AJPB 2021, Vol 3, No 2, 6-11
**DOI:** https://doi.org/10.54987/ajpb.v3i2.635

HIBISCUS PUBLISHER

ASIAN JOURNAL OF PLANT BIOLOGY

Website: http://journal.hibiscuspublisher.com/index.php/AJPB/index

AJPB VOL 3 NO 2 2021
*Artemisin-docked plasmepsin V*

# Anti-Protozoan Activities of *Stachytarpheta angustifolia* on Some Haematological Parameters in Wistar Rats

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**HISTORY**

Received: 4th Oct 2021
Received in revised form: 15th Nov 2021
Accepted: 15th Dec 2021

**KEYWORDS**

*Stachytarpheta angustifolia*
*E. tenella*
*Wistar Rats*
Anti-protozoa
Haematological

**ABSTRACT**

This study focuses on the anti-protozoan activities of *Stachytarpheta angustifolia* (Tarkajiya; Hausa, Devil's coach whip; English) on haematological parameters of Albino Wistar rats which is an unexplored study area. The work is aimed at the determination of the effects of S. angustifolia on Wistar Rats, when exposed to herbal extract on the haematological parameters of Wistar Rats infected with *E. tenella* Biomarkers. The plant was obtained whole; dried under the shade, made into a powdered form and aqueous extraction method carried by maceration technique. After infecting the experimental animals with the parasites; *E. tenella*, the following respective doses of 750 mg and 1500 mg were administered to the rats in groups of 3 and 4. Results obtained were analyzed using Analysis of Variance (ANOVA). It was discovered that no significant harmful effect on the rats was recorded, but 60 % of the parasites were killed. This work demonstrated that the herbal extract killed the parasites but induced minimal stress to the animals as shown by the low haematological parameters in the study.

## INTRODUCTION

Research on herbal medicine has been in existence since time immemorial and it is still ongoing. Further the knowledge that plants derived compounds could also serve as therapeutic weapons available to man to fight various ailments has made plants a sine qua non to lives. "Herbal remedies" are relied upon for the treatment of all sorts of diseases, recently. Many studies have suggested that flavonoids like rutin, kaempferoin, apigenin and so on, are well-known for its anti-inflammatory, anti-allergic, anti-thrombotic, hepato-protective, anti-spasmodic and anticancer properties [1].

Consequently, demand for the herbal formulation is increasing daily. As the half of the world suffering from bacterial and Helminthes infection, the source of infection being very common due to poor sanitation, poor family hygiene, malnutrition, and crowded living conditions [2].
The use of herbal medicine to treat and manage ailments continues unabatedly to date in most Nigerian communities as well as in the other developing countries. Its (medicinal plants) use is also increasingly relied on in the industrialized societies, which has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies. Plants have been used as medicine for millennia, and out of estimated 250,000 to 350,000 plant species identified so far, about 35,000 are used worldwide for medicinal purposes. It has been confirmed by WHO [3], that herbal remedies serve the health needs of about 80 percent of the world's population; especially for millions of people in the vast rural areas of under-developed countries.
Meanwhile, according to Kong [4] consumers in developed countries are becoming disillusioned with modern healthcare and are seeking alternatives. The recent resurgence of plant remedies results from several factors like: the effectiveness of plant medicines, the side effect of most modern drugs, and the development of science and technology. It has been estimated that in the mid-1990s over 200 companies and research organizations worldwide are screening plant and animal compounds for medicinal properties. Several drugs used in

modern medicine have come from medicinal plant studies, like taxol/paclitaxel, vinblastine, vincristine, topotecan, irinotecan, etoposide, teniposide, etc [4]. Consequently, attention is now focused in the exploration of herbal remedies as alternatives in the treatment of infectious diseases since pathogens have been found to develop multiple resistances to most of the currently used synthetic antibiotics [5].

