Chemical and Biological Screening of the Leaves of *Stachytarpheta cayennensis* (l. VAHL).

Okah Pius Otom, S.K. Okwute

Department of Chemistry, University of Abuja, Nigeria

Abstract: ***Stachytarpheta cayennensis*** **belongs to the family Verbenaceae; it is a species of flowering and is applied by many traditional healers to treat a host of diseases including diabetes, dysentery pain, wounds, eye infections, bacterial infections, chicken pox, measles, blood pressure, toothache and malaria. The leaf of** ***Stachytarpheta cayennensis*** **was studied to evaluate its chemical constituents and to confirm its traditional medicinal use. The phytochemical screening of the leaves of this plant revealed the presence of carbohydrates, steroids, terpenoids, tannins, saponins, alkaloids and flavanoids. The antimicrobial screening against some selected human pathogens; showed that the extract was sensitive to** ***Escherichia coli, Staphilococcusaureus, Pseudomonas aeruginosa, Micrococcus luteus and Candida albicans.*** **The Gas Chromatography/Mass Spectrometry (GC-MS) analysis of the steam distillate showed 32 peaks. This led to the identification of several compounds with valuable biological activities including enzo[d]isothiazol-3-one ($C_8H_7O_3$) a disinfectant, a platelet aggregation inhibitor and a xenobiotic, dodecanoic acid ($C_{12}H_{24}O_2$) which is an antibacterial agent and an algal metabolite, hexanol, (Z)-9-tricosene,phthalic acid, octade-9-enoic acid, heptacosan-1-ol. The Gas Chromatography/Mass Spectrometry (GC-MS) analysis revealed that the steam distillate is mainly composed of oxygenated hydrocarbons, predominantly esters and carboxylic acids, a few long chain hydrocarbons and alcohols. The crude methanol extract of the leaf was also subjected to antimalarial screening at daily doses of 200, 400 and 800mg extract/kg body weight of mice infected with plasmodium berghei in a four-day treatment. The percentages of suppression of the parasitaemia at the doses of 200, 400, and 800 mg extract/kg body weight were, respectively, 36.62%, 50.36%, and 61.06%. The findings on antiplasmodial activity of this plant justify the traditional use by local populations against malaria. The findings therefore, suggest that there is an indication that** ***S.cayennensis leaves*** **contain phytochemicals that may be linked to its beneficial effects on health. Thus, the isolation of the active compounds from this plant is suggested for possible antimalarial drugs.**

Keywords: **Stachytarpheta Cayennensis, Phytochemicals, Antimicrobial activity, Antimalarial activity, Gas Chromatography/Mass Spectrometry.**

I INTRODUCTION

Medicinal plants are various types of plants used for medicinal purposes. They are used to attempt to maintain good health. Medicinal plants are often referred to as medicinal herbs. Nowadays, herb refers to any part of the plant like fruit, seed, stem, bark, flower, leaf, stigma or a root, as well as a non-woody plant. Earlier, the term "herb" was only applied to non-woody plants, including those that come from trees and shrubs. These medicinal plants are also used as food, flavonoid, medicine or perfume and also in certain spiritual activities [1].Many medicinal plant species have been investigated in the search for the active constituents that make them effective in treatment of various ailments [2], but generally there is still a demand to find more information concerning the therapeutic potential of plant species

The active constituents of plants have only relatively recently been isolated. The active constituents in plants are the chemicals that have a medicinal effect on the body. They include phenols, tannins, flavonoids, saponins, carbohydrates, alkaloids, phytosterols etc. These naturally occurring chemical compounds are called phytochemicals. In many places, as a dietary accessory they are comfortably accessible but dormant health advantages of phytochemicals are only reachable from the utilization of whole plant. Phytochemicals are beneficial to boost up immunolatory responses and also provide immunity against many diseases; some are known to reveal medicinal and physiological activities [3], [4], [5].

Crude extracts and dry powder samples from medicinal plants and their species have become the object of interest for the development and preparation of alternative medicines [6], [7], [8], [9]. This has drawn attention to urgent need to search for herbal medicinal plants with potential medicinal properties on the basis of their ethno-medicinal uses and also for their isolation.

Furthermore, bioactive compounds from medicinal plant materials possess high antimicrobial activity and thus used as potential antimicrobial agents. [10], [11].

Some herbal remedies may be safe and effective for the treatment of malaria. Nevertheless, scientific proof is needed before herbal remedies can be recommended on a large scale. In remote settings with poor resources where modern antimalarials are not steadily available, research can provide an evidence base for traditional medicine, to inform local treatment choices.

Considering the potentiality of plants as sources for antimalarial and antimicrobial drugs, systematic screening for bioactive compounds could possibly lead to the development of therapeutic agents.

The aim of this study is to evaluate the leaves of *Stachytarpheta cayennensis* for its chemical characteristics, antimicrobial activity and antimalarial activity.

