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MAJOR PHYTOCONSTITUENTS IN THE AQUEOUS LEAF EXTRACT OF *TITHONIA DIVERSIFOLIA* (*HEMSL. A. GRAY*) INDICATED ANTIMALARIA POTENTIALS

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ABSTRACT

Objective

The aqueous leaf extract of *Tithonia divers folia* **(*Hemsl. A. Gray*)** was screened for Phyto-constituents with anti-malaria potentials.

Methods

The leaf was air-dried in the shade for 15 days, pulverized and extracted with water. The aqueous leaf extract was lyophilised with a yield of (16.89%$^{w}/_{v}$) and subjected to Phytochemicals analyses using standard methods.

Results

The qualitative and quantitative phyto-constituents screening revealed the presences of alkaloids, flavonoids, quinones, gallate, glucosides, peptides, terpenes and xanthones, in which alkaloids (265.00±0.04), flavonoids (64.00±0.05) and quinones (44.02±0.04) were highly concentrated. Further analyses of fractionates of the phyto-constituents in the aqueous leaf extract recorded papaverine (67.32±0.01) and reservarine (21.16±0.01) as the major alkaloids, while glycosylflavonoids (25.13±0.02) was the main flavonoids and quinlenone (25.00±0.01) was the major quinones.

Conclusion

From the foregoing, it can be hypothesised that the aqueous leaf extract of *T. diversifolia* can serve as a good anti-malaria regime, as the active ingredients in most orthodox anti-malaria medicines are derivatives of the phyto-consistuents identified in our study.

KEYWORDS: Aqueous Leaf Extract, *Tithonia Diverifolia*, Phyto-Constituents, Anti-Malaria Potentials, Hypothesised and Good Anti-Malaria Regime

INTRODUCTION

Malaria is a major public health problem due to the development of resistance by the most lethal causative parasitic species, *Plasmodium falciparum* to the mainstay drugs like chloroquine [1, 2]. Malaria was reported to be the cause of death of about 1-2 million people each year, in which 300-500 million new clinical cases of malaria are reported annually [3]. Human malaria, transmitted by female anopheles mosquitoes is caused by four species of *Plasmodium*, which are *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. However, the most cases of malaria and deaths are caused by *P. falciparum* [4]. The development of resistance to mainstay drugs, such as chloroquine, pyrimethamine etc and controlled use of new artemisinin analogues, have created an urgent need to discover new antimalarial agents. Thus, new drugs with

unique structures and mechanism of action are urgently required to treat sensitive and drug-resistant strains of malaria.

Nature remains an ever evolving source for compounds of medicinal importance. There are recent advances in antimalarial drug discovery from natural sources, including plant extracts, and compounds isolated from plants, bacteria, fungi and marine organisms. These compounds offer new and novel scaffolds for development as antimalarials [5, 6]. In fact, a recent analysis, [7] showed that 80% of the 122 plant-derived drugs studied were related to their original ethnopharmacological purposes. Attempts have been made to provide an exhaustive compilation on structural features of antimalarials isolated from natural sources and to provide insights into their properties, including advantages and limitations in malaria chemotherapy.

Tithonia diversifolia (*Hemsl. A. Gray*) is an impressive member of the sunflower family, *Asteraceae* (compositae). The specific name '*diversifolia*' means 'separated leaf', from the *Latin* 'diversus' (divergent) and 'folium' (leaf). *Tithonia diversifolia* is a woody herb or succulent shrub, a perennial native of Mexico and Central America and is cultivated for its beautiful flowers and enormous size and was probably introduced into West Africa as an ornamental plant [8]. *T. diversifolia* has been a subject of research interest because of its various indigenous medicinal uses in many countries.