Plants contain untapped reservoir of bioactive compounds that can be used directly as well as 'lead' compounds for synthetic compounds. These compounds have wide applications such as found in natural pesticides of plant origin which their active agents have been developed and are currently in use e.g. Calabar bean (*Physostigma venenisum*), used traditionally as an ideal poison from where methyl carbamate insecticides were developed; and pyrethrum insecticides from the flower of *Chrysanthemum cinerariaefolium* extract, which was discovered because of its local use to control insect pest [6].

The root of *Lonchocarpus* is a source of rotenone used as poison to stun fish [7]. This therefore points out that the whole parts of the plant; fruits, flowers, leaves, stems, bark and roots can be potential sources of active agents which can be employed for diverse uses. These parts of plant contain secondary metabolites known as phytochemicals of which extracts can contain active compounds that have potentials for use in the development of natural active products [8], [9], [10], and [11]. Herbal remedies have many traditional medicinal claims and are employed in the treatment of diseases of diverse origins.

According to Mian-Ying [12], herbal and natural products of folk medicine have been used for centuries in every culture throughout the world. Scientists and medical professionals have shown increased interest in this field as they recognize the true health benefits of these remedies. "Let food be your medicine and let medicine be your food" was advised by the father of medicine, Hippocrates, over two millennia ago. It's still true today that "you are what you eat."
Studies on the antibacterial activities of *S. angustifolia* have since been studied by authors such as [16], however, this work focused anti-protozoan activities of *S. angustifolia.*

***Stachytarpheta angustifolia***
*Stachytarpheta angustifolia* (**Fig. 1**) is a valuable medicinal plant and remedies prepared with it are used locally as anti-infection (antibacterial) agent. The leaves on squeezing produce a foamy juice and the local herbalists probably use it in the treatment of various diseases including sexually transmitted diseases (STDs) [13]. *S. angustifolia* is a seasonal weed growing along the banks of rivers and streams, constituting menace in the farmlands during the rainy seasons, in the southern parts of Nigeria. It is also popularly used by children and local farmers because of its foamy nature as soap for bathing in the locality where it is available.

The preliminary phytochemical analysis of the extract indicated positive results for polyphenols and triterpenoid saponins. For a plant or herbal preparation containing active organic principles to be identified for use in the traditional medicine, a systemic approach is required for the evaluation of efficacy and safety through experiment and clinical findings [14].

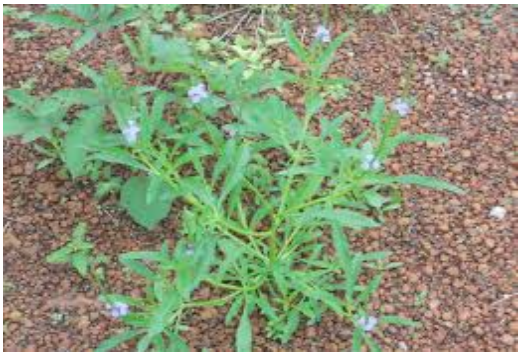

**Fig. 1.** Stachytarpheta angustifolia Vahl (picture culled from NRCSP Database USDA 2015)**.**

**Parasites and *Eimeria***
Parasitic protozoa affect animals and man causing considerable suffering and poor growth. Effective anti-protozoan drugs, used for treatment and the control of these infections, must therefore, have selective toxic effects on the parasites. Unfortunately, with the increased use of these compounds, anti-protozoan resistance has appeared and increased in frequency [15]. It is against this backdrop, that the researcher felt that there is a need for deeper study and research into herbal drugs, with a view to finding a more potent and cheaper anti-protozoan drug for use by the cmmon man. The aim of this study is to investigate the anti-protozoan activities of *Stachytarpheta angustifolia* on some haematological parameters in albino wistar rats. This can be obtained through the following objectives:
**a.** To determine the effects of *Starchytarphyta angustifolia on* Albino Wistar Rats infected with *Eimeria tenella*.
**b.** To evaluate the effect(s) of *S. angustifolia* on haematological parameters of Wistar albino rats infected with *Eimeria tenella.*
**c.** To evaluate the role of the crude extract on the rats' biological system(s).