II. MATERIALS AND METHODS

Plant Materials Collection

The leaves of *Starchytapheta cayennensis* were collected from Gwagwalada, Abuja-Nigeria in the month of July, 2018 and botanically identified by Mr Lateef Akeem, a Botanist, at National Institute for Pharmaceutical Research and Development (NIPRD). One voucher specimen was deposited in the Herbarium of NIPRD under the number NIPRD/H/6914.

Experimental Methods

Preparation of Crude Plant Extracts

The leaves were plucked from the stem and washed, then dried at room temperature for three weeks. The dried leaves were ground into powder using mortar and pestle in the laboratory. The powdered plant was then stored in a dry airtight containers for subsequent analysis.

241g of the dried and powdered leaf of *Stachytarpheta cayennensis* was exhaustively macerated in 98% methanol for 8 days. The liquid extract obtained was concentrated in rotary evaporator. The yield was 16.6%.

Extraction of Essential Oils

The collected fresh leaves of *Stachytarpheta cayennensis* were washed to remove the dirt on the surface of the leaves. It is to make sure that no any other impurities stick to the leaves. The extraction was carried out using only fresh leaves. 162g of fresh leaves of *Stachytarpheta cayennensis* was subjected to steam distillation. The extraction was performed within the period of 6 hours. The essential oil was then separated from the distillate using separating funnel with dichloromethane as solvent, the yield was 0.3%.

Gas Chromatography-Mass Spectrometry (GC-MS) of Essential Oils

For the determination of the chemical constituents contained in the essential oils of *Stachytarpheta cayennensis* leaves, Gas Chromatography-Mass Spectrometry (GC-MS) machine was used. This machine, model SHIMADZU GCMS - QP2010 PLUS, MSD Triple-Axis Selective Detector is available in Laboratory of SHIMADZU training center for analytical instrument, Lagos. The machine used helium as the carrier gas at a constant pressure of 100.2kPa and Column Oven Temperature of 60.0^{o}C. The Injection Temperature was 250.00^{o}C, the total flow was 39.4 mL/min and the Column Flow was 1.61mL/min.

Phytochemical Analysis

Phytochemical analysis was carried out to detect the presence of some secondary metabolites. The methods used by [12], [13], [14] were used to analyse the bioactive components in the plant extract. The phytochemical tests carried out in this work include alkaloids, saponins, glycosides, tannins, steroid, terpenes and flavonoids.

Test for Tannins: The powdered sample of the plant leaves (0.5g) was boiled in 20 mL of distilled water in a test tube and then filtered. The filtration method used here is the normal method, which includes a conical flask and filter paper. The 0.1% $FeCl_3$ is added to the filtered samples. Brownish-green coloration was observed.

Test for Alkaloids:

Mayer' s Test

To a few ml of plant sample extract, two drops of Maye's reagent are added along the sides of test tube. Appearance of white creamy precipitate was observed.

Test for steroids: One milliliter of the extracts was dissolved in 10 mL of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence.

Test for terpenoids: Five milliliter of methanolic extract is mixed with 2 mL of $CHCl_3$ in a test tube. Three milliliter of concentrated $H_2SO_4$is carefully added to the mixture to form a layer. An interface with a reddish brown coloration was formed.

Test for Carbohydrates:

Molish' s Test

To 2 ml of plant sample extract, two drops of alcoholic solution of α- naphthol are added. The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of test tube. A violet ring was observed.

Test for Flavonoids: The stock solution (1 mL) was taken in a test tube and added few drop of dilute NaOH solution. An intense yellow colour was appeared in the test tube. It became colourless when on addition of a few drop of dilute acid.

Test for Saponins: The extract (50 mg) is diluted with distilled water and made up to 20 ml. The suspension is shaken in a graduated cylinder for 15 minutes. About two cm layer of foam was observed.

Thin Layer Chromatographic Analysis

Several plates measuring 10cm x 4cm were used for the thin layer chromatography (TLC) with silica gel as the adsorbent.

Preparation of Solvent System

Two transparent glass tanks containing different solvent systems were prepared according to the ratio given below.

Tank I: Contains chloroform and methanol in the ratio of 9:1.

Tank II: Contains hexane and ethylacetate in the ratio of 4:1

Spotting, Development and Detection of Spots by Analytical TLC

With the aid of Pasteur pipettes, a spots of the crude extract was made on a base line measured 1.5cm from the edge of the

plate. The spots were allowed to dry in air for five minutes before developing in the solvent tanks which were shaken for five minutes and kept for thirty minutes for saturation to occur.

Two plates, each were placed in tanks with the spotted end made to face downward. The depth of the developing solvent was less than the spotted height. The tanks were sealed and allowed to stand undisturbed until the solvent front traversed the length of the plate to 1cm of the top of the coated portion of the plates. The plates were removed and the solvent front marked with a pencil. The plates were allowed to stand for three minutes before detecting the spots.

