant astringent, vermicide and treatment of all skin diseases. Extracts of *Cassia alata* leaves have been reported to possess analgesics, anti-bacterial, anti-inflammatory, fungicidal, hypoglycemic, laxative, and oxytocic, and wound healing activity etc. (Mohideen *et al*., 2005).

Afzelia africana is widely distributed in Africa and Asia (Keay *et al*., 1964). It is a large tree with a spreading crown; its height varies from 10-20 m. Scaly grey-brown bark, with a pink or red slash. It grows well in watered sites with a deep sandy soil and can adapt to lateritic soils. [Burkill *et al*, 1995]. It is a hard wood, heavy, durable, termite-proof, light brown to red-brown in colour, excellent timber. It is used in human medicine as a febrifugal, analgaesic, anti-hemorrhageic, laxative, emetic, emmenagogic and aphrodisiac. Pods are rich in ashes used for making soap. The leaves are rich in nitrogen and are used to enrich the soil. (Katende *et al*., 1995)

From the investigations conducted on *A. africana* by some scientists, the plant exhibited bioactive activities which included antimicrobial, anti-inflammatory, antimalarial, analgesic and trypanocidal activities. Akah *et al*. (2007) studied the anti-inflammatory and analgesic properties of extract from *A. africana* on rats. The extract inhibited topical oedema as well as paw oedema induced in these experimental rats. Furthermore, Akah *et al*. (2007) also investigated the antimicrobial effects of the extract on *Staphylococcus aureus* and *Bacillus subtilis* which were found to be susceptible to the extract while *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhii* were not susceptible to the extract. Etawodi (2005) investigated the trypanocidal activities of the leaves and stem bark extract of *A. africana* on *Trypanosoma brucei*. The protozoan was inhibited by the action of the extract. Powdered root of *A. africana* mixed with millet beer has been found to serve as treatment for hernia among some tribes in Cote d'Ivoire (Dalziel, 1937). Agbelusi *et al*. (2007) screened some chewing sticks aqueous extract which include *Afzelia africana* against microbial isolates from mouth washings of some patients. Only two out of nine chewing stick aqueous extracts were found not to inhibit the growth of some of the microbial isolates and these exclude *A. africana*.

The main aim of this research is to give scientific credence to the use of *Afzelia africana* and *Cassia alata* for the treatment of malaria.

2. MATERIALS AND METHODS

2.1 Plant material:

The stem bark of *Afzelia africana* and the leaves of *Cassia alata* were collected in February, 2014 from the Physique garden of the Faculty of Pharmacy and Pharmaceutical Sciences, KNUST.

2.2 Extraction and phytochemical screening:

2.2.1 Extraction of the plant species:

The plant materials, thus the leaves of *Cassia alata* and the stem bark of *Afzelia africana* were air dried for 2 to 3 weeks at ambient temperature, grounded and stored in airtight containers at -20ºC. Organic extracts was prepared by soaking about 80 grams of ground plant material in about 500 ml of ethanol.

The organic was macerated for 72 hours in the organic solvent and a sonicator was used to further ground plant in the solution in order to further break the dissolved particles, this aided better extraction. Filtration followed, using vacuum filtration through a white and clear cloth. It was then placed in an oven of 60ºC for 72hours. The dried extracts were weighed, transferred into 20 ml tubes and stored at -20 ˚C for further analysis.

2.2.2 Preliminary Phytochemical analysis:

The preliminary phytochemical analysis of the plant extracts was carried out using the thin - layer chromatography (TLC). The standard screening test using standard procedure was utilized for detecting the active constituents (Harborne, 1984).

The results of the screening indicated the presence of tannins, alkaloids, flavonoids, saponins, steroids and saponins.

2.3 *Invitro* test for antimalarial activity (WHO Mark III Test assay):

2.3.1 Collection of blood samples for in vitro sensitivity test:

This study was conducted at Noguchi Memorial Institute for Medical Research located on the campus of the University of Ghana, Legon. It was carried out between the months of January to September 2014. A cultured parasite strain, 3D7 *P. Falciparum* strain was used for the study.

