RESEARCH ARTICLE

PHYTOCHEMICAL ANALYSIS OF HYPTIS SUAVEOLENS EXTRACT AND ITS EFFECTS ON THE GROWTH OF DISEASE-CAUSING PARASITES

Racheal M. Agenyi
Department of Chemistry, Kogi State College of Education, Ankpa, Kogi State, Nigeria.

Plants extracts contain many essential natural synthesized chemical compounds with considerable potentials for medicinal exploitation and application. There has been a growing concern about the adverse effects of mosquito repellants and the need to search for natural and environment-friendly Mosquito repellants. Synthetic insecticides and their associated toxicity issues and the growing incidence of insect resistance have inspired novel insecticides. The present study analyzed the phytochemical extract of *H. Suaveolens* its effect on mosquito pupa. A total number of pupa (320) was poured into a 200ml glass beaker; twenty pupae each per three hours was introduced into a glass beaker containing different extract concentrations (ppm) of *Hyptis suaveolens*. Twenty pupae were introduced in contrast to a glass beaker containing 100ml of distilled water treated with 1ml of acetone used as treated control. Twenty pupae were introduced into 100ml of distilled water and used as untreated control. The result reveals that *Hyptis suaveolens* possesses inhibitory activity against *Anopheles gambiae*.

**Manuscript Info**

**Received:** 20 March 2021  
**Final Accepted:** 24 April 2021  
**Published:** May 2021

**Key words:** -  
H. Suaveolens, Mosquito, Pupa, Phytochemical

**Abstract**

Plants extracts contain many essential natural synthesized chemical compounds with considerable potentials for medicinal exploitation and application. There has been a growing concern about the adverse effects of mosquito repellants and the need to search for natural and environment-friendly Mosquito repellants. Synthetic insecticides and their associated toxicity issues and the growing incidence of insect resistance have inspired novel insecticides. The present study analyzed the phytochemical extract of *H. Suaveolens* its effect on mosquito pupa. A total number of pupa (320) was poured into a 200ml glass beaker; twenty pupae each per three hours was introduced into a glass beaker containing different extract concentrations (ppm) of *Hyptis suaveolens*. Twenty pupae were introduced in contrast to a glass beaker containing 100ml of distilled water treated with 1ml of acetone used as treated control. Twenty pupae were introduced into 100ml of distilled water and used as untreated control. The result reveals that *Hyptis suaveolens* possesses inhibitory activity against *Anopheles gambiae*.

**Introduction:** -  
Plant extracts are a rich source of naturally synthesized chemical compounds (Muthukrishnan et al., 2014). The concept of developing drugs from plants in the indigenous medical system is much older (Mishra et al., 2011). The traditional population uses *Hyptis suaveolens* several parts of the world to treat illnesses (Barbosa et al., 2013; Jesus et al., 2013; Vera-Arzave et al., 2012). It has been reported to relieve respiratory and gastrointestinal infections, indigestion, cold, pain, fever, cramps, skin diseases, gastric ulcers, and inflammatory disorders (Machado et al., 2021). The extracts from *H. suaveolens* show considerable potential for medicinal exploitation and application (Li et al., 2020). For instance, the plant has been found helpful in antimicrobial and antioxidant activity (Das et al., 2017; Gavani&Paarakh, 2008; Nantitanon et al., 2007), parasitical cutaneous diseases (Pachkore & Dhale, 2011), neuroprotective activities (Ghaifari et al., 2014), antiviral activity (Kothandan & Swaminathan, 2014), antymycobacterial and cytotoxic activities (Aremu et al., 2020; Mandal et al., 2007; Prawatsri et al., 2013; Satish et al., 2010), antiplasmodial activity (Chukwujekwu et al., 2005), antifungal (Moreira et al., 2010), wound healing activity (Bayala et al., 2020; Shenoy et al., 2009), antidiarrhoeal activity (Shaikat et al., 2012), antinociceptive (Santos et al., 2007). However, biological attributes of the plant have been well documented owing to its good medicinal value due to the presence of essential oils, alkaloids, flavonoids, phenols, saponins, terpenes, and sterols. In traditional medicine, the plant leaves are applied as insectifuge because of their intense aroma, especially against mosquitoes.
Mosquitoes are a significant global public health concern, with a concomitant increase in people at risk of infection (Guégan et al., 2018). It has become the oldest human enemy and represents a significant threat to human health because of its ability to vector pathogens that cause diseases like Dengue fever, Dengue hemorrhagic fever, Malaria, Japanese encephalitis, and Filariasis that afflict millions of people worldwide. (Lawler and Lanzaro 2005). Mosquito-borne diseases are associated with significant global health burdens (Lee et al., 2019).