Spots on the developed plates were detected using UV light type A 409 (wavelength 250nm) and spraying with freshly prepared mixture of methanol and concentrated sulphuric acid (90 %:10% v/v). The colours and retentions factors obtained were noted.

Preparation of Test Organisms

The microorganisms used namely *Staphylocuccusaureus, Escherichia coli, Pseudomonas aeruginosa, Micrococcus luteus* and *Candida albicans* were obtained from stock cultures in the Microbiology Laboratory of the University of Abuja, Abuja, Nigeria. They were sub-cultured and identified based on their colonial morphology, microscopic appearance and specific biochemical reactions. The test organisms were sub cultured in 10ml broth each and incubated at 37℃ for 24 hours. After 24 hours, the organisms were sub cultured into a fresh Mueller Hinton Agar and autoclaved for 3 hours which was used for all analyses.

Antimicrobial Screening of Crude Methanol Extract of Leaves

Freshly prepared sterile Mueller Hinton Agar was poured into sterile, labeled Petri dishes and allowed to set. A sterile swab was dipped into the adjusted inoculum of the test organism and is used in streaking the surface of the nutrient agar to form a microbial lawn. The surface of the medium was allowed to dry for 3 minutes and sterile 5mm cork borer was used to bore holes on agar plates, the base of each hole was sealed with a molten agar to avoid diffusion extract under agar. Some milligrams of each concentration were dispensed into each hole. The plates were left undisturbed for about 15 minutes before and then incubated at 37^{o}C for 24 hours. The diameters of zones of inhibition were measured using a transparent ruler in millimeters.

In Vivo Antimalarial Activity Essay

In Vivo Suppressive Test with Plasmodium Berghei.

Evaluation of the *in vivo* antimalarial activity was performed based on the Peters 4-day suppressive test [15].

Animal

Young adult white mice, of both sexes, weighing 18–22g were used (appendix B). They were obtained from the National Institute for Pharmaceutical Research and Development (NIPRD), Idu Abuja. They were maintained on standard animal pellets and water. Animals were acclimatized to the laboratory conditions, supplied with food and water for 5 days before being used for the test. The animals were handled according to the National and International Guidelines for Handling of Laboratory Animals.

The Parasite

The chloroquine - sensitive Plasmodium berghei used in the study was obtained from the Department of Pathogen Molecular Biology, Ahmadu Bello University, zaria–Kaduna, Nigeria.

Preparation of Infected red Blood Cells Suspension

Donor mice with high parasitemia were anesthetized by diethyl ether; blood was collected by cardiac puncture and diluted with sterile normal saline (0.9% w/v sodium chloride) to make a suspension of 1×10^8 infected red blood cells (iRBCs) per ml, which was used to infect test mice. This was prepared by determining both the percentage parasitaemia and the erythrocytes count of the donor mouse.

Inoculation of Parasites and Administration of Extracts

Each mouse was infected by injection via tail vein with 2×10^7iRBCs in 0.2 ml suspension of 1×10^8iRBCs per ml. The extract was dissolved in distilled water. Chloroquine was dissolved in normal saline. Three hours post infection, the mice were randomly allocated into groups of 6 mice each (appendix B): Negative control group received solvent (5 mg/kg/day), positive control group received chloroquine (10 mg/kg/day), and treatment groups received different doses of extracts (200, 400, or 800 mg/kg/day). Dosing was done orally, once daily, starting on the day of infection and continued for a total of four daily doses. Body weight was recorded daily while parasitemia was determined on day 4.

Determination of Parasitemia and Percentage Suppression at Day 4

On day 4, tail blood smear was fixed with absolute MeOH and then stained with 10% Giemsa-stain at (pH 7.2) for 20 min. The parasites were examined under microscope at ×100 oil immersion. Percentage parasitemia was determined by counting the infected erythrocytes in at least 1000 total erythrocytes (infected plus non-infected erythrocytes).The mean percentage suppression of parasitemia for each extract was calculated as follows:

$$\% \text{ suppression} = \frac{\text{mean \% parasitemia in negative control mice} - \text{mean \% parasitemia in treated mice}}{\text{mean \% parasitemia in negative control mice}} \times 100$$

Statistical Analysis

Percentage parasitemia suppression and survival time in days were presented as mean ± standard deviation for each group. The mean percentage parasitemia on day 4 and the mean were analyzed statistically using the Student's t-test to identify the differences between treated groups and negative control

group. The difference was considered statistically significant at $P < 0.05$.