2.3.2 Washing of Human (O^+ erythrocyte) blood for parasite culture:

Human O^+ non- sickling blood was collected in Vacutainer tubes containing citrate phosphate dextrose (CPD). This was mixed well and stored at 4ºC for more than 24 hours to suspend the RBCs and remove plasma and antigens that may compete with the RBCs. The blood was afterwards transferred into 50 ml conical tubes using sterile pipettes. This was then centrifuged at 2000 rotations per minute (rpm) for 7 minutes (Kubota 1120, Japan). The plasma and buffy coat (supernatant) containing white blood cells were discarded into a waste flask containing bleach. About 3 ml of red blood cells at the bottom of the tube was transferred in to 15 ml conical tubes containing 7 ml of RBC Wash Media (RWM).

The red blood cells were washed by centrifuging tubes at 2000 rpm for 7 minutes. The process of washing was repeated three times and the RBC was ready to be used for parasite culturing.

2.3.3 Establishing Malaria Parasite Culture:

The cultivation of the asexual erythrocytic stage of *Plasmodium falciparum* used for the study was based on a modification of the technique described by Trager and Jensen (1976). Frozen 3D7 chloroquine sensitive strain of *P. falciparum* stored in a cryotube was picked from liquid nitrogen and thawed by adding parasite thawing mix to the tube and centrifuging at 2000 rpm for 7 minutes. The thawing mix was prepared by dissolving 3.5 grams of NaCl in 100ml of deionised water.

Five millilitres of complete parasite media (CPM) and 200µl of the washed RBC were dispensed into a pre-labelled parasite culture flask (Becton Dickinson labware, USA). Two hundred microlitres of the thawed parasite was added to the flask and flushed with mixed gas containing 5.5% carbon dioxide, 2% oxygen and 92.5% nitrogen for 30 seconds. The culture flask was incubated at 37 ^{o}C.

2.3.4 Daily maintenance of Malaria Parasite Culture:

Culture flask with 3D7 parasites was maintained by the daily change of culture media and preparation of thin RBC smears to monitor parasite growth. The smears were prepared by pipetting 5µl of the infected RBC's unto a frosted glass slide and spreading the drop to a feathered edge with a second slide held an angle of 40 to 45 degrees. After air drying, the slide was fixed with 98% (v/v) methanol by dipping the slide in the methanol for about 5 seconds and leaving it to dry. The slide was then stained with 10% fresh Giemsa for about 10 minutes allowed to dry and examined under the light microscope using the x100 oil immersion objective. The number of infected RBC's and non-infected RBC's in each field were counted and recorded. Enough fields were counted to make up to at least a total of 1000 RBC's. The percentage parasitemia in the flask corresponding to the slide examined was calculated using the formula below:

$$\%\ Parasitemia = \frac{(\text{Number of infected RBC's})\text{x}100\%}{(\text{Total RBC's})}$$

The level of parasitemia in culture flask was reduced or sub-cultured whenever parasitaemia rose above 5%.

2.3.5 Procedure for the stock preparation of drug:

1mg of the drug samples was weighed. It was then dissolved in 1000µl of distilled water, vortex to re-dissolve particles of the sample. It was then filtered in a cryo preserved tube using a 0.22micropore filter and 1ml syringe. It was then labeled and store in a refrigerator at -20^{o}C. This was done under sterile conditions to prevent contaminations.

2.3.5.1 Preparation of serial dilution from the stock of 1mg/ml:

A concentration of 100µg/ml was prepared by dissolving 100µg of each stock of the plant extract in a 1ml of medium (CPM +2% NHS) differently. A four-fold dilution was prepared from the 100µg/ml concentration. From the newly prepared concentration, a four-fold dilution was prepared and from that another was prepared until six different concentrations were prepared. This was done for the two plants extracts and the control drug which was artesunate-amodiaquine. Specific concentrations used were 100, 25, 6.25, 1.56, 0.39, 0.098µg/ml.

