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Antımalarıal Analysıs Of Alkaloıdal Extract From Root Bark of *Maytenus Senegalensis* (LAM.) Exell

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Abstract

The search for new antimalarial compounds has been necessitated by Plasmodium falciparum's resistance to standard antimalarial drugs. *Maytenus senegalensis* LAM, is a plant used in treatment of malaria in Nigeria. In this study, the alkaloid extracts of root bark of *Maytenus senegalensis* were assessed for their anti-plasmodial, analgesic, and anti-inflammatory effects. Plasmodium berghei was inoculated into 15 mice assigned to 5 groups of 3 mice each. Groups I and II were treated with 200mg/kgbw and 300mg/kgbw alkaloids extracts respectively, while 200mg/kgbw of the herbal standard (Artemisia annua) was administered to group III. Group IV was gavaged with 5mg/kgbw of chloroquine phosphate and group V received normal saline. Analgesic and anti-inflammatory effects were evaluated by the Eddys hot plate method in mice and egg albumin induced paw oedema in rats respectively. The crude alkaloid extract suppressed parasitemia in mice by 58.88%, with a slight but non-significant p value decrease in packed cell volume (PCV) in groups treated with alkaloidal extracts. Inhibition of paw oedema (30%), showed no significant difference from that seen in the crude extract (54.66%). The effect of the alkaloidal extract on the response time to thermally induced pain in test animals was significantly different from that of the crude extract. Therefore *M. senegalensis* can be effective in the management of malaria with an accompanying analgesic effect, and results of this study demonstrats alkaloids from M. senegalensis as an active compound showing promising antimalarial effect.

1. INTRODUCTION

Malaria is a parasitic disease transmitted by the bites of Anopheles mosquitoes infected with Plasmodium species, four of which infect humans: Plasmodium falciparum (the most deadly one), Plasmodium vivax, Plasmodium malariae and Plasmodium ovale. The disease primarily affects tropical and subtropical areas, where the temperature and rainfall are suitable for the development of vectors and parasites [1]. According to World Malarial Report Malaria and WHO, malaria has a greater morbidity and mortality than any other infectious diseases of the world. About 3.2 billion People (almost half of the world's population) is at risk of malaria. In 2019, 229 million cases of malaria were recorded globally, which resulted in 438,000 deaths. 94% of the deaths occurred in sub Saharan Africa, with 23% of the total death being in Nigeria alone. 67% of deaths from malaria are children under the age of 5. It is estimated that malaria cost endemic countries up to 1.3% of GDP and since the year 2000, malaria has caused sub Saharan countries US$300 million each year for case management alone [2].

There are many possible reasons for this situation, including: the emergence of resistance to chloroquine and other known antimalarial drugs [3], resistance of parasite vectors to insecticides, demographic growth with subsequent worsening of living and infrastructure standards in endemic areas. "Malaria is both a disease of poverty and a cause of poverty"[4]. Most affected populations have little access to western medicine and therefore turn towards the

use of traditional medicinal plants for their primary healthcare. However, few clinical studies have been conducted to evaluate plant safety and efficacy in the treatment of malaria [5].

The search for new antimalarial compounds from plants is a contribution to the safety and efficacy evaluation of plant extracts used in traditional medicine; it may provide quality control parameters for the production of traditional phyto-medicines and also a source of lead compounds to be developed into new drugs against resistant Plasmodium falciparum infection *Maytenus senegalensis* (Lam.). is an African shrubs or tree that grows under the common name of Red spike-thorn, which belong to the celastraccae family, growing in the semi desert regions of Asia and tropical regions Africa. Its roots and bark are traditionally used in the folk medicine of some African regions for the treatment of a number of ailments, including chest pains, rheumatism, snakebites, diarrhoea, eye infection, and dyspepsia(indigestion). It is also used as an antibacterial, antimicrobial and antibiotic. An extract of the roots and barks is used for severe headaches, an analgesic, for skin rashes, muscle spasms, excessive sweating, fevers, parasitic intestinal infections, as an anti-inflammatory, for arthritis and muscle pain, for nausea, vomiting and diarrhea The leaves are used for malaria, yellow fever, and trypanosomiasis [6]. It is also used for fertility problems, venereal diseases, pneumonia, epilepsy, and as a tonic [7]. Following the traditional use of the plant in Sudan, Kenya, Tanzania and Nigeria, it was demonstrated that leaf, root and stem bark extracts of *M. senegalensis* possess in vitro antiplasmodial, anti-leishmanial, and antibacterial activities [6].