There has been a growing concern about the adverse effects of Mosquito repellants. There has been an increasing need to search for natural and environment-friendly mosquito repellants (Singh et al., 2011). Synthetic insecticides and their associated toxicity issues and the growing incidence of insect resistance have inspired novel insecticides. Plant extracts may be alternatives sources that constitute a rich source of bioactive compounds that are biodegradable and environmentally friendly. Research has shown that H. suaveolens an invasive weed with insecticidal properties (Benelli et al., 2012; Devi Priya, 2016; Sharma et al., 2019). Thus, the current study intends to explore the inhibitory role of H. suaveolens aqueous extract on the pupa of mosquitoes.

**Materials and Method: -**

**Collection of Anopheles mosquito pupa**

The anopheles’ mosquito pupa was collected from stagnant waters at various locations in Kogi state using dipper and pipette as described. The collected pupa was free from tadpoles which may cause its mortality. The pupa was reared in a clean white plastic bowl covered with a fine and clean mosquito net.

**Plant source**

The plant Hyptis suaveolens leaves were collected and confirmed by a Botanist. The leaves were washed with distilled water and sliced into small pieces, and dried.

**Preparation of Crude Extract**

**Soxhlet Extraction**

The crude extraction of Hyptis suaveolens was done using the Soxhlet extraction technique as describe by Zygler et al. (2012). The coarse powder (200g) of Hyptis suaveolens leaves was gained by pounding the air-dried leaves with a mortar and pestle. The coarse powder was transferred into the Soxhlet extractor column and measured into the flat bottom flask and the Soxhlet. A reflux condenser was inserted into the Soxhlet, and rubber hoses connected from the condenser to the circular water. The setup was placed in a heating mantle, and the mantle was connected to the main connection.

**Preparation of Test Concentration**

Plant crude extract was prepared through a single dilution method by mixing it with distilled water, each in a sterile glass beaker. Treated control was prepared and kept safe before pupa inhibitory bioassay.

**Pupa Inhibitory Bioassay**

Pupa bioassay will be prepared according to a standard procedure provided by the world health organization, guideline for laboratory and field testing of mosquito pupacide (WHO, 2005). The pupa was transferred through strainers of droppers to labeled sterile glass beakers of concentrations. The pupa inhibitory activities of each extract concentration were evaluated by counting the number of dead pupae for the period. Pupa was confirmed dead when no movement is observed and no response to a stimulus when touched with a pasture pipette. The dead pupa was carefully removed from the setup and placed in a clean filter paper, counted, and recorded at some intervals.

**Result: -**

Tables containing the number of dead and live pupa against each concentration were made. Degree activities were evaluated by plotting the number of dead pupae against concentration. The percentage of dead and alive for every interval will be determined.

**Table 1:** - Table showing the effect of different concentrations of the crude extracts of H. suaveolens on Anopheles gambiae pupa at a different time interval.

| Extract concentration | Number of pupa exposed | Number of pupa died in 3hours | Number of dead pupa in 9hours | Number of dead pupa in 12hours (PPM) |
|-----------------------|------------------------|-------------------------------|-----------------------------|----------------------------------|
| Treated Pupa          | 20                     | 0                             | 0                           | 0                                |
Untreated pupa  | 20 | 0 | 0 | 0
50ppm         | 20 | 2 | 5 | 11
100ppm        | 20 | 3 | 6 | 15
150ppm        | 20 | 5 | 8 | 17
200ppm        | 20 | 9 | 11| 19

Table 2: - Table showing the effect of different concentrations of extracts (ppm) and percentage % death and alive in 3 hours.

| Extract concentration (ppm) | Number of pupa exposed | Number of pupa died in 3 hours | (%) dead | (%) alive |
|-----------------------------|-------------------------|--------------------------------|----------|-----------|
| Treated Pupa                | 20                      | 0                              | 0        | 0         |
| Untreated pupa              | 20                      | 0                              | 0        | 0         |
| 50ppm                       | 20                      | 2                              | 10       | 90        |
| 100ppm                      | 20                      | 5                              | 15       | 85        |
| 150ppm                      | 20                      | 5                              | 25       | 75        |
| 200ppm                      | 20                      | 9                              | 45       | 55        |