2.3.6 Establishment of antiplasmodial activity of plant samples:

The WHO Mark III Microtest assay ((WHO, 2001, Ngemenya *et al*., 2006) was employed in the antiplasmodial investigation. The process was carried out when parasite growth stabilized, with ring or early trophozoite stage being the most predominant. The assay was performed in triplicates in 24- well microtitre plates.

Each well of the 24- microtiter plate (Greiner Bio-one, Belgium) was filled with 900µl of infected RBC culture of 1% parasitemia. Hundred microlitres each of the sterile dilution was added to each well. A drug free control was also prepared. This well contained 100µl CPM instead of the drug a test sample. The prepared plate was placed in a modular incubator chamber (Billlups Rothemberg, Carlifonia) and flushed with mixed gas made up of 5.5% carbon dioxide, 2% oxygen and 92.5% nitrogen for about 6 minutes. The plate was incubated at 37^{0}C for 48 hours.

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After 48 hours, thin RBC smears corresponding to each well were prepared and air dried. The slides were fixed with 98% (v/v) methanol and the stained with 10% fresh Giemsa for 10 minutes and left to dry. The slides were then examined under a light microscope using the oil immersion x100 objective lens. The number of infected RBC's and non-infected RBC's in each field were counted and recorded. Enough fields were counted to make up to at least a total of 1000 RBC's. The percentage parasitemia for each slide that corresponded to each well was calculated.

Based on the percentage parasitemia calculated, the percentage parasite inhibition of each concentration of the test samples was calculated using the drug-free control as 100% growth. Percent inhibition was calculated from the formula below:

%Inhibition= [(% Parasitemia of drug-free control) - (% parasitemia of test sample)] (%parasitemia of drug-free control)

A graph of percentage inhibition against log of concentration of the sample was plotted and the IC_{50} value was determined.

2.3.7 Statistical analysis:

Growth inhibition due to each extract, defined as the difference between the percentage parasitaemia of each treatment group and the corresponding positive control was calculated as follows (Ene *et al*., 2009). Total parasitaemia was calculated in arbitrary unit as the area under the curve (AUC). The IC_{50} of each plant extract was determined using regression equations of best fit plotted as percentage inhibition against log of concentration. Graph Pad Prism for Windows version 5.0 (Graph Pad Software, San Diego, CA, USA) was used for the statistical analyses.

3. RESULTS AND DISCUSSION OF THE STUDY

3.1 Results:

The extracts of the two plants were tested on trophozoites, mainly ring forms. At each of the six concentrations of all the extracts there was some reduction in the number of parasitized cells. The basic measurement of antimalarial activity used in this study was the reduction in number of parasitized cells in the test cultures compared to control at 48 hours of incubation. Of the two plants, as compared to the standard drug, *Afzelia africana* was the most effective with an IC_{5O} value of 2.695µg/ml and a maximum percentage inhibition of 80.91.*Cassia alata* was not as effective as compared to *A. africana* but showed a significant effect with IC_{50} of 17.270µg/ml and maximum percentage inhibition of 65.62. The Inhibition Concentration (IC_{50}) of each plant extract was determined using regression equations of best fit plotted as percentage inhibition against log of concentration. Graph Pad Prism for Windows version 5.0 (Graph Pad Software, San Diego, CA, USA) was used for the statistical analyses.

Table of Inhibition Concentration (IC_{50}) of *A. africana* and *C alata* compared to a standard drug (Artesunate amodiaquine).