III. RESULTS AND DISCUSSION

Results

Extraction and TLC of Crude Extract

The leaves were subjected to extraction with methanol and the extract subjected to TLC using silica gel precoated plate with different solvent systems, UV lamp (λmas 366+254mm) and the Spray reagent: 90ml conc.H_2SO_4 + 10ml C_2H_5OH. The results are as shown in table I

Table I
TLC Analysis of the Methanolic Leaves Extract of *Stachytarpheta cayennensis*

Solvent	Colour when viewed	Colour after spraying	Number of components	R_f Values
Hexane:ethylacetate (4:1)	Red	Purple	4	0.88
				0.83
				0.75
				0.57
Chloroform:methanol (9:1)	Red	Purple	4	0.91
				0.87
				0.70
				0.54

Key: R_f= Retention Factor

Phytochemical screening

The result of phytochemicals analysis on the methanol leaves extract of Stachytarpheta cayennensis are presented in table II.

TableII: Results For Phytochemical Analysis

Compound	Test	Inference
Carbohydrates	Morlish's test	+
Steroids	Chloroform/sulphuric acid test	+
Tepenoids	Chloroform/sulphuric acid test	+
Tannns	Ferric chloride test	+
Saponin	Frothing test	+
Alkaloids	Mayer's Test	+
Flavonoids	Sodium hydroxide Test	+

Antimicrobial Activity

The antimicrobial screening results for the crude methanol extracts are presented in table III.

Table III: Antimicrobial Activity Screening Of the Leaf Extract of Stachytarpheta cayennensis

Sample	Diameter of Zone of Inhibition (mm)					
	Concentration of extract (mg/cm^3)	Escherichia coli	Staphiloccusaureus	Pseudomonas aeruginosa	Micrococcus luteus	Candida albicans
extract	120	26	24	20	28	26
	100	18	18	14	22	20
	80	10	16	4	18	16
	60	8	04	2	10	9
Penicillin	0.4	30	50	30	40	35

Key: mm=millimeter, mg=milligram.

The GC/MS analysis of the steam distillate

The steam distillate was subjected to GC/MS. The gas chromatogram and the MS data are as shown in figure 1 and table IV respectively.

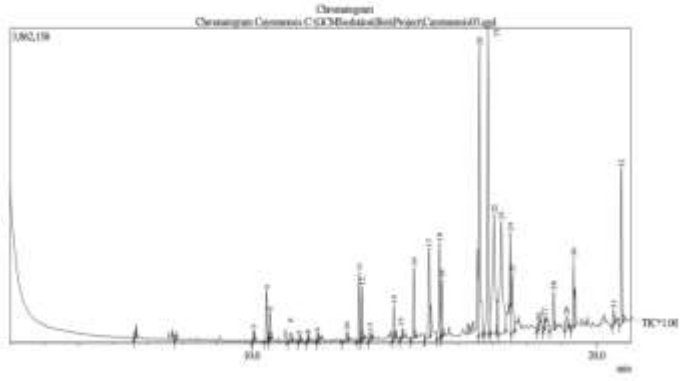

Figure1: Gas chromatogram of steam distillate.

Table IV: GC-MS of Volatile Components of the Leaves of *Stachytarpheta cayennensis*

Name	(Rt)	(RI)	Molecular formula	MM	Peak Area %	Peak height %
2-methoxy-1,3,5-trimethylBenzene,	6.617	1209	$C_{10}H_{14}O$	150	0.08	0.14
4,5-Dimethylnonane	7.791	986	$C_{11}H_{24}$	156	0.02	0.05
Dodecanoic acid	10.058	1570	$C_{12}H_{24}O_2$	200	0.41	0.60
1-hexanol	10.433	1854	$C_{16}H_{34}O$	242	2.16	2.67
Hexadecane	10.533	1612	$C_{16}H_{34}$	226	1.13	1.56
1,2-Benzisothiazol-3(2H)-one	11.117	0	$C_8H_7O_3$	197	0.46	0.45
n-Pentadecylcyclohexane	11.392	2172	$C_{21}H_{42}$	294	0.26	0.32