The Celastraceae family is a source of important bioactive secondary metabolites. Alkaloid amines such as cathine often occur in this botanical family as also, rarely, benzylisoquinolide alkaloids. Compounds isolated from the Maytenus genus include the ansa macrolide, maytansine, and related macrolides such as nor-maytansine, maytanprine and maytanbutine [8]. Two of the well-known chemicals are mayteine and maytansine - alkaloids long documented with antitumor activitity and which occur in other Maytenus plants as well. Other isolated compounds include spermidine alkaloids (celacinnine and celallocinine) and nicotinoyl sesquiterpene alkaloids (maytoline and maytolidine) as reported by Pistelli et al.,2008 [9]. Alkaloids are one of the major classes of natural products that exhibit antimalarial activity. Indeed, quinine, the first antimalarial drug, belongs to this class. Over 100 alkaloids from higher plants were reported to demonstrate significant antimalarial activity in studies published from 1990; some of these were more potent than chloroquine [10].
These reports necessitated the in-vivo evaluation of the anti-plasmodial, analgesic and anti-inflammatory potentials of alkaloidal extracts of *M. senegalensis.*

2. MATERIALS AND METHOD

2.1 Chemicals and Reagents

All chemicals and reagents used were of analytical grade . Some of the chemicals used for this study include 99% methanol (BDH), Commercial Kit for analysis of biochemical parameters (Randox laboratories London). Chloroquine Sulphate Standard (Sigma Aldrich).

2.2 Plant Materials

The plant *Maytenus senegalensis* was identified by traditional herbalists and collected from Bida, Niger state. The plant was authenticated by botanist, from Department of Biological Sciences, Federal university of Technology Minna. The plant was then allowed to dry at room temperature and kept in the laboratory in the Department of Biochemistry, Federal University of Technology, Minna for analysis.
Artemisia annua, a lead plant in the treatment of malaria, from which several compounds have been derived, one of which is Artemisinin. Isolated from the plant as the most potent antimalarial

drug against chloroquine resistant Plasmodium falciparum malaria [11]. The plant was used for comparative studies.

2.3 Animals Adult Swiss albino mice were obtained from National Veterinary Research Institute (NVRI), Vom, Plateau State of Nigeria. The animals were fed ad libitum with standard mice feed and maintained under standard conditions of humidity, temperature and 12 hours light /darkness cycles. The animals were allowed to acclimatize for three weeks in the laboratory before the commencement of the study.

2.4 Parasites

The parasite, Chloroquine sensitive Plasmodium berghei (NK-65) was obtained from the National Institute of Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria and kept alive at the Departmental laboratory of Biochemistry, Federal University of Technology Minna by parasite serial passage in mice every four days.

2.5 Data Analysis

Data analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan multiple range test(DMRT). Data were expressed as mean ± SEM of triplicate determinations. Significance was considered at $p<0.05$.

2.6 Methods

Preparation of the Plant Extract

The plant materials were dried at room temperature and broken down into coarse form using mortar and pestle. The samples were further reduced to fine powder using electric blending machine. Hot exhaustive Soxhlet extraction of the plant material was carried out with 99% methanol at 65^{o}C for 4 hours. The resultant extract was concentrated to dryness under reduced pressure using rotary evaporator as described by [12]. The extract was stored in tightly stoppered sample bottles and kept in a refrigerator until required for assays. The Artemisia annua was extracted using cold Maceration technique.

Qualitative Phytochemical Analysis of the Plant Samples

Qualitative phytochemical analysis of the plant samples was carried out using standard methods for phytochemical properties according to the methods of Harbone [13], Trease and Evans [14], and Sofowora [15] as described by Geetha and Geetha [16], for the detection of phyto-constituents present in the plant.

Extraction of Total Alkaloids

Extraction of Alkaloids The extraction of the alkaloid was done by the continuous extraction method using the Soxhlet apparatus as described by Gonzalez et al., [17]. Three hundred grams (300g) of ground *Maytenus senegalensis* root bark was weighed and packed in a cheese cloth bag which served as an extraction thimble. The thimble was then placed into a suitable jar with cover. The sample was moistened with sufficient amount of 95% ethanol. This was made alkaline with sufficient quantity of ammonia T.S. and mixed thoroughly. The sample in the thimble was macerated overnight and then placed in the Soxhlet extractor on the next day. Sufficient amount of 95% ethanol was placed in the solvent flask. The sample was extracted for about 3 – 4 hours. The ethanol extract was filtered and was concentrated in a Soxhlet distilling apparatus at 60oC. The crude alkaloid extract was further treated with 1.0 N hydrochloric acid. This was filtered and the filtrate was collected. The filtrate was alkalinified with ammonia T.S. and placed in a separatory funnel. Measured quantities of chloroform was added into the separatory funnel, mixed and shaken for about five times and allowed to separate into two layers. The lower layer of chloroform contained the alkaloids and the upper layer the aqueous portion. The upper layer was extracted until the last chloroform extract was found negative to Dragendorff's reagent. The