In Nigeria, the decoctions of its various parts are used for the treatment of fever, diabetes mellitus, sore throat, malaria, jaundice, liver menstrual pains among others [9, 10, and 11]. [12, 13] reported the *in vivo* anti-malaria and mosquito repellent capabilities of the methanolic and oil extract of *Tithonia diversifolia* leaf. Therefore, there are the urgent needs to find alternative therapies that are not only effective against resistant malaria but are also readily available and affordable, to compensate for the expensive orthodox anti-malaria medicines. This study evaluated the preliminary anti-malaria capabilities of the aqueous leaf extract of *Tithonia diversifolia* by the determination of the concentration of some major phyto-constituents with antimalaria properties.

MATERIALS AND METHODS

Plant Material

Fresh plant of *Tithonia diversifolia* was collected within the premises of Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Oyo state, Nigeria. The plant was identified at the Department of Crop Science, LAUTECH, Ogbomoso and a voucher specimen was deposited at the herbarium in the Department.

Chemical Reagents

All the chemicals and reagents used in the study were of analytical grade and were purchased from the Bristish Drug House (BDH) Poole England and Sigma Aldrich Chemical Co. Inc., Milwaukee, Wis., U.S.A.

Preparation of Crude Extract

The leaf of the fresh plant of *Tithonia diversifolia* was picked without the stalk. The leaf was rinsed in distilled water and spread in the shade to air dry for 15 days. The dried leaf was ground into fine powder, using a domestic grinder. The leaf powder was weighed and extracted with distilled water in ratio 1: 5 by shaking in an orbital shaker for 12 hours. The filtrates were pulled together and reconstituted to solid in a lyophilizer with a yield of 16.89 % $^{w}/_{v}$.

Leaf Analyses

Screening of the Phytochemicals Constituents

The analyses of the Phytochemicals constituents in the aqueous leaf extract of *Tithonia diversifolia* were performed according to standard method as described by [14, 15, and 16] for alkaloids, flavonoids, terpeniods, cardiac glycosides, anthraquinones, peptide, gallic, ellagic, chalcones, coumarin and pantothenol.

Determination of Percentage Composition of the Phytochemicals Constituents

The percentage concentrations of the phyto-constituent were determined by the methods described by [17] for coumarin and caffeic, while that of pantothenol was determined by [18]. Chalcones, xanthones, terpenes, glycosides and peptides by [19], quinones by [20], alkaloids by [21], flavonoids by [22] and phenolics (ellagic and gallic acids) by [19].

Determinations of the Fractionate Compositions of Some of the Phytochemicals

The quinones fractionate was screened further for quinoline and quinlenone [20], while the alkaloids fractionate was screened for papeverine, cocaine and reserverine [17], loustarin [19] and the flavonoids fractionate was screened for glucosy flavonoids and gluco flavonoids [17].

Statistical Analyses

The results were expressed as mean ± standard deviation of three determinations and student t test was performed to determine the significant mean values at 95% confidence level ($p<0.05$).

RESULTS

The results were presented as mean ± standard deviation of three determinations and mean values bearing different superscripts denote significant differences ($p<0.05$). The results obtained in the qualitative, quantitative and fractionate Phytochemicals analyses of the aqueous leaf extract of *Tithonia diversifolia* are presented in Tables 1 and 2. The phyto-constituents identified in the leaf extract revealed the presence of alkaloids, flavonoids, quinones, gallate, glucosides, peptides, terpenes and xanthones in the order of decreasing concentration (Table 1). In table 2, the result obtained in the quantification of the fractions of the phyto-constituents in the leaf extract revealed that papaverine and reservarine were the major alkaloids, glycosylflavonoids was the main flavonoids and quinlenone was the major quinones.

Table 1: Some Phytochemicals in the Aqueous leaf Extract of *Tithonia Diversifolia*

Phytochemicals	Identification	Concentration (Mg/100g)
Alkaloids	+++	265.00 ± 0.04
Caffeinic acid	-ve	0.00 ± 0.00
Chalcones	-ve	0.00 ± 0.00
Coumarines	-ve	0.00 ± 0.00
Flavonoids	++	64.00 ± 0.05
Gallic acid	+	24.01 ± 0.01
Glucosides	+	18.02 ± 0.01
Pantothenol	-ve	0.00 ± 0.00
Peptides	+	16.01 ± 0.01
Quinones	++	44.02 ± 0.04
Terpenes	+	16.18 ± 0.03
Xanthones	+	1.44 ± 0.00

Values are means ± SEM; n=3. Values bearing different alphabets are significantly different ($p<0.05$).