Percentage (%) dead = \( \frac{n \text{ dead after treatment}}{n \text{ in control}} \times 100 \)
(Percentage (%) alive = 100 – percentage (%) dead)

Table 3: - Table showing the different concentrations of extracts (ppm) and percentage % death and alive in 6 hours.

| Extract concentration (ppm) | Number of pupa exposed | Number of pupa died in 6 hours | (%) dead | (%) alive |
|-----------------------------|-------------------------|--------------------------------|----------|-----------|
| Treated control             | 20                      | 0                              | 0        | 0         |
| Untreated control           | 20                      | 0                              | 0        | 0         |
| 50ppm                       | 20                      | 5                              | 25       | 75        |
| 100ppm                      | 20                      | 6                              | 30       | 70        |
| 150ppm                      | 20                      | 8                              | 40       | 60        |
| 200ppm                      | 20                      | 11                             | 55       | 45        |

Table 4: - Table showing the different concentrations of extracts (ppm) and percentage % death and alive in 9 hours.

| Extract concentration (ppm) | Number of pupa exposed | Number of pupa died in 9 hours | (%) dead | (%) alive |
|-----------------------------|-------------------------|--------------------------------|----------|-----------|
| Treated control             | 20                      | 0                              | 0        | 0         |
| Untreated control           | 20                      | 0                              | 0        | 0         |
| 50ppm                       | 20                      | 9                              | 45       | 55        |
| 100ppm                      | 20                      | 10                             | 50       | 50        |
| 150ppm                      | 20                      | 13                             | 65       | 35        |
| 200ppm                      | 20                      | 15                             | 75       | 25        |

Table 5: - Table showing the different concentrations of extracts (ppm) and percentage % death and alive in 12 hours.

| Extract concentration (ppm) | Number of pupa exposed | Number of pupa died in 12 hours | (%) dead | (%) alive |
|-----------------------------|-------------------------|--------------------------------|----------|-----------|
| Treated control             | 20                      | 0                              | 0        | 0         |
| Untreated control           | 20                      | 0                              | 0        | 0         |
| 50ppm                       | 20                      | 11                             | 55       | 45        |
| 100ppm                      | 20                      | 15                             | 75       | 25        |
| 150ppm                      | 20                      | 17                             | 85       | 15        |
| 200ppm                      | 20                      | 19                             | 95       | 5         |

Discussion: -
Natural pesticides, especially those derived from plants, are more prosing and effective in mosquito (pupa) control(Amer & Mehlhorn, 2006). *Hyptis suaveolens* were found to have some pupa inhibitory activity against
mosquito pupa at different concentration rates in part per million (ppm), as shown in table 1. The result showed the net change in the death rate of pupa with a subsequent increase in its concentration compared to control. A total number of pupa (320) was poured into a 200ml glass beaker; twenty pupae each per three hours was introduced into a glass beaker containing different extract concentrations (ppm) of Hyptis suaveolens. Twenty pupae were introduced in contrast to a glass beaker containing 100ml of distilled water treated with 1ml of acetone used as treated control. Twenty pupae were introduced into 100ml of distilled water and used as untreated control.

Table 1 shows the effect of different concentrations of crude extracts of Hyptis suaveolens on Anopheles Gambiae larvae at different time intervals. After 12 hours, no motility was recorded for treated and untreated control. In the third (3) hours, ±2 pupa was recorded dead against a concentration of 50 ppm. After three hours, twenty (20) pupa was introduced into another glass beaker containing 50 ppm of crude extract and allowed to stand. In six (6) hours, ±5 pupa was recorded dead. The same procedure was repeated for the 9th and 12th hours, respectively. In the 9th hour, ±9 pupa was recorded dead, and in the 12th hour, ±11 pupa was recorded dead. The same procedure was repeated for concentrations 100 ppm, 150 ppm, and 200 ppm. At concentration 100 ppm, in 3 hours, 6 hours, 9 hours and 12 hours, ±3, ±6, ±10, and ±15 pupa was recorded dead, respectively. At concentration 150 ppm, in 3 hours, 6 hours, 9 hours, and 12 hours, ±5, ±8, ±13 and ±17 pupa was recorded dead. At concentration 200 ppm, in 3 hours, 6 hours, 9 hours and 12 hours, ±9, ±11, ±15, and ±19 pupa was recorded dead. Number of dead for pupa that died in 3 hours, 6 hours, 9 hours, and 12 hours were calculated by dividing the number of pupae dead after treatment with several controlled (20) and multiplying with 100%, and percentage alive for the remaining pupa was calculated by subtracting percentage dead from 100%. This procedure was used in the following tables. The inhibitory activity was found to be higher against the pupa at the highest concentration rate of 200 ppm.