combined chloroform extract was concentrated in Soxhlet distilling apparatus at 60°C and evaporated in water bath maintained at that temperature until semi-dry. The residue was weighed and percentage yield was calculated using the formula:

$$\% \text{ Yield} = \frac{\text{Weight of the alkaloidal residue}}{\text{Weight of the ground } M.\ senegalensis \text{ root bark}} x\ 100$$

In vivo Anti-plasmodial screening of alkaloid extract.

This was carried out using Rane's test as described by Jigam et al [18]. On the first day (D_0), 15 mice were inoculated with 0.2ml normal saline, which contains $1.0x10^7$ P. berghei parasitized erythrocytes. The 15 mice were then divided into 5 groups of 3 animals each. Drugs and extracts were administered orally. 2 groups (Group 1 and 2) received graded doses of test extract and 1 group (Group 3) received similar concentration of graded doses of Artemisia annua (as herbal control). Group 4 was administered 20ml/kgbw of physiological saline as negative control and Group 5 received 5mg/kgbw of chloroquine phosphate as positive control. The treatment started 72 hours after parasitemia challenge. The extracts were given once daily for 4 days. Blood was collected from the tail vein of each animal using heparinized capillary tube. Blood films were made and allowed to dry, the dried blood films were then fixed with methanol and subsequently stained with Geimsa's stain for 25 minutes, then washed with phosphate buffer and allowed to dry. The slides were microscopically examined using x100 magnification in oil immersion. The percentage inhibition of parasitemia was calculated for each test concentration by comparing the parasitemia in infected controls with those that received different concentrations of the extracts.

$$\% \ Parasitemia = \frac{No.\ of\ parasitized\ RBC}{Total\ No.\ of\ RBC}\ X\ 100$$

$$\%Inhibition = \frac{mean\ parasitemia\ of\ -VE\ control - mean\ parasitemia\ of\ treated\ group}{mean\ parasitemia\ of\ -VE\ control} X\ 100$$

Determination of Packed Cell Volume

The method described by Ogbadoyi et al., [18] was used. The Blood was collected from tail of each mouse in heparinized micro-hematocrit capillary tubes and sealed by crystal seal. The samples were then placed in a micro-hematocrit centrifuge with the sealed ends outwards. The blood was centrifuged at 12,000 rpm for 15 minutes. The packed cell volume (PCV) of each mouse was measured before infection and on day 7 after infection.

Anti-Inflammatory Activity of Alkaloidal Extracts.

The anti-inflammatory activity of the extract was tested using egg albumin induced paw oedema in mice [20] as described by [19]Adult mice were divided six per each treatment group and used for the analysis. Inflammation was induced by the injection of 0.01ml egg albumin into the sub-planter surface on the right hind paw 30 min after administering the extracts. The increase in volume(cm3) of the hind paw was measured with a digital Vernier caliper before and at 20 min interval after the injection of egg albumin for a period of 2 h. Control mice received an equivalent amount of normal saline while ASA (150 mg/kg.bw) served as reference. The percentage inhibition of oedema was calculated for each dose.

$$\%Inhibition = \frac{mean\ paw\ oedema\ \ of\ -VE\ control - mean\ paw\ oedema\ of\ treated\ group}{mean\ parasitemia\ of\ -VE\ control} X\ 100$$

Analgesic Activity of Alkaloidal Extract

Hot-plate test: The hot plate test was used to measure analgesic activity by the method of Eddy

[21]as described by Williamson et al., [22]. In this experiment, the hot plate was maintained at 55 ± 0.50C. All animals were selected 24 hour prior to experimentation and the animals were selected on the basis of their normal reaction time i.e. pain response to the hot plate with a minimum and maximum of 2-15 sec respectively. In order to avoid the damage to the paws of the animals, the time standing on the plate was limited to 20 sec. Acetyl Salicylic Acid (Aspirin) 150mg/kgbw was administered intraperitoneally as a reference standard. 30 min after administration of vehicle methanol extract (400 and 800 mg/kgbw), animals were placed individually on to the hot plate and the time from placing the animal on the hot plate to jumping of the animal from the hot plate was recorded as the reaction time or latency of the pain response.

