

**Preliminary Phytochemical Screening of *Spilanthes uliginosa*,
Ocimum basilicum, *Hyptis spicigera* and *Cymbopogon citratus* leaf
extracts and Haematological Changes of Mice Infected with Malaria Parasite**

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Abstract: Plants have been an alternative remedy in prevention and treatment of many diseases globally. Malaria is a life-threatening infectious disease that remains a major global health problem. Preliminary phytochemical screening of *Spilanthes uliginosa*, *Ocimum basilicum*, *Hyptis spicigera* and *Cymbopogon citratus* leaf extracts and haematological changes of mice infected with malaria parasite were investigated. The percentage yield of the leaf extracts showed that the results in the order of *Ocimum basilicum*, *Hyptis spicigera*, *Cymbopogon citratus* and *Spilanthes uliginosa* with 17.33%, 15.33%, 14.00% and 10.67% respectively. The result of phytochemical screening revealed the presence of alkaloids, glycosides, phenols, saponins, flavonoids and tannins with absence of steroids. The results also indicated a dose-dependent significant reductions ($P < 0.05$) in packed cell volume (PCV) in parasitized treated mice. Dose-dependent significant elevations ($P < 0.05$) were observed in hemoglobin levels and white blood cell (WBC) counts of parasitized treated mice.

Key words: *Spilanthes uliginosa* • *Ocimum basilicum* • *Hyptis spicigera* • *Cymbopogon citratus* • Malaria

INTRODUCTION

The parasite plasmodia are the major cause of infectious disease malaria. World malaria report stated that malaria remained one of the major public problems in Sub-Sahara Africa [1]. The groups of people mainly at risk of this infection are children under the age of five years and pregnant women [2]. Over 729 children per 100,000 children less than five years in Nigeria die annually, the disease is prevalent in tropical and sub-tropical regions and is a major obstacle to economic advancement of the regions posing people to poverty and disease [3]. Malaria is one of the leading contributors to the unacceptably high maternal mortality rate in the world today especially in developing countries. In pregnant women, malaria often results in anemia and eventually delivery of low-birth-weight babies as a result of impaired nutrient transport across the placenta. Other complications of malaria in pregnancy include delivery of preterm babies, spontaneous abortion and early neonatal deaths among others.

The liver is an important organ involved during the hepatic stage of the malaria parasite's life cycle,

where malaria sporozoites develop into merozoites. The merozoites are then released into the circulation and enter the erythrocytic stage. In the erythrocytic stage, parasitized red blood cells (PRBCs) become sequestered in small blood vessels. The degraded haemozoin pigment is then engulfed by local tissue macrophages, such as Kupffer cells and alveolar macrophages. Common histopathological findings of the liver in *P. falciparum* malaria include reactive Kupffer cells, retention of haemozoin pigment and minimal PRBC sequestration [4-5]. Malaria infection has been shown to cause severe damage to RBC since the parasites derived its nutrients from hemoglobin [6] thereby causing loss of blood quality [7-9].

This study evaluated the Packed Cell Volume, Hemoglobin and White Blood Cell levels of mice with severe plasmodium berghei malaria.

MATERIALS AND METHODS

Collection and Preparation of *Spilanthes Uliginosa* (Sw) Leaf Extract: Whole plant of *Spilanthes uliginosa* (Sw) was collected from Ogboji-Agoutu Ezzagu in Inyaba

Development Centre of Ebonyi State, Nigeria. Dr (Mrs) Nnamani, K. of the Department of Applied Biology of Ebonyi State University, Abakaliki graciously identified the plants. Apparently healthy leaf of the plant was removed from plant stalk, rinsed with clean water and shade dried to a constant weight. The dried plant sample was ground to fine powder with grinding machine, packaged in glass jars and stored at 4°C until analysis.

Extraction of Plant Materials: Exactly 150 g of powder samples of *Spilanthes uliginosa*, *Ocimum basilicum*, *Hyptis spicigera* and *Cymbopogon citratus* were soaked in 500 ml of ethanol each for 24h. They were filtered into a graduated beaker and exposed to mild heat at 40°C in water bath until a semi solid extracts were obtained.