**Conclusion:**

Plant extracts in insect/mosquito control are an effective pest control method and help minimize the aggressive growth of mosquitoes. The result reveals that Hyptis suaveolens possesses inhibitory activity against Anopheles gambiae. Therefore, the result contributed to the literature by laying the ground for further analysis of the bioactive constituents of Hyptis suaveolens extract and its systemic effects on target mosquitoes. This may enable the application of the extract as a pupa inhibitor in a considerable area of aquatic habitats or breeding sites in and around human dwellings for effective control of vector mosquitoes.

**Funding**

Tetfund funds the research

**References:**

1. Aremu, O. I., Ajala, T. O., Ajeh, I. J., & Ekere, K. E. (2020). The pharmaceutical and antimicrobial properties of dermatological formulations containing Hyptis suaveolens (L.) poit (Lamiaceae) aerial extract. Acta Pharmacuetica Scientifica, 58(3). https://doi.org/10.23893/1307-2080.APS.05820
2. Amer, A., & Mehlhorn, H. (2006). Repellency effect of forty-one essential oils against Aedes, Anopheles, and Culex mosquitoes. Parasitology Research, 99(4). https://doi.org/10.1007/s00436-006-0184-1
3. Barbosa, L. C. A., Martins, F. T., Teixeira, R. R., Polo, M., & Montanari, R. M. (2013). Chemical variability and biological activities of volatile oils from Hyptis suaveolens (L.) Poit. In Agriculturae Conspectus Scientificus (Vol. 78, Issue 1).
4. Bayala, B., Nadembe, C., Guenné, S., Buñay, J., Zohoncon, T. M., Djigma, F. W., Yonli, A., Baron, S., Figueredo, G., Lobacarro, J. M. A., & Simpore, J. (2020). Chemical composition, antioxidant and cytotoxic activities of Hyptis suaveolens (L.) poit. Essential oil on prostate and cervical cancer cells. Pakistan Journal of Biological Sciences, 23(9). https://doi.org/10.3923/pjbs.2020.1184.1192
5. Benelli, G., Flamini, G., Canale, A., Molfetta, I., Cioni, P. L., & Conti, B. (2012). Repellence of Hyptis suaveolens whole essential oil and major constituents against adults of the granary weevil Sitophilus granarius. Bulletin of Insectology, 65(2).
6. Chukwujekwu, J. C., Smith, P., Coombes, P. H., Mulolland, D. A., & van Staden, J. (2005). Antiplasmodial diterpenoid from the leaves of Hyptis suaveolens. Journal of Ethnopharmacology, 102(2). https://doi.org/10.1016/j.jep.2005.08.018
7. Das, I., Panda, M. K., Rath, C. C., & Tayung, K. (2017). Bioactivities of bacterial endophytes isolated from leaf tissues of Hyptis suaveolens against some clinically significant pathogens. Journal of Applied Pharmaceutical Science, 7(8). https://doi.org/10.7324/JAPS.2017.70818
8. Devi Priya, M. (2016). A review on the pharmacology and phytochemistry of folklore medicinal plant Hyptis suaveolens (L.) Poit. International Journal of Basic, Applied and Innovative Research.

9. Gavani, U., & Paarakh, P. M. (2008). Antioxidant activity of Hyptis suaveolens Poit. International Journal of Pharmacology, 4(3). https://doi.org/10.3923/ijp.2008.227.229

10. Ghaffari, H., Ghassam, B. J., Chandra Nayaka, S., Ramachandra Kini, K., & Prakash, H. S. (2014). Antioxidant and neuroprotective activities of Hyptis suaveolens (L.) Poit. Against oxidative stress-induced neurotoxicity. Cellular and Molecular Neurobiology, 34(3). https://doi.org/10.1007/s10571-013-0016-7

11. Guégan, M., Zouache, K., Démichel, C., Minard, G., Tran Van, V., Potier, P., Mavingui, P., & Valiente Moro, C. (2018). The mosquito holobiont: fresh insight into mosquito-microbiota interactions. In Microbiome (Vol. 6, Issue 1). https://doi.org/10.1186/s40168-018-0435-2