Dichloroacetic acid, 4-hexadecyl ester	11.625	2259	$C_{18}H_{34}C_{l_2}O_2$	352	0.25	0.36
2,3,7-Trimethyldecane	11.908	1121	$C_{13}H_{28}$	184	0.31	0.45
Octadecanoic acid	12.750	2167	$C_{18}H_{36}O_2$	284	0.52	0.51
1-nonadecene	13.117	1900	$C_{19}H_{38}$	266	2.70	3.64
Eicosane	13.208	2009	$C_{20}H_{42}$	282	1.97	2.85
(Z)-9-Tricosene	13.433	2315	$C_{23}H_{46}$	322	0.36	0.44
Phtthalic acid	14.125	2732	$C_8H_6O_4$	166	1.32	1.85
3-Ethyl-5-(2'-ethylbutyl)octadecane	14.392	2413	$C_{26}H_{54}$	366	0.11	0.15
hexadecanoic acid methyl ester	14.708	18781	$C_{17}H_{34}O_2$	270	2.99	3.79
l-(+)-Ascorbic acid 2,6-dihexadecanoate	15.150	4765	$C_{38}H_{68}O_8$	652	4.94	4.76
1-nonadecene	15.450	1900	$C_{19}H_{38}$	266	4.12	5.04
Eicosane	15.51	2009	$C_{20}H_{42}$	282	2.27	3.09
9-Octadecenoicacid, methyl ester, (E)-	16.608	2085	$C_{19}H_{36}O_2$	296	14.60	15.24
Octadecanoic acid	16.850	2077	$C_{19}H_{38}O_2$	298	14.37	16.14
Octadec-9-enoic acid	17.025	2175	$C_{18}H_{34}O_2$	282	12.33	6.34
Octadecanoic acid 2-(2-hydroxyethoxy)ethyl ester	17.233	2694	$C_{22}H_{44}O_4$	372	12.02	5.94
1-heptacosanol	17.500	2948	$C_{27}H_{56}O$	396	2.20	3.86
9-Hexacosene	17.500	2614	$C_{26}H_{52}$	364	3.35	3.10
Pentadecanal	18.367	1701	$C_{15}H_{30}O$	226	0.55	0.47
Sulfurous acid, octadecyl 2-propyl ester	18.493	2668	$C_{21}H_{44}O_3S$	376	0.57	0.70
Eicosanoic acid, methyl ester	18.758	2276	$C_{21}H_{42}O_2$	326	1.92	2.07
18,19-Secoyohimban-19-oic acid, 16,17,20,21-tetradehydro-16-(hydroxymethyl)-, methyl ester, (15.beta.,16E)-	19.142	2849	$C_{21}H_{24}N_2O_3$	352	1.06	0.69
Heptacosanol	19.333	2948	$C_{21}H_{42}O_2$	396	3.01	3.77
Methyl-3-hydroxy-2-tetradecyloctadecanoate	20.483	3567	$C_{33}H_{66}O_3$	510	0.80	0.83
1,2-Benzenedicarboxylic acid, diisooctyl ester	20.725	2704	$C_{24}H_{38}O_4$	390	6.80	8.14

Key: Rt=retention time, R_I=retention index, MM= molecular mass\

In vivo antimalarial activity of the crude methanolic extract

The crude methanol extract was screened for antimalarial activity using 4-day suppression test against chloroquine sensitive *P. berghei* infected mice. The level of suppression of the extract at concentrations of 200 mg/kg/day, 400 mg/kg/day and 800 mg/kg/day following the 4-day test was 36.61, 50.36 and 61.06%, respectively as shown in V and VI.

Table V: *In Vivo* Antimalarial Activity Of Methanol Extract Of The Leaves Of Stachytarpheta cayennensis At Different Doses On *Plasmodium Berghei.*

Treatment	Mean percentage parasitemia at day 4±SD (n=6)	Mean percentage suppression of parasitemia at day 4	Treatment	Mean percentage parasitemia at day 4±SD (n=6)
I	Extract	800	16.37±0.58	61.06
II	Extract	400	20.87±1.42	50.36
III	Extract	200	26.65±1.55	36.61
IV(-ve ctrl group)	Distilled water	1ml	42.67±3.00	0.00
V(+ve ctrl group)	Chloroquine	10	0.00	100

KEY: +ve ctrl group=positive control group group treated with standard drug -chloroquine), -ve ctrl group= negative control group (group treated with distilled water), SD = standard deviation

Table VI: Effect Of Methanolic Leaves Extracts Of S. Cayennensis On Body Weight Of *P. Berghei* Infected Mice.

Test Substace	Dose(mg/kg)	Body weight	Body weight
Extract	800	20.00±1.00	21.00±1.00
Extract	400	18.00±2.00	17.00±2.00
Extract	200	15.33±1.52	13.67±2.08
Distilled water		20.00±1.00	15.00±1.67
Chloroquine	10	19.33±1.55	22.00±1.86

Key: mg = milligram, kg = kilogram, Do = first day, D4 = fourth day

IV DISCUSSION

The phytochemical screening of the leaves of this *S. cayennensis* revealed the presence of carbohydrates, steroids, terpenoids, tannins, saponins, alkaloids and flavanoid. The presence of steroids and terpenoids in S. cayennensis contradicts the observation of Edeoga who reported that steroid and terpenoid were absent in this plant [16]. However, the presence of steroids and terpenoids in *S. cayennensis'* leaves has also been reported bysome other researchers[17]. Steroids reduce the production of inflammatory chemicals in order to minimize tissue damage and also reduce the activity of the immune system by affecting the function of white blood cells [18]. This may be the reason the leaves of *S. cayennensis* are used to treat various ailments such as inflammation, pain, hepatic and renal disorder [19], [20], [21] and [22]. The presence of terpenoidsin *S. cayennensis* has also been reported