3. RESULTS AND DISCUSSIONS

Table 1 presents the percentage yield of crude and alkaloidal extract of *M. senegalensis*, and also shows the percentage yield of A. *annua*, which was used as herbal standard.

Table 1. Percentage yield of plant extracts used in this study

Plant	Weight of Powedered sample (g)	Weight of extract (g)	Percentage Yield (%)
***Maytenus senegalensis* (Crude)**	300	38	12.6
***Maytenus senegalensis* (Alkaloid)**	300	1.1	0.37
Artemesia annua	50	8.7	17.4

Table 2. Phytochemical content of *Maytenus senegalensis*

Phytochemicals	Results
Alkaloids	+
Flavonoids	+
Phenols	+
Tannins	+
Glycosides	+
Terpenes	+
Steroids	-
Saponins	+
Anthraquinones	+

Phytochemical constituents are integral part of medicinal plants, and are responsible for their numerous bioactivities. The phytochemical screening of *M. senegalensis* showed the presence of Alkaloids, Flavonoids, Phenols, Tannins, Glycosides, Terpenes, Steroids, Saponins, And Anthraquinones (Table 2). This agrees with phytochemical analysis of other Maytenus species which also reports the presence of Alkaloids, Flavonoids, Tannins, Phenols e.t.c. [23]. The traditional use of *M. senegalensis* for the treatment of malaria can be attributed to the presence of certain phytochemicals that constitute the bioactive principles in the plant, some of the secondary metabolites detected in this study have been implicated in anti-plasmodium activities. The anti-plasmodium activities of some plants have been attributed to the presence of alkaloids, flavonoids and terpenes all of which are contained in this plant.

Alkaloids are compounds that have been used extensively in the treatment of malaria. For instance, Quinine, which is a cinchona alkaloid and belongs to the aryl amino alcohol group of drug has been a mainstay of malaria treatment for decades. Alklaoids are very basic compound and therefore, always present as a salt, due to its basic nature, it is usually found in the food vacuoles of P. falciparum. The theorized mechanism of action for quinine and related anti-malarial drugs is that these drugs are toxic to the malaria parasite. Specifically, the drugs interfere with the parasite's ability to break down and digest hemoglobin. Quinine has rapid schizonticidal action against intra-erythrocytic malaria parasites. It also has analgesic properties [24].

Table 3. Curative anti-*plasmodial* activity of alkaloidal extracts of *M. Senegalensis*

Treatment	**Dose mg/kgbw**	**Parasitemia** ($\bar{X} \pm$ **SEM**)	**Inhibition (%)**
M. senegalensis Alkaloid	200	21.33 ±1.64^{c}	58.88
M. senegalensis Alkaloid	300	21.53 ±2.66^{c}	58.49
A. annua	200	17.93 ±2.35^{b}	65.43
CQR	5	13.13 ±4.26^{a}	74.69
N.S	20	51.87 ±10.27^{d}	-

Values are mean ± SEM of 3 determinations. NS= Normal Saline CQR = Chloroquine
Values along the same column with different superscripts are significantly different. (p< 0.05)

Table 4. Packed cell volume of *Plasmodium* infected mice

Treatment	**Dose**	**PCV (%) Before**	**PCV (%) After**
Alkaloid	200	55.33±5.49^{a}	47.33±3.48 ^{a}
Alkaloid	300	44±5.51 ^{a}	39±5.13 ^{a}
A. annua	200	41.33±6.64 ^{a}	51.67±3.84 ^{a}
Chloroquine	5	46.33±4.26 ^{a}	52.67±5.24 ^{a}
Normal saline	20	50.33±2.73 ^{a}	31.33±1.45^{b}

Values are mean ± SEM of 3 determinations. NS= Normal Saline CQR = Chloroquine
Values along the same column with different superscripts are significantly different. (p< 0.05)

The alkaloidal extract of *M. senegalensis* when administered to animals in the infected groups, showed a progressive reduction in parasitemia with time (Table 3). There was no significant difference (p>0.05) in the anti-plasmodial effects of the two doses used for treatment, this may Imply that the minimum dose required to produce anti malaria activity was exceeded in this study. The high anti-plasmodial activity of the alkaloidal extract from the root bark of *M. senegalensis* possibly explains its widespread use in herbal medicine especially in crude form. After two dosing, all doses of extract reduced parasitemia. However, activity was observed from the first dose of chloroquine administered. The delay in the activity of extract may indicate the need for a loading dose, or the extract may have delayed onset of action. It could also indicate the need for more frequent administration per day.