Preliminary Phytochemical Screening

Extracts Preparation: The leaves of the plants; *Spilanthes uliginosa* (Sw), *Ocimum basilicum*, *Hyptis spicigera* and *Cymbopogon citratus* were shade dried at room temperature and reduced to a coarse powder in a mill. The powder was extracted with absolute ethanol. The extracts were recovered by distillation under reduced pressure at 49°C in a Buchi rotavapour. The extracts were then dried to solid forms in vacuum dessicator and used for qualitative analysis before quantitative analyses were carried out.

- Concentrations of Alkaloids Were Determined Using (Harborne, 1973) [7].
- Glycosides were analyzed using (Okwu and Joshia, 2006) [8].
- Concentrations of Phenols Were Determined Using (Amin *et al.*, 2013) [9].
- Saponins were analyzed using (Amin *et al.*, 2013) [9].
- Flavonoids were determined using (Boham and Kocipai, 1994) [10].
- Tannins were determined using (Amin *et al.*, 2013) [9].
- Steroids were analyzed using Sofowara, 1993) [10].

Experimental Animals: Thirty Swizz albino mice aged 2 months weighing 17-34 g of both sexes were obtained from Chris King Animal Farm of Nnamdi Azikiwe University Awka, Anambra State and transferred to Animal House of Department of Biochemistry, Ebonyi State University, Abakalki. The animals were

housed in metal cages under controlled conditions and acclimatized for 7 days under standard environment conditions and fed *ad-libitum* on their normal diets.

Rodent Parasite (*Plasmodium Berghei* NK65):

The rodent parasite was sourced from National Institute for Medical Research (NIMR), Lagos, Nigeria and maintained alive in mice by continuous intraperitoneal passage in mice after every 5 days. The re infected mice were moved to the Animal House of Department of Biochemistry, Faculty of Biological Science, Ebonyi State University Abakaliki where the study was carried out. Prior to the start of the study, one of the infected mice was kept and observed to reproduce signs of diseases similar to human malarial infection.

Inoculation of Animals: The mice were infected with parasites consisting of 1×10^7 of *P. berghei* parasitized erythrocytes per ml. This was carried out by determining both the percentage parasitaemia and erythrocytes count of the donor mouse and diluting the blood with phosphate buffer saline pH 7.4 in proportions indicated by both determinations. Each mouse was inoculated intraperitoneally on day 0 with 0.2 ml of infected blood containing 1×10^7 *P. berghei* parasitized red blood cells. Parasitaemia was assessed by thin blood film made by collecting blood from the cut tip of the tail and this was stained with Geimsa stain (Odeghe *et al.*, 2012).

Experimental Design: The mice were injected intraperitoneally with standard inoculums of 1×10^7 *P. berghei* NK 65 infected erythrocytes on the first day. Seventy two hours later, the mice were divided into 5 groups namely; A, B, C, D, E and F of 6 mice each. Groups B, C, D and E were subdivided into three (3): B₁, B₂, B₃, C₁, C₂, C₃, D₁, D₂, D₃, E₁, E₂ and E₃. The subgroups were treated with the extracts of *Spilanthes uliginosa* (Sw), *Ocimum basilicum*, *Hyptis spicigera* and *Cymbopogon citratus* each for five (5) consecutive days with 200, 400 and 800 mg/kg body weight via oral intubation daily respectively. Two control groups, A and F were used. The negative control (A) was treated daily with 5 ml/kg normal saline while positive control group (F) was treated with 5 mg/kg body weight of chloroquine. All groups were given water and fed *ad libitum*. On the sixth day, mice were starved overnight, sacrificed and liver as well as bloods were collected for histological and haematological analysis.

Statistics: The results obtained were expressed as mean \pm standard deviation of mean (SDE) for six times determination. A one – way analysis of variance (ANOVA) for a completely randomized design and Duncan's multiple range tests were used to analyze experimental data. Values were considered significant at $P < 0.05$.

RESULTS

Results of Percentage Yields of the Ethanol Leaf Extracts:

The percentage yields of ethanol leaf extracts of *Spilanthes uliginosa* (Sw), *Ocimum basilicum*, *Hyptis spicigera* and *Cymbopogon citratus* leaves were shown in Table 1.