by some other researchers [23]. Important therapeutic uses of terpenoids include antifungal, antiviral, antihyperglycemic, anti-inflammatory, antioxidants (increase the concentration of antioxidants in wounds and restore inflamed tissues by increasing blood supply), antiparasitic, immunomodulatory, and as skin permeation enhancer [24]. This may be the reason why it is used traditionally to treat diabetes, to reduce stress [25] and externally to treat wounds and toothache [26]. From table II, it can also be seen that the leaves of *Starchytapheta cayennensiss* contain alkaloids, this conform to the report of [23]. Alkaloids rank among the most efficient therapeutically significant plant substance. Pure isolated alkaloids and their synthetic derivatives are used by ethnomedicinal practitioners for their analgesic, antispasmodic and bactericidal effects [27].The alkaloid content of *Starchytapheta cayennensiss* may be the reason for its use in the treatment of cough, wounds, fever and malaria, rheumatism and skin infections [28], [29].

Flavonoids, glycoside and tannins which were also found to be present the leaves of *S. cayennensis* have hypoglycemic activities [30], [31], which is also in line with its use traditionally to treat diabetes [32].

The leaves were subjected to extraction with methanol and the extract subjected to TLC using silica gel precoated plate with different solvent systems, UV lamp (λmas 366+254mm) and the Spray reagent: 90ml conc.H_2SO_4 + 10ml C_2H_5OH. The results are as shown in table I. TLC analysis of the methanolic extract of *Stachytarpheta cayennensis* gives an impressive result that indicates the presence of a number of phytochemicals. Various phytochemicals gives different Rf values in different solvent system. This variation in Rf values of the phytochemicals provides a very important clue in understanding of their polarity and also will helps in selection of appropriate solvent system for separation of pure compounds by Column Chromatography. The selection of appropriate solvent system for a particular plant extracts can only be achieved by analyzing the Rf values of compounds in different solvent system.

The antimicrobial activities of the crude extracts exhibited dose dependent inhibition against all tested human pathogens with zones of inhibition ranging from 2 to 28mm at various concentrations as shown in table III.The crude extract has the highest inhibition of 28mm on *Micrococcus luteus* at 120mg/cm^3 and the lowest inhibition of 2mm on *Pseudomonas aeruginosa*at 60mg/cm^3. From the result it shows that the higher the concentration, the higher the activity. Similar result was reported by [23] but on the contrary, their extracts show no significant activity on C. albican at all concentration.

The Gas Chromatography/Mass Spectrometry (GC-MS) analysis of the steam distillate showed 32 peaks, the chromatogram is shown figure 1. This led to the identification of several compounds with valuable biological activities including enzo[d]isothiazol-3-one ($C_8H_7O_3$) which has a role as a disinfectant, a platelet aggregation inhibitor, and a xenobiotic, dodecanoic acid ($C_{12}H_{24}O_2$) which has a role as an antibacterial agent and an algal metabolite, hexanol, (Z)-9-tricosene, phthalic acid, octadec-9-enoic acid, Heptacosan-1-ol. The Gas Chromatography/Mass Spectrometry (GC-MS) analysis revealed that the steam distillate is mainly composed of oxygenated hydrocarbons, predominantly esters and fatty acids, a few long chain hydrocarbons and alcohols (table IV).

The 4-day suppressive test results indicated that the methanolic extract of the leaves of *S. cayennensis* had prominent antiplasmodial activity against chloroquine sensitive *P. berghei* infected mice. The level of suppression of the extract at concentrations of 200 mg/kg/day, 400 mg/kg/day and 800 mg/kg/day following the 4-day test was 36.61, 50.36 and 61.06%, respectively. This gives lower percentage suppression than that reported by J [33]. Similar work done by [33] gives a dose dependent chemosuppressive effect of 64.6%, 77.42% and 78.2% for 90,180 and 270 mg/kg/day doses.

The antiplasmodial activity observed in this study may have resulted from metabolites which could be acting singly or in synergy with one another to exert the observed antimalarial activity. Previous studies showed that terpenoids, steroids, flavonoides, fatty acids, exhibited antiplasmodial activity in different antimalarial assays, [34], [35], [36] and [37].

V. CONCLUSION AND RECOMMENDATIONS

Conclusion

The phytochemical screening of the leaves of this plant revealed the presence of carbohydrates, steroids, terpenoids, tannins, saponins, alkaloids and flavanoids. Thus, the study has provided biochemical basis for ethno pharmacological uses of the plant in the treatment and prevention of various diseases and disorders. The leaf extract has activity against *Escherichia coli, Staphilococcusaureus, Pseudomonas aeruginosa, Micrococcus luteus and Candida albicans.* The Gas Chromatography/Mass Spectrometry (GC-MS) analysis of the steam distillate showed 32 peaks, this led to the identification of several compounds with valuable biological activities, it revealed that the steam distillate is mainly composed of oxygenated hydrocarbons, predominantly esters and carboxylic acids, a few long chain hydrocarbons and alcohols. The results of this study have also shown that the methanolic leaf extract of *Stachytarpheta cayennensis* possesses antimalarial activity as seen in its ability to suppress *Plasmodium berghei* infection. This justifies the traditional usage of this plant as malarial remedy.