The antimalarial activity of *M. senegalensis* could be attributed to the alkaloid content of the plant. However, it is possible that a mixture of secondary metabolites present in the plant which would consist of Terpenoids, Phenolic compounds, Alkaloids, and Flavonoids etc. are accountable for the observed activity. Reports from literature of different plant species have reported these metabolites as having different extent of antimalarial activity. [25, 26, 27].

The PCV was measured to ascertain the degree to which the crude alkaloid is able to prevent hemolysis caused by rising parasitemia levels. In this study, both doses of alkaloidal extract of M.*senegalensis* allowed a slight reduction in PCV (Table 4), with no statistically significant

difference in the initial and final PCV in the groups treated with the alkaloidal extract. The underlying cause of anemia includes the following mechanisms: the clearance and/or destruction of infected RBC, the clearance of uninfected RBC, and erythropoietic suppression and dyserythropoiesis [28]. All these mechanisms have been shown to be connected in human and in mouse malarial anemia.

Table 5. Anti-inflammatory effect of alkaloidal extract of *M. senegalensis.*

Treatment	Dose mg/kgbw	Paw edema ($\bar{X} \pm$ SEM)	Inhibition (%)
Alkaloid	200	5.11±0.40 ^{c}	30.00
Alkaloid	300	5.12±0.37 ^{c}	29.86
Crude	400	3.51 ±0.35 ^{b}	51.92
Crude	800	3.31±0.27 ^{b}	54.66
ASA	150	3.00±0.16 ^{a}	70.96
NS	20ml/kgbw	7.30±0.60 ^{d}	-

Values are mean ± SEM of 3 determinations. Values along the same column with different superscripts aresignificantly different. (p< 0.05). ASA = Acetyl Salicylic Acid. NS = Normal Saline

The alkaloidal extract showed relatively low activity against inflammation when compared with the crude extract and standard drug (Table 5). Since Evidence for the anti-inflammatory properties of flavonoids, saponins, terpenes and anthraquinones have been provided by several studies using different models of inflammation [29. 30, 31, 32]. Perhaps the secondary metabolite responsible for the level of anti-inflammatory effect shown by the crude was not the alkaloid but other phytochemicals. Like Flavonoids, which are anti-oxidants that also possess anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation [33].

Table 6. Analgesic effect of Alkaloidal extract of *M. senegalensis*

Treatment	Dose (mg/kgbw)	Time (mins) 0	15	30	45	60
Alkaloid	200	3.30±0.432^{a}	3.60±0.193^{b}	3.80±0.297^{b}	4.26±0.290^{b}	4.20±0.065^{b}
Alkaloid	300	3.20±0.266^{a}	3.60±0.405^{b}	3.40±0.326^{b}	3.70±0.313^{b}	3.90±0.311^{bc}
ASA	150	3.60±0.241^{a}	7.00±0.795^{a}	7.30±0.578^{a}	9.60±1.526^{a}	11.60±0.201^{a}
NS	20	3.00±0.930^{a}	2.90±0.221^{c}	3.00±0.359^{c}	2.70±0.363^{c}	2.30±0.489^{c}

Analgesic Effect

This study showed the crude alkaloidal extract of *M. senegalensis* significantly delayed the reaction time of thermally induced pain (Table 6). This model is selective for centrally acting analgesics and indicates narcotic involvement with opioid receptors [34, 35], suggesting that it is a centrally acting analgesic. This observation is consistent with other studies involving Gindarudine, a morphine alkaloid extracted from *Stephia glabra* which also showed significant analgesic effect when tested by the same method [36, 37]. Alkaloids and its derivatives are known to have significant analgesic activities through the modulation of Camp [38].

4. CONCLUSION

The result of this study has shown that the crude alkaloidal extract of *M. senegalensis* root bark exhibits promising antimalarial activity. It has also shown that the alkaloidal extract of *M. senegalensis* can be a useful analgesic, though it possesses much lower anti-inflammatory effect, which could be attributed to other phytochemicals present in the plant. The results of this study justifies its use in traditional medicine as an antimalarial agent.

The *M. senegalensis* root bark extract has shown promising anti malaria and analgesic activity, the next step would be to isolate, identify and characterize the active alkaloid. The mechanism by which this alkaloid exerts its effect can also be determined.

Conflict of Interest

The authors declare that there is no conflict of interest regarding this paper

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