Results of Phytochemical Contents of Plant Leaves: The results indicated that all the plant leaves contained alkaloids, glycosides, phenolics, saponins, flavonoids and tannins while steriods were absent in all the plant leaves.

Results of the Effects of Ethanol Leaf Extract on the Haematological, Packed Cell Volume and White Blood Cell Profile of the Treated and Untreated Mice: The Packed Cell Volume (PCV) values, Hemoglobin (Hb) levels and White Blood Cell (WBC) counts of the treated animals are shown in Figures 1, 2 and 3.

The results of PCV values for the treated and untreated mice were shown in Figure 1. The results showed significant reductions ($P < 0.05$) in the level of PCV for all the extracts at all the varying doses when compared to the negative control. There were no significant difference ($P > 0.05$) between the effects of the standard drug and the extract of *S. uliginosa* (Sw), *H. spicigera* and *C. citratus* at dosages of 400 and 800 mg/kg. However, there were marked significant differences ($P < 0.05$) between the effects of the standard drug and the extracts of *O. basilicum* at all dosages of *S. uliginosa* (Sw), *H. spicigera* and *C. citratus* 200 mg/kg. The extracts of *S. uliginosa* (Sw), *H. spicigera* and *C. citratus* at dosages of 400 and 800 mg/kg had no significant differences when compared with each other while *O. basilicum* had significant ($P < 0.05$) changes at all doses used when compared to each other.

The results of hemoglobin levels for the treated and untreated mice are shown in Figure 2. The results showed significant elevation ($P < 0.05$) in the level of Hb for the extracts of *O. basilicum*, *H. spicigera* and *C. citratus* at all

Table 1: Percentage Yields of the Leaf Extracts

Plant Leaves	Percentage Yields (%)
<i>Spilanthes uliginosa</i> (Sw)	10.67
<i>Ocimum basilicum</i>	17.33
<i>Hyptis spicigera</i>	15.33
<i>Cymbopogon citratus</i>	14.00

the varying doses except *S. uliginosa* (Sw) at 200 when compared to negative control. There were no significant differences ($P > 0.05$) between the effects of the standard drug and the extracts of *S. uliginosa* (Sw) at only 200 mg/kg and of *O. basilicum*, *H. spicigera* and *C. citratus* at all doses used. The extracts of *S. uliginosa* (Sw) and *O. basilicum* at all doses used caused a significant difference when compared to each other while that of *H. spicigera* and *C. citratus* at 200 and 400 mg/kg had no significant differences ($P > 0.05$).

The results of WBC counts for the treated and untreated mice are shown in Figure 20. The results showed that there are significant elevation ($P < 0.05$) in the WBC count for all the extracts of *S. uliginosa* (Sw) and *C. citratus* at all varying doses used except the extracts of *O. basilicum* at 200 and 400 mg/kg and that of *H. spicigera* at 200 mg/kg when compared to negative control. There was no significant difference ($P > 0.05$) between the effects of the standard drug and the extracts of *O. basilicum* at only 800 mg/kg and *H. spicigera* at only 200 mg/kg while the extracts of *S. uliginosa* (Sw) and *C. citratus* caused significant difference ($P < 0.05$) at all given doses. The extracts of *S. uliginosa* (Sw) and *O. basilicum* at 200 and 400 mg/kg and that of *H. spicigera* at 400 and 800 mg/kg did not show any significant difference ($P > 0.05$) in WBC counts when compared to each other. However, the extract of *C. citratus* at all given doses caused a significant difference ($P < 0.05$) when compared to other doses.

DISCUSSION

The percentage yield of the organic extract for all the plants (Table 1) compare favourably with that reported for other medicinal plants in Nigeria [11-16]. The differences in the percentage yields of these plant materials could be attributed to oil constituents of the different plant leaves. Similar findings have been documented by Offor, (2011) [16]. WHO (2005) [17] reported that active ingredient in plants can dissolve in solvents such as ethanol and water etc and that these dissolved constituents may be some of the active ingredients present in the plant materials that are used in the treatment and prevention of diseases.