The outcome of this study will provide baseline information to both governmental and non-governmental organizations which will be useful in the effective formulation and further control of parasitic protozoans' diseases. It will also provide the measures and strategies for control, prevention and reduction of this health problem when applied to human and animal populations after due re-assessments. The study is justified because so far from reviewed literatures, it reveals that, this work will be the first research of its kind conducted to evaluate the scientific basis for the activity of S*tachytarpheta angustifolia* as an anti-protozoan. However, study on both ethanolic and aqueous herbal extracts as an antibacterial has been done for a long time [7].

## MATERIALS AND METHODS

**Study area and periods**
The study area is Gombe State located in the Northeastern part of Nigeria. Plants were collected from Kwadon area of Gombe State. The plant was lodged and identified in the Biological Sciences Department, Taxonomy Unit via a voucher/ Herbarium number 102.

**Materials: Source of drugs and drying method**
Plants obtained from Kwadon area after drying were ground using pestle & mortar, and sieved into coarse particles and a giant electric blender was used to make it into a finely ground powder.

**Source of Experimental Animals**
a.Adult Wistar Rats (rattus norvegicus) of either sex were used 100 – 180 g
b.The rats were obtained and reared in the Department of Human physiology Animals' house at the Laboratory of the College of Medical Sciences Gombe State University.
c.They were housed in standard environmental condition of temperature, humidity and fed a fixed standard diet from vital feeds brand from Jos Plateau State Nigeria and were given water ad libidum.

**Extraction of plant material**
The extraction process was by the Maceration extraction procedure to form an aqueous crude extract.

**Experimental design and Animal treatment protocol**

**Animals' Grouping**
**Group I**; control group (passive control):- Animals were fed standard diet, they were not inoculated with parasites or feed crude herbal extracts.
**Group II**; (Active control):- Animals in this experimental group were inoculated with parasites only.
**Group III**; (Test 1):- The animals were inoculated with the parasite for same incubation period of 2 – 7 days and 750 mg of extract were administered.
**Group IV**; (Test 2):- Animals in this group were inoculated with the parasite for the incubation period and 1500 mg of the aqueous extract administered.
**Group V**; (Test 3):- Animals in this group were inoculated with parasites and were given a standard anti-protozoan drug and the animals were monitored for the same incubation period.

After induction of the parasites, the animals were under constant observation for the first 30 minutes, 1 hour, 4 hours, 12 hours, 24 hours, 3 days, 7 days and subsequently on the eighth day of the incubation period, the experimental animals were observed for the presence of the parasites in their droppings (stool microscopy) and by observing some clinical signs and symptoms of the disease process. And blood samples were collected for Haematological analysis. And the animals were then induced with the crude herbal extract orally via gastric intubation and later on, each individual animal was studied for the presence of the parasite by microscopic analysis in each individual's droppings and the rats were euthanised for blood and organs extraction.

## RESULTS

**Phytochemical Analysis of crude plant extract:**

**Qualitative Analysis**
Preliminary phytochemical screening tests showed the presence of Alkaloids, Saponins, Tannins, Flavonoids, Anthraquinones, Steroids positive, Glycosides, Phlobotannins, and Resins (**Table 1**).

**Quantitative Analysis**
The quantitative analysis of the crude extract showed that it contains some amounts of alkaloids, saponins, flavonoids, anthraquinones, steroids, glycosides, phlobotannins, but tannins and resins were proved to be negative as can be seen on **Table 2**.

**Reaction of Rats after induction with crude herbal extracts ($LD_{50}$)**
**Table 3** shows the relative doses of the extract administered to the three groups of rats in this experimental $LD_{50}$ group, the extracts were administered in ascending doses from 750 mg for all the members of first group of rats, 1000 mg of the extract was served to group two rats, while, 1500 mg was given to the rats in group three.