Recommendation

Medicinal plants are important for drug discoveries and development. The findings from thisresearch therefore, suggest that there is an indication that *S.cayennensis leaves* contain chief phytochemicals and bioactive compounds that may be linked to its beneficial effects on health. Thus, the isolation of the active compounds from this plant is suggested for possible antimalarial drugs.

REFERENCE

[1] MahtabAlam Khan (2016), Introduction and Importance of Medicinal Plants and Herbs,Zahid publishers.

[2] C.N.Muthaura, J.M.Keriko, S.Derese, A.Yenesew, G.M.Rukunga(2011). Investigation of some medicinal plants traditionally used for treatment of malaria in Kenya as potential sources of antimalarial drugs. Experimental ParasitologyVolume 127, Issue 3, Pages 609-626

[3] R, Khare RK, Singhal A. (2017). Qualitative Phytochemical Screening of Some Selected Medicinal Plants of Shivpuri District (MP). Int J Life SciScienti Res 3: 844-847.

[4] Grover N, Patni V (2013). Phytochemical characterization using various solvent extracts and GC-MS analysis of methanolicextract of Woodfordiafruticosa (L) Kurz. Leaves. Int J Pharma PharmaceuticSci 5: 291-295.

[5] Ayoola GA, Coker HA, Adesegun SA, Adepoju-Bello AA, Obaweya K, et al. (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Trop J Pharmaceutic Res 7: 1019-1024.

[6] Baygar H, Sagdic O, Ozkan G, Karadogan T. (2004) Antibacterial activity and composition of esseintial oils from *Origanum*, *Thymbra* and *Satureja* species with commercial importance in Turky. Food Control. 15:169-72.

[7] Marino M, Bersani C, Comi G. (2001). Impedance measurements to study the antimicrobial activity of essential oils from *Lamiaceae* and *Compositae*. Int J Food Microbiol. 67:187-95.

[8] Oke B, Aslim C, Ozturk S, Altundag G. (2009). Essential oil composition, antimicrobial and antioxidant activities of *Saturejacuneifolia* Ten. Food Chem. 112:874-9.

[9] Rota MC, Herrera A, Martinez RM, Sotomayor JA, Jordan MJ. (2008). Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* esseintial oils. Food Control. 19:681-7

[10] Duraipandiyan, M. Ayyanar, and S. Ignacimuthu. (2006). Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India," BMC Complementary and Alternative Medicine, vol. 6, no. 35.

[11] Doriane E Djeussi, Jaurès AK Noumedem, Jackson A Seukep, Aimé G Fankam, Igor K Voukeng, Simplice B Tankeo, Antoine HL Nkuete, Victor Kuete. (2013). Antibacterial activities of selected edible plants extracts against multidrug- resistant Gram-negative bacteria," BMC Complementary and Alternative Medicine, vol. 13, no. 164.

[12] K. Karthishwaran, S. Mirunalini, G. Dhamodharan, M. Krishnaveni and V. Arulmozhi (2010), Phytochemical Investigation of Methanolic Extract of the Leaves of Pergulariadaemia, journal of biological science 10(3) 242246.

[13] K.SahiraBanu, Dr. L.Cathrine (2015), General Techniques Involved in Phytochemical Analysis .International Journal of Advanced Research in Chemical Science (IJARCS) Volume 2, Issue 4, April 2015, PP 25-32 ISSN 2349-039X (Print) & ISSN 2349-0403.

[14] RahmanGul, Syed Umer Jan, Syed Faridullah, SamiullahSherani, and NusratJahan(2017), Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from Ephedra intermedia Indigenous to Balochistan. The Scientific World Journal, Volume 2017, Article ID 5873648.

[15] Peters W. The chemotherapy of rodent malaria, XXII. The value of drug-resistant strains of P. Berghei in screening for blood schizontocidal activity. Ann Trop Med Parasitol. 1975;69:155–71.

[16] H.O Edeoga, D.E Okwu, B.O Mbaebie. Phytochemical constituent of some Nigerian medicinal plants. Africana Journal of Biotechnology, vol. 4(7) pp. 685-688, july 2005.

[17] Alice CB, Vargas VM, Silva GA, Siqueria NC, Schapoval EE, Gleeve J. Screening of plants used in south Brazilian folk medicine. J Ethnopharmacol. 1991;35:165–71.

[18] William Ericson-Neilsen, MDand Alan David Kaye, MD, PhD (2014), Steroids: Pharmacology, Complications, and Practice Delivery Issues, Ochsner J. 2014 Summer; 14(2): 203–207. PMCID: PMC4052587, PMID: 24940130.