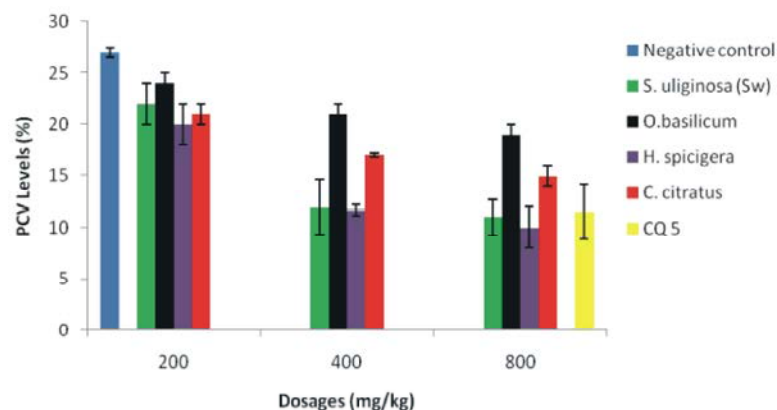


Fig. 1: The results of PCV values (%) of the treated and untreated mice. Bars bearing the same letters (dosage by dosage for each plant) are not significantly different from each other ($P < 0.05$)

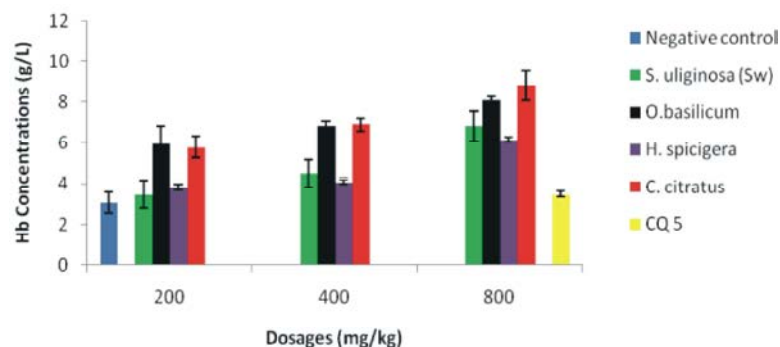


Fig. 2: The results of hemoglobin levels (g/l) of the treated and untreated mice. Bars bearing the same letters (dosage by dosage for each plant) are not significantly different from each other ($P < 0.05$)

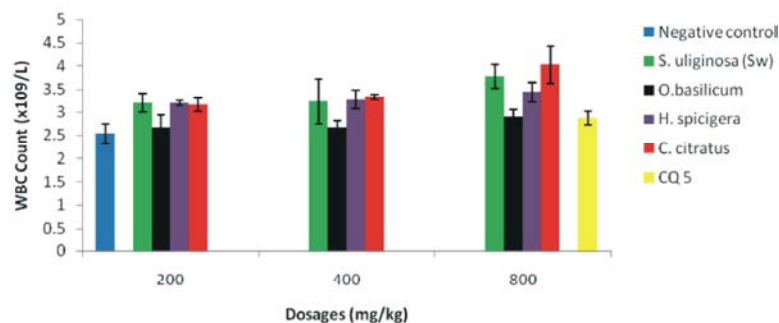


Fig. 3: The results of WBC counts ($\times 10^9/L$) of the treated and untreated mice. Bars bearing the same letters (dosage by dosage for each plant) are not significantly different from each other ($P < 0.05$)

Table 2: Results of phytochemical screening of ethanol extracts of *Spilanthes uliginosa* (Sw), *Ocimum basilicum*, *Hyptis spicigera* and *Cymbopogon citratus*.

Phytochemicals	<i>S. uliginosa</i> (Sw)	<i>O. basilicum</i>	<i>H. spicigera</i>	<i>C. citratus</i>
Alkaloids	+	+	+	+
Glucosides	+	+	+	+
Phenolics	+	+	+	+
Saponins	+	+	+	+
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Steroid	Nd	Nd	Nd	Nd