Key: +++ (Present in appreciable amount), ++ (Moderately present), + (Present in trace amount) and -ve (Absent).

Table 2: Concentrations of the Fractions of Some of the Phyto-Constituents in the Leaf Extract of *Tithonia Diversifolia*

Phytochemicals	Concentration (mg/100g)
Alkaloids:	
Loustarine	17.10 ± 0.02
Cocaine	6.02 ± 0.01
Papaverine	67.32 ± 0.01
Reservarine	21.16 ± 0.01
Flavonoids:	
Glycosylflavonoid	25.13 ± 0.02
Glycoflavonoid	16.08 ± 0.00
Quinones:	
Quinoline	16.21 ± 0.04
Quinlenone	25.00 ± 0.01

Values are means ± SEM; n=3. Values bearing different alphabets are significantly different ($p<0.05$)

DISCUSSIONS

Plants, especially the ones with reported ethno medicinal uses, have been the primary source of medicines for early drug discovery. In fact, [7] reported that about 80% of the 122 plant-derived drugs they studied were related to their original ethno medicinal purposes. The result obtained in the qualitative and quantitative Phytochemicals analyses of the aqueous leaf extract of *Tithonia diversifolia* indicated that the leaf extract could be a potential regime for the treatment of malaria and inflammatory disorders. The further quantification of some fractions of the phyto-constituents with renowned anti-malaria properties strengthened the indicated anti-plasmodia capabilities of aqueous leaf extract.

For example, the concentration of alkaloids in the aqueous leaf extract supported the previous report of [9], in which most antimalarials were found to be alkaloids based. Many alkaloids have been reported to possess anti-plasmodia activities by targeting the apicoplast, which is a plastid-like organelle in the plasmodium. The fractions of the alkaloids content are important anti-malaria bioactive. In an *in vitro* studies reported by [23, 24], protein biosynthesis in the plasmodium was inhibited at the level of translation by the selective binding of some lactone alkaloids, such as papaverine and reservation on the plasmodium ribosomes. [25] implicated the anti-plasmodia role some peptides an *in vitro* plasmodia growth study.

On the other hand, flavonoids, such as the glycosylflavonoids and glycoflavonoids and some gallates have been reported to inhibit the intra-erythrocytic growth of two strains of human malaria parasite *Plasmodium falciparum* [26]. Terpenes in general were reported to exhibit anti-plasmodia activities by the inhibition of H^+- ATPase pumps present in the plasma membrane and food vacuole of the plasmodium, the inhibition of the parasite growth by erythrocyte membrane modification [27, 28, 29]. Xanthones and quinones are renowned for their antiplasmodial activities. [30] Reported the inhibitory activities of quinones, such as quinlenone and quinoline, on the mitochondrial electron transport and respiratory chain by reduced oxygen consumption in the plasmodia or the blocking the polymerization of toxic heme released during hemoglobin proteolysis in intraerythrocytic *Plasmodium falciparum* [31, 32]. The result of the phyto-constituents in the aqueous leaf extract of *Tithonia diversifolia* is in agreement with the previous work, in which methanolic and volatile oil

extracts of *Tithonia diversifolia* leaf indicated antiplasmodial activities and mosquito repellant capabilities [12, 11].

CONCLUSIONS

The phyto-constituents in the aqueous leaf extract of *T. diversifolia* makes the plant a good anti-malaria candidate for the treatment of malaria and also, an alternative source of bioactive ingredients in the development of new orthodox medicines in malaria therapy. However, further study is required on the *in vivo* anti-plasmodia activities of the different fractions of the phyto-constituents of *T. diversifolia*.

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