**Table 1.** Qualitative analysis of crude plants extracts.

| S/N | Test | Observation | Inference |
|---|---|---|---|
| i. | 0.5g Crude Extract + 5ml 1% (aq) dil. HCl + Dragengoff's Reagent + Heat for 5mins. | Yellow Precipitate formed | Alkaloids are positive (++) |
| ii. | 0.5g Crude Extract + 5ml Distilled Water + Shake vigorously + warm mixture at $50^0$C for 10 minutes. | Foamy frothy Solution formed | Saponins are Positive (+) |
| iii. | 5g Crude Extract + 10ml Distilled Water + Stir + Filtering with Whatman filter paper + 2-3ml Ferric Chloride gradually to filtrate | Sample remained yellowish | Tannin is Negative (-ve) |
| iv. | 0.5g Crude Extract + 2ml NaOH + Conc. $H_2SO_4$ | Solution remained unchanged | Indication Flavonoids are positive (+) |
| v. | 0.5g Crude Extract + 10ml Dil. $H_2SO_4$ + Boil + Filter with Whatman paper + 5ml ether to filtrate + shake + stand until it separates, then add Ammoniacal Solution | Green flouroscent colour appears | Anthraquinones are positive (+) |
| vi. | 0.5g Crude Extract + Chloroform + $H_2SO_4$ | A red Layer formed | Steroids are positive (+) |
| vii. | 0.5g Crude Extract + $H_2O$ + Heat + HCl + Benedicts Solution | Brown Precipitate formed | Glycosides are positive (+) |
| viii. | 0.5g Crude Extract + $H_2O$ 1% HCl + Heat | A reddish precipitate formed | Phlobotannins are positive (++) |
| ix. | 2ml Crud Extract solution(ii) + 2ml Acetic Acid + Conc. $H_2SO_4$ | No change in colour | Presence of Resins is Negative (-) |

**Table 2.** Quantitative analysis of plant extracts.

| Substance | Amount |
|---|---|
| Alkaloids | ++ |
| Saponins | + |
| Anthraquinones | + |
| Steroids | + |
| Glycosides | ++ |
| Phlobotannins | ++ |
| Tannins | - |
| Resins | - |

**Table 3.** Reaction of rats after induction with crude herbal extracts in $LD_{50}$ group.

| | | Time for Reaction | | | | |
|---|---|---|---|---|---|---|
| Rat Group | Dose | 30 min | 1 hour | 4 hours | 8 hours | 24 hours |
| Group 1 | 750mg | Normal activities | Normal activities | Normal activities | Normal activities | Normal activities |
| Group 2 | 1000mg | Slowed activity, feeding Slowly | Purring of hairs | One Rat died | Others were normal | Others were normal |
| Group 3 | 1500mg | Wrighting, not feeding and purring | Started feeding slowly with weak activities | Very weak and slow reaction | Weak but feeding all Rats died | Nil and |

**Hematological Analysis Results**
There is no significant difference ($P > 0.05$) in all the values between group 1(control) with all the other groups. This means the extract and or standard drug (Metronidazole) administered to the rats did not significantly damage their respective liver cells to distort its physiology (**Table 4**).

**Hematological Parameters in Relation to the Groups**
**Table 5** shows the mean values for WBC, NEU, LYM, and MON values for all the groups one to five (1 – 5), except in the Neutrophils' column where groups 3 and 5 indicated significant increase in the number of Neutrophils up to 2 folds. This may likely be as a result of increased infection rate or higher response to the presence of parasites in the two groups

### Hematological parameters in relation to the groups

**Table 6** shows the Hematological parameters for Eos, Bas, Rbcs, and Hb, these results indicate no significant difference in almost all the values except for Basophils in group four (4) and may also not be unconnected with the rat's increased response to the presence of parasites in their system. Otherwise, all other values showed no significant difference.