[19] Burkill HM. Stachytarpheta cayennensis: The useful plants of West Africa. Royal Botanical Gardens: Keids; 1966.

[20] Weniger B, Robineau L. ElementsparaunaFarmacopeaCaribena, La Habana. SeminarioTramil. 1988;3:243–5.

[21] Rodriguez SM, Castro O. Evaluation farmacologica y quimica de Stachytarphetajamaicensis(verbenaeae) Revista de Biol Trop. 1996;44:353–9.

[22] Cano JH, Volpato G. Herbal mixture in the traditional medicine of Eastern Cuba. J Ethnopharmacol. 2004;90:293–316.

[23] Okoye TC, Akah PA, Ezike AC, Uzor PF, Odoh UE, Igboeme SO, Onwuka UB, Okafor SN, (2014), Immunomodulatory effects of Stachytarpheta cayennensis leaf extract and its synergistic effect with artesunate. BMC Complement Altern Med. 2014 Oct 5;14:376. doi: 10.1186/1472-6882-14-376.

[24] Priyanka P. Brahmkshatriya, Pathik S. Brahmkshatriy, (2013) Terpenes: Chemistry, Biological Role, and Therapeutic Applications, Natural Products pp 2665-2691.

[25] Adebajo, A.C., et al. (2007). Hypoglycaemicconstituentsof Stachytarpheta cayennensis leaf. Planta Med 73(3) 241-50.

[26] DeFilipps, R. A.; Maina, S. L.; &Crepin, (2014) J.MedicinalPlantsoftheGuianashttp://botany.si.edu/bdg/medicinal/index.html,Smithsonian Museum publishers.edicinal plant consumption". J EthnobiolEthnomed. 8: 43. doi:10.1186/1746-4269-8-43. PMC 3549945. PMID 23148504.

[27] Ritu Soni1, SakshiJaiswal, JyotiKiran Bara ,Dr.ParulSaksena,(2016), The Use of Rauwolfiaserpentina in Hypertensive Patients Department of Biotechnology, Sri Sathya Sai College Bhopal, Madhya Pradesh, India. Journal of Biotechnology and Biochemistry (IOSR-JBB) ISSN: 2455-264X, Volume 2, Issue 5, PP 28-32.

[28] Leslie Taylor (2005), The Healing Power of Rainforest Herbs.

[29] Iwu. I. C., Onu. U.L., Chijioke-okere. M., Ukaoma. A. A., Azorji. J. N. (2016) GC-MS, Phytochemical and Antibacterial Analysis of Pentaclethramacrophylla Leaf. The International Journal of Science and Technology 4 (7) pp 151-159.

[30] Oliver, B., (1980): Oral hypoglycaemic plants in West Africa. J. Ethnopharmacol., 2: 119-127.

[31] Cherian, S. and K.T. Augusti, 1995. Insulin sparing action of leucopelargonidin derivative isolated from *Ficusbengalesis* Linn. Indian J. Exp. Biol., 33: 608-611.

[32] AdebajoAC, OlawodeEO, Omobuwajo OR, Adesanya SA, Begrow F, Elkhawad A, Akanmu MA, EdradaR, Proksch P, Schmidt TJ, Klaes M, Verspohl EJ. (2007) Hypoglycaemic constituents of Stachytarpheta cayennensis leaf. 73 (3). pp. 241-250.

[33] Jude E. Okokon, EtteEttebong, and Bassey S. Antia, (2008). In vivo antimalarial activity of ethanolic leaf extract of Stachytarpheta cayennensis.Indian J Pharmacol. 40(3): 111–113. doi: 10.4103/0253-7613.42303.

[34] Tonaa L, Cimanga RK, Mesia K, Musuamba CT, De Bruyne T, Apers S, Hernans N, Van Miert S, Pieters L, Totte J, Vlietinc AJ, (2004). In vitro antiplasmodial activity of extracts and fractions from seven medicinal plants used in the Democratic Republic of Congo. J. Ethnopharm. P. 27– 32.

[35] Yenesew A, Induli M, Derese S, Midiwo JO, Heydenreich M, Peter MG, Akala H, Wangui J, LiyalaP, Waters NC, (2004). Anti-plasmodial flavonoids from the stem bark of Erythrinaabyssinica. Phytochemistry. 65:3029–32.

[36] Kraft C, JenettSiems K, Siems K, Jakupovic J, Mavi S, Bienzle U, Eich E, (2003). In vitro antiplasmodial evaluation of medicinal plants from Zimbabwe. Phyto. Res. ;17:123–8.

[37] FideleNtie-Kang, Pascal AmoaOnguéné, Lydia L Lifongo, Jean Claude Ndom, Wolfgang Sippl & Luc Meva'aMbaze (2014). The potential of anti-malarial compounds derived from African medicinal plants, part II: a pharmacological evaluation of non-alkaloids and non-terpenoids. Malaria journal.