Table 1: Phytochemical screening for the leaves of *C. alata* and the stem bark of *a. africana* adopted from (Veerachari and Bopaiah, 2012) and (Simon and Jegede, 2013)

Plants and Herbal Remedies	IC_{50}values (µg/ml)
Artesunate-amodiaqiune, AT	0.313
Afzelia africana, AA	2.695
Cassia alata, CA	17.270

Table 2:

Phytochemical test	Ethanol extract of *cassia alata* leaves	Ethanol extract of the stem back *afzelia africana*
Alkaloids	++	++
Tannin	++	++
Saponin	-	++
Anthraquinone	+	++
Flavonoids	+	++

Carbohydrates	++	++
Steroids	++	++
Terpenoids	++	+
Present ++	Moderately present +	Absent -

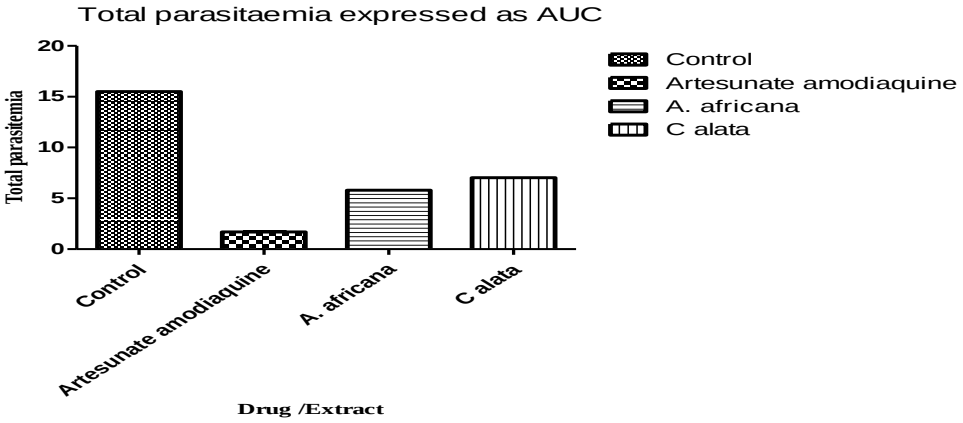

FIG.1: A bar chart showing the total parasitaemia of the various extract compared with the negative control.

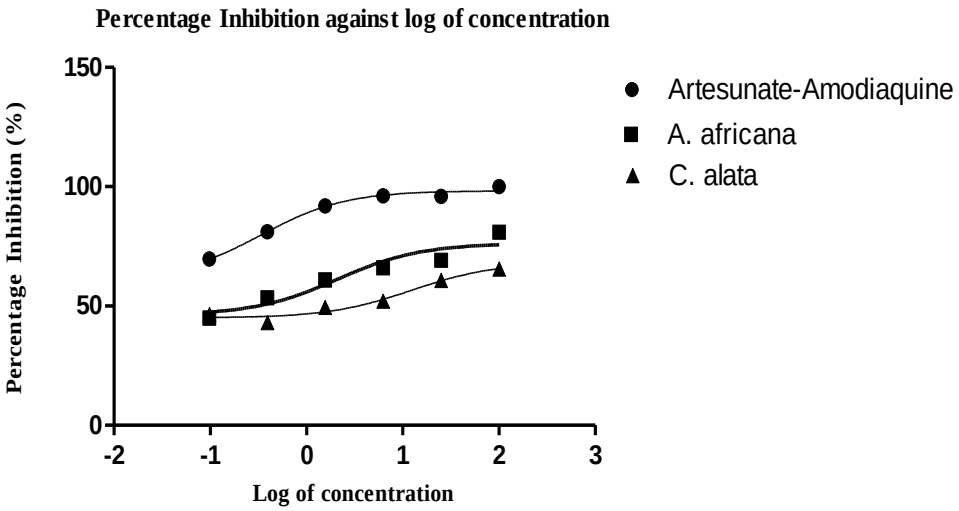

Fig.2: A graph showing the percentage inhibition against log of concentration of the standard drug and the various plant extracts

3.2 Discussion:

In Ghana, traditional medicines remain one of the most important remedies for treatment of malaria. Since plant materials remain an important source of medicine in the fight against malaria (Aderounmu, 2007). But because the very antimalarial drugs in used today such as quinine and artermisinin were isolated from plant, there is still the need of diving to research into plant medicine (Wang *et al*., 2007). Malaria poses a major health problem to more than half of the world. Ghana, a sub-Saharan African country with a population of approximately 25 million people of which more than half live in malaria endemic areas, is moving towards elimination of malaria.