**Table 4.** Haematological parameters analysis.

| | Passive control | Active Control | Test 7500mg | 1; Test 1500mg | 2; Standard drug |
|---|---|---|---|---|---|
| RBC | 9.20 ± 0.56 | 8.64 ± 0.51 | 8.23 ± 1.05 | 9.47 ± 0.25 | 8.61 ± 1.19 |
| HGB | 16.17 ± 0.57 | 14.93 ± 0.71 | 14.20 ± 0.87 | 15.77 ± 0.55 | 14.40 ± 1.70 |
| HCT | 53.60 ± 2.95 | 52.33 ± 2.08 | 56.23 ± 4.53 | 60.33 ± 4.16 | 49.83 ± 5.06 |
| MCV | 58.17 ± 1.55 | 60.83 ± 1.37 | 69.40 ± 2.60 | 63.93 ± 2.48 | 58.07 ± 4.56 |
| MCHC | 30.27 ± 0.70 | 28.40 ± 0.70 | 25.30 ± 0.52 | 26.07 ± 0.81 | 28.93 ± 1.46 |
| WBC | 8.08±0.67 | 8.75±0.70 | 11.28±2.54 | 10.80±2.10 | 9.52±3.68 |
| Eosin-ophils | 0.16 ± 0.10 | 0.62 ± 0.32 | 0.35 ± 0.23 | 0.76 ± 0.13 | 0.42 ± 0.21 |
| Baso-phils | 0.22 ± 0.14 | 0.35 ± 0.21 | 0.45 ± 0.22 | 1.41 ± 0.25 | 0.80 ± 0.53 |
| Neut-rophils | 1.18±0.24 | 1.69±0.33 | 3.79±1.33 | 15.75±15.28 | 28.00±22.34 |
| Lymp-hocyte | 5.85±0.57 | 6.60±1.91 | 7.74±3.17 | 7.49±2.88 | 6.70±2.95 |
| MCHC | 30.27±0.70 | 28.40±0.70 | 25.30±0.52 | 26.07±0.81 | 28.93±1.46 |
| Plat-elets | 889.00 ± 218.89* | ± 839.00 426.82 | ± 754.67 49.07 | ± 827.00 35.59 | ± 624.67 ± 274.14* |

**Table 5.** Haematological parameters in relation to the variables per $X10^3/\mu$.

| Group | White blood cells | Neutrophils | Lymphocytes | Monocytes |
|---|---|---|---|---|
| 1 | 8.067 | 1.180 | 5.853 | 0.637 |
| 2 | 8.747 | 1.687 | 6.600 | 0.853 |
| 3 | 11.277 | 3.787* | 7.743 | 0.827 |
| 4 | 10.803 | 1.575 | 7.490 | 0.967 |
| 5 | 9.520 | 2.800* | 6.697 | 1.423 |

**Table 6.** Haematological parameters in relation to the variables per $X10^3/\mu L$.

| Group | Eosinophils | Basophils | Red Blood Cells | Haemoglobins |
|---|---|---|---|---|
| 1 | 0.157 | 0.220 | 9.197 | 16.167 |
| 2 | 0.620 | 0.347 | 8.640 | 14.933 |
| 3 | 0.350 | 0.447 | 8.233 | 14.200 |
| 4 | 0.763 | 1.410* | 9.467 | 15.767 |
| 5 | 0.417 | 0.797 | 8.613 | 14.400 |

### Hematological Parameters in Relation to the Groups

Table 7 shows the hematological mean values for MCV, HCT, MCHC, and PLT for the rats in group one to five (1 - 5) and the results showed no significant difference across all the groups. This indicated that there was no significant difference in the Blood cells' Mean corpuscular volume, concentration and the platelets volume.

**Table 7.** Haematological parameters in relation to the variables per $X10^3/\mu$.

| Group | MCV | HCT | MCHC | Platelets |
|---|---|---|---|---|
| 1 | 58.167 | 53.467 | 30.267 | 889.000 |
| 2 | 60.833 | 52.533 | 28.400 | 839.000 |
| 3 | 69.400 | 56.133 | 25.300 | 754.667 |
| 4 | 63.933 | 60.533 | 26.067 | 827.000 |
| 5 | 58.067 | 49.800 | 28.967 | 624.667 |

Note: NB:- MCV; Mean corpuscular volume; HCT; Haematocrit concentration test; MCHC; Mean corpuscular haemoglobin concentration.