Nd = Not detected, + = present

The preliminary phytochemical screening of leaves of *Spilanthes uliginosa* (Sw), *Ocimum basilicum*, *Hyptis spicigera* and *Cymbopogon citratus* revealed the presence of alkaloids, glycosides, phenols, saponins, flavonoids and tannins in variable amount and absence of steroid. The results disagree with the report of Omeri *et al.* (2011) [17] who reported that the extracts of *Hyptis spicigera* contained steroids. The results (Figure 1) indicated that the leaf extracts contain appreciable high levels of numerous phytochemicals and this confirms the ethnomedical relevance of the plants in their use for the management and treatment of diseases. The differences in the phytochemical constituent of the plants could be attributed to differences in species variation (Dean, 2002) [17-19]. The presence of these phytochemicals showed high level of their possible medicinal and dietary values. Although, some of these analyzed constituents of the leaves may be completely harmful to both man and farm animals and some are species specific as observed in the case of tannins and glucosides (Ajah *et al.*, 2010) [20]. Some of these active components have been demonstrated to possess anti-nutritional effects, following their ability to reduce palatability and digestibility of feedstuff (Idorenyin *et al.*, 2013) [18].

There was a general increase in physical activities of the mice treated with the extracts when compared with the parasitized untreated group. The results showed that the physical activities of the extracts-treated mice were better (more active) and this could be due to ameliorating effect of the plant extracts in malaria infection. The observed effect of the extracts may be attributed to some chemical components of the extracts such as alkaloids and saponins which have bactericidal and antispasmodic effects as well as antioxidant compounds which help to protect the animals against the damaging effects of reactive oxygen species imposed by malaria parasites [19-22]. This result disagrees with the report of Gregor (1997) [23] who reported that the ethanol extract of *Ocimum basilicum* within one week of its administration caused a general decrease in physical activities of the animals. The reason(s) responsible for the differences are not clear but may suggest differences in species variation and locations.

Decrease in PCV levels below normal values in the treated groups suggested anemia and reduced oxygen carrying capacity (Figure 1). The results therefore, strongly suggest that the extracts could be toxic in mice especially if the animals are exposed to the extracts for long period of time. Decreased serum hemoglobin levels in the parasitized untreated group (Figure 2) may be due

to increased degradation of hemoglobin by the parasite to provide nutrients needed to sustain its growth and multiplication [24]. An increase in the level of WBC in the treated groups was observed (Figure 3) and this observation showed the principal function of phagocytosis which is to defend against invading micro-organisms by ingesting and destroying them, thus contributing to cellular inflammatory processes will be enhanced (Adedapo *et al.*, 2005) which may account for its antibacterial activity [25].

REFERENCES

1. Tripathi, K.D., 2013. Essentials of medical pharmacology. 7th Edition, Jaypee Brothers Medical Publishers (P) Ltd. New Delhi, pp: 816-835.
2. Uraku, A.J., A.N.C. Okaka, U.A. Ibiam, K.N. Agbafor, N.A. Obasi, P.M. Ibiam Ajah, O.U. Obasi and F.N. Nwalo, 2015. Antiplasmodial Activity of Ethanolic Leaf Extracts of *Spilanthes uliginosa*, *Ocimum basilicum* (Sweet Basil), *Hyptis Spicigera* and *Cymbopogon citratus* on Mice Exposed to *Plasmodium berghei* Nk 65. International Journal of Biochemistry Research and Review, 6(1): 28-36.
3. Ugwu P.C. Okechukwu, Nwodo F.C. Okwesili, Joshua E. Parker, Odo E. Christian, Bawa Abubakar, Ossai, C. Emmanuel and Adonu C. Cyril, 2013. Anti-malaria and Hematological Analyses of Ethanol Extract of *Moringa oleifera* Leaf on Malaria Infected Mice. International Journal of Pharmacy and Biological Sciences, 3(1): 360-371.
4. Uraku, A.J., A.N.C. Okaka, U.A. Ibiam, K.N. Agbafor, C. Okoye and M.E. Ogbanshi, 2015. Use of Nigerian Medicinal Plants Protected Liver from Injury in *Plasmodium berghei* Infected Mice. British Journal of Medicine and Medical Research, 6(9): 926-934.
5. Sowjanya, M., K.K. Kalyan and K. Sunita, 2013. Assessment of biochemical variations and splenomegaly during *Falciparum* malaria in mice model. Bioscan, 8(3): 925-929.
6. Haruna, Y., H.O. Kwanashie, J.A. Anuka, S.E. Atawodi and I.M. Hussaimi, 2013. *In-vivo* anti-malarial activity of methanol root extract of *Securidaca longepedunculata* in mice infected with *Plasmodium berghei*. International Journal of Modern Biology and Medicine, 3(1): 7-16.
7. Harborne, J.B., 1998. Textbook of Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. 5th Edition. Chapman and Hall Ltd., London, pp: 21-72.