Afzelia Africana and *Cassia alata* are one of combined plant extracts used in the Sekyere Afram plains for the treatment of malaria. The two extracts have been found from previous survey to exhibit promising antimalarial activity but it's activity has not been reported and authenticated in literature. The survey has revealed that the leaves part of the plant after boiling can eradicate the *plasmodium faciparum*. Phytochemical screening of the stem bark of *Afzelia africana* plant extract revealed the presence of alkaloids, tannins, saponin, flavonoids, glycosides, steroids.

Multiple efficacy parameters for *in vitro* antimalarial activity have been proposed. For crude extracts, IC50 values should certainly be below 100 mg/mL although most promising antimalarial extracts exhibit IC-50 values under 10 mg/mL .(Cos *et al*, 2001 , Deharo *et al*, 2005, Benoit *et al*, 2006).

To estimate the potential of molecules or extracts to inhibit parasite growth without toxicity, the selectivity index (SI) was introduced. Low SI indicates that the antiplasmodial activity is probably due to cytotoxicity rather than activity against the parasite themselves. In contrast, high SI should offer the potential of safer therapy. We decided to define 4 as minimal SI value to validate a safe antimalarial use, whereas, SI greater than 10 and IC50 values under 10 mg/mL should be promising sources of antimalarial molecules. (Krettli *et al*, 2009, Muñoz *et al*, 2007, Soh *et al*, 2007, Zihiri, *et al*, 2005).

The standard drug, Artesunate-amodiaquine was the most active with an Inhibitory Concentration (IC_{50}) of 0.313µg/ml this was followed by *Afzelia africana*, and *Cassia alata* with IC_{50}values of 2.954µg/ml, and 17.270µg/ml respectively. This suggests the reason behind the combined usage of the aqueous decoction of *Afzelia africana* stem bark and the leaves of *Cassia alata* for the treatment of malaria in the Sekyere central district and other areas of the Ashanti region.

4. CONCLUSION

The result of the study has authenticated the combined usage of *Afzelia africana* and *Cassia alata* for the treatment of malaria.

The results of this study have shown that the methanolic extract of *Afzelia africana* exhibited higher antimalarial activity with an IC-50 of 2.954ug/ml whilst that of *Cassia alata* also exhibited activity with IC-50 of 17.270ug/ml. Further work is suggested to isolate, identify and characterize the active principles from the plants.

REFERENCES

[1] Aderounmu, A. O. (2007). *The antimalarial activity of the crude organic extracts of four commonly used Nigerian medicinal plants.* Adeolas Malaria Research Files Word Press.

[2] Agbelusi GA., Odukoya OA., Otegbeye AF. (2007). *In vitro* screening of chewing stick Extracts and sap on oral pathogens: Immune compromised infection. Biotechnology, vol 6(1): pp 97-100.

[3] Akah PA., Okpi O., Okoli CO. (2007). Evaluation of the anti-inflammatory, analgesic and antimicrobial activities of *Afzelia africana*. Niger. J. Nat. Prod. Med., 11: 48-52.

[4] Atawodi SE. (2005). Antioxidant potential of African medicinal plants. Afr. J. Biotechnol., 4(2): 128-133.

[5] Benoit-Vical F.(2005). Ethnomedicine in malaria treatment. I Drugs.Vol. 8 pp 45-52.

[6] Burkill HM. 1995. The useful plants of West Tropical Africa. vol. 3. Caesalpinioideae, pp. 50-177. Royal Botanic gardens, Kew. 857 pp.