### Lethal Dose ($LD_{50}$) Rats Group

Table 8 shows the Histology result; liver and kidney morphology report of the $LD_{50}$ rats in groups 3, 4 and 5, which indicated marked sinusoidal congestion and areas of centrilobular necrosis and steatosis. Group 2 rats showed mild steatosis, which is the abnormal retention of lipids within cells and there was marked centrilobular congestion in the group that is; groups 1 and 2. The result of the $LD_{50}$ indicated that at high concentrations the extract is quite harmful especially, on liver tissues.

**Table 8.** Lethal dose ($LD_{50}$) rats group.

| Rat group | Liver | Kidney |
|---|---|---|
| Grp 1 Rat 1 | Congestion | Necrosed |
| Grp 1 Rat 2 | Congestion | Necrosed |
| Grp 1 Rat 3 | Steatosis | Necrosed |
| Grp 2 Rat 1 | Steatosis | Necrosed |
| Grp 2 Rat 2 | Steatosis | Normal |
| Grp 2 Rat 3 | Steatosis | Necrosed |
| Grp 3 Rat 1 | Steatosis | Necrosed |
| Grp 3 Rat 2 | Steatosis | Necrosed |
| Grp 3 Rat 3 | Steatosis | Necrosed |

## DISCUSSION

The crude extract of *S. angustifolia* vahl was analyzed using qualitative and quantitative phytochemical methods and was found to contain the following bioactive components; alkaloids ++, saponnins +, anthraquinones +, steroids +, glycosides ++, phlobotannins ++, flavonoids -, and tannins. The Histology report has shown that *Stachytarpheta angustifolia* vahl has slightly less lethal effects on the liver and kidney tissues when administered in small doses, but when administered in higher doses it is deleterious and harmful to biological system, because there is marked centrilobular congestion in group 2 and 3. Also rat 2 in group 5 showed mild steatosis, which is the abnormal retention of lipids within liver cells. Furthermore, marked sinusoidal congestion and areas of centrilobular necrosis and steatosis were observed in the $LD_{50}$ group because of their exposure to high doses of crude extracts; 1000 – 1500 mg.

*Stachytarpheta angustifolia* were reported to be inactive, having shown to produce IC50 values greater than 50 µg/mL when tested on plasmodium sp [44]. A similar study has shown that extracts of *Stachytarpheta angustifolia* revealed remarkable total antioxidant capacities of upto (>800 mg/g AAE) [44]. Noteworthy is extracts of *Stachytarpheta angustifolia* have been reported to possess antibacterial, antioxidant, immunomodulatory, and antidiabetic properties [45, 46]. Although no study has reported on the activity of *Stachytarpheta angustifolia* on *Eimeriasis*; this is in other words called; coccidiosis while coccidia can infect a wide variety of animals, including humans, birds, and livestock, they are usually species-specific. One well-known exception is toxoplasmosis caused by *Toxoplasma gondii* [8].

## CONCLUSION

The research is work a positive indicator pointing to the fact that in the nearest future, cheaper and more readily available method(s) of parasitic control for both humans and domestic animals from the local community would abound and that the herbal extract *Stachytarpheta angustifolia* is a potential beneficial herb that can fill the existing gap between the highly expensive synthetic drugs and the relatively cheap herbal extracts. There is need for further phytochemical study and characterization of the herbal plant extract to itemize the various

bioactive components which are found in the roots, stem, and leaves of the plant. Therefore, the study on *Stachytarpheta angustifolia* should be further taken in a more advanced level to isolate the primary and secondary bioactive components responsible for its anti-parasitic and antibiotic properties. Governmental and Non-Governmental Donor agencies should get involved in the funding of further advanced study on the herbal plant extract.

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