8. Okwu, D.E. and C. Josiah, 2006. Evaluation of the chemical composition of two Nigerian medicinal plants. *African Journal of Biotechnology*, 5(4): 357-36.
9. Amin, M.M., S.S. Sawhney, M.M.S. Jassal, 2013. Qualitative and quantitative analysis of phytochemicals of *Taraxacum officinale*. *Wudpecker Journal of Pharmacy and Pharmacology*, 2(1): 001-005.
10. Boham, A.B. and A.C. Kocipai, 1994. Flavonoid and condensed tannins from leaves of *Hawaiian vaccinium* and *Vicalycinium vaticulum*. *Pacific Science*, 48: 458-463.
11. Sofowora, A., 1993. Medicinal Plants and Transitional Medicine of Africa. Spectrum Book Ltd., Ibadan, pp: 1-37.
12. Sofowora, E.A., 2004. Medicinal Plant in Africa. 2nd Edition. John and Wiley and Sons Ltd. New York, pp: 54.
13. Odeghe, O.B., A.A. Uwakwe and C.C. Monago, 2012. Antiplasmodial activity of methanolic stem bark extract of *Anthocleista grandiflora* in mice. *International Journal of Applied Science and Technology*, 2(4): 142-148.
14. Ilekwe, A.S., 2000. Biochemical techniques. 5th Edition, Onakib Ventures, Nigeria, pp: 122-125.
15. Ayadin, E.J., K.J. Fahrettin, K. Hulusi, T. Yalcyn and U. Muzzaffer, 2003. Perspective of natural products in medicine. *Journal of Pharmacology*, 45: 127-135.
16. Offor, C.E., 2011. Comparative biochemical and toxicological studies on the Effects of leaves extracts of *Vernonia amygdalina* and *Azadirachta indica* on albino rats. P.hD thesis submitted to the Department of Biochemistry, Ebonyi State University, Abakaliki, pp: 48-60.
17. WHO, 2005. Principles of traditional medicine. 9th Edition, American Press, New York, pp: 124-128.
18. Omori, E.O., M.M. Richard, A. Lawrence and O.O. Paul, 2011. Evaluation of methanolic extracts of six medicinal plants used by herbal practitioners in central province-Kenya. *International Journal of Pharmaceutical Sciences and Research*, 2(4): 867-874.
19. Dean, G.S., 2002. Physiological significance of flavanol glycosides. *Journal of Biological Science*, 30: 24-29.
20. Aja, P.M., A.N.C. Okaka, P. Onu, U. Ibiam and A.J. Uraku, 2010. Phytochemical composition of *Talinum triangulare* (water leaf) leaves. *Pakistan Journal of Nutrition*, 9(6): 527-530.
21. Idorenyin, U., O. Samson, B. Eno-obong and N. Ukeme, 2013. Chronic consumption of combined Extracts of *Abelmoschus esculentus* and *Piper guineense* induced hepatotoxicity in wistar rats: histopathological study. *International Journal Pharmacology and Biomedical Science*, 4(2): 73-77.
22. Achuba, F.I., 2005. Effect of vitamins C and E intake on blood lipid concentration, lipid peroxidation, superoxide dismutase and catalase activities in rabbit fed petroleum contaminated diet. *Pakistan Journal of Nutrition*, 4(5): 330-335.
23. Gregor, F.T., 1997. Effect of traditional medicines on liver enzymes. *Journal of Traditional Medicines*, 25: 317-324.
24. Adedapo, A.A., A.Y. Adegbayibi and B.O. Emikpe, 2005. Some clinico-pathological changes associated with the extract of the leaves of *Phyllanthus amarus* in rats. *Phytotherapeutic Research*, 19: 971-976.
25. Adedapo, A.A., O.A. Omoloye and G. Ohore, 2007. Studies on the toxicity of an aqueous extract of the leaves of *Abrus precatorius* in rats. *Onderstepoort Journal of Veterinary Research*, 74: 31-36.