[7] Cos P, Vlietinck AJ, Berghe DV, Maes L. (2006) Anti-infective potential of natural products: how to develop a stronger *in vitro* 'proof-of- concept'. J Ethnopharmacol. Vol.106: pp 290-302.

[8] Dalziel JM. (1937). The useful plants of West Tropical Africa. Crown Agents for the colonies, London. p. 612.

[9] Deharo E, Bourdy G, Quenevo C, Muñoz V, Ruiz G, Sauvain M. A (2001). Search for natural bioactive compounds in Bolivia through a multidisciplinary approach. Part V. Evaluation of the antimalarial activity of plants used by the Tacana Indians. J Ethnopharmacol. Vol 77: pp 91-8.

[10] Ene AC, Atawodia SE, Ameh DA, Ndukwe GI, Kwanashie HO. Bioassay-guided fractionation and *in vivo* antipasmodial effect of fractions of chloroform extract of *Artemisia macivera*. 2009; 112: 288-94.

[11] Harborne, J. (1984). *Phytochemical Methods.* Chapman and Hall, London-New York. Pp. 120.

[12] Katende A.B., A. Birnie and B. Tengnas (1995). Useful trees and shrubs for Uganda: Identification, propagation and Management for agricultural and Pastoral communities. Regional soil conservation unit (RSCU) Technical handbook no. 10. Regional soil conservation unit, RSCU/SIDA, Nairobi, Kenya.

[13] Keay RWJ., Onochie CFA., Stanfield DP. (1964). Nigerian Trees. Vols 1 and 2; Dept. of Forest Research, Ibadan. p. 334- 495.

[14] Krettli AU.(2009) Antimalarial drug discovery: screening of Brazilian medicinal plants and purified compounds. Expert Opin Drug Discov. Vol. 4: pp 95-108.

[15] Muñoz V, Sauvain M, Bourdy G, Arrázola S, Callapa J, Ruiz G, *et al*.(2007) A search for natural bioactive compounds in Bolivia through a multidisciplinary approach. Part III. Evaluation of the antimalarial activity of plants used by Alteños Indians. J Ethnopharmacol. Vol.71: pp 123-31.

[16] Ngemenya, M. N., Akam, T. M., Yong, J. N., Tane, P., Fanso-Free, S. N. Y., Berzins, K. & Titanji, V. P. K. (2006). Antiplasmodial activities of some products from *Turrenthus africanus* (Meliaceae). *African Journal of Health Sciences*, Vol. 13: pp 33 - 39.

[17] Sofowara A., (1993). Medicinal plants and Traditional medicines in Africa chichester john, Willey and sons, New York; p 256.

[18] Soh PN, Benoit-Vical F. (2007). Are West African plants a source of future antimalarial drugs? J Ethnopharmacol. Vol. 114: pp 130-40.

[19] Trease G E. Evans WC. (1983). Textbook of pharmacognosy, 12th edition (Balliere, Tindall, London), pp. 57 -59; pp 343 -383.

[20] Wang, M.-W., Hao, X., & Chen, K. (2007). Biological screening of natural products and drug innovation in China. *Philosophical Transactions of the Royal Society Biological Sciences*, 362, 1093-1105. Retrieved from http://rstb.royalsociety publishing. Org.

[21] WHO (2001*). In vitro* micro test (Mark III) for the assessment of the response of *Plasmodium falciparum* to chloroquine, mefloquine, quinine, amodiaquine, sulfadoxine/pyrimethamine artemisinin. Geneva; WHO.CTD/MAL/ 97, -20.

[22] Zihiri GN., Mambu L., Guédé-Guina F., Bodo B., Grellier P. (2005). *In-vitro* antiplasmodial activity and cytotoxicity of 33 West African plants used for treatment of malaria. J. Ethnopharmacol. Vol. 98:281-5.

[23] Mohideen S, E. Sasikala and P.Aruh.A, (2005). Pharmacognosy of *Cassia alata* Linn – leaves vol: XXIV (4) Pages 192 – 198.