12. Jesus, N. Z. T., Falcão, H. S., Lima, G. R. M., Caldas Filho, M. R. D., Sales, I. R. P., Gomes, I. F., Santos, S. G., Tavares, J. F., Barbosa-Filho, J. M., & Batista, L. M. (2013). Hyptis suaveolens (L.) Poit (Lamiaceae), a medicinal plant protects the stomach against several gastric ulcer models. Journal of Ethnopharmacology, 150(3). https://doi.org/10.1016/j.ijep.2013.10.010

13. Kothandan, S., & Swaminathan, R. (2014). Evaluation of in vitro antiviral activity of Vitex Negundo L., Hyptis suaveolens(L) poit., Decalepishamiltonii Wight & Arn., to Chikungunya virus. Asian Pacific Journal of Tropical Disease, 4(S1). https://doi.org/10.1016/S2222-1808(14)60424-2

14. Lee, W. S., Webster, J. A., Madzokere, E. T., Stephenson, E. B., & Herrero, L. J. (2019). Mosquito antiviral defense mechanisms: A delicate balance between innate immunity and persistent viral infection. Parasites and Vectors, 12(1). https://doi.org/10.1186/s13071-019-3433-8

15. Li, R., Tang, G., Liu, X., Li, J., Wang, D., & Ji, S. (2020). An ethnopharmacological review of Hyptis suaveolens (L.) Poit. In Tropical Journal of Pharmaceutical Research (Vol. 19, Issue 7). https://doi.org/10.4314/tjpr.v19i7.29

16. Machado, F. D. F., Formiga, R. de O., Lima, G. R. de M., de Jesus, N. Z. T., Alves Júnior, E. B., Marinho, A. F., Tavares, J. F., Santos, F. A., Viana, A. F. S. C., Araújo, A. A., de Araújo Júnior, R. F., Pellizzon, C. H., & Batista, L. M. (2021). Hyptis suaveolens (L.) Poit protects colon from TNBS-induced inflammation via immunomodulatory, antioxidant and anti-proliferative mechanisms. Journal of Ethnopharmacology, 265. https://doi.org/10.1016/j.ijep.2020.113153

17. Mandal, S., Mondal, K., Dey, S., & Pati, B. (2007). Antimicrobial activity of the leaf extracts of Hyptis suaveolens (L.) Poit. Indian Journal of Pharmaceutical Sciences, 69(4). https://doi.org/10.4103/0250-474x.36946

18. Mishra, S. B., Verma, A., Mukerjee, A., & Vijayakumar, M. (2011). Anti-hyperglycemic activity of leaves extracts of Hyptis suaveolens L. Poit. in streptozotocin induced diabetic rats. Asian Pacific Journal of Tropical Medicine, 4(9). https://doi.org/10.1016/S1995-7645(11)60175-2

19. Moreira, A. C. P., Lima, E. de O., Wanderley, P. A., Carmo, E. S., & de Souza, E. L. (2010). Chemical composition and antifungal activity of Hyptis suaveolens (L.) Poit leaves essential oil against Aspergillus species. Brazilian Journal of Microbiology, 41(1). https://doi.org/10.1590/S1517-83822010000100006

20. Muthukrishnan, P., Jayaprabha, B., & Prakash, P. (2014). Mild steel corrosion inhibition by aqueous extract of Hyptis Suaveolens leaves. International Journal of Industrial Chemistry, 5(1). https://doi.org/10.4103/0250-014-0005-9

21. Nantitanon, W., Chowwanapoophon, S., & Okonogi, S. (2007). Antioxidant and antimicrobial activities of Hyptis suaveolens essential oil. Scientia Pharmaceutica, 75(1). https://doi.org/10.3797/scipharm.2007.75.35

22. Pachkore, G. L., & Dhale, D. A. (2011). Pharmacognostic Evaluation of Hyptis suaveolens (L. Poit) Lamiaceae. Elements, 3(3), 05–10.

23. Prawatsri, S., Suxsamrarn, A., Chindaduang, A., & Rukchaisirikul, T. (2013). Abietane diterpenes from Hyptis suaveolens. Chemistry and Biodiversity, 10(8). https://doi.org/10.3923/ijp.2008.227.229

24. Santos, T. C., Marques, M. S., Menezes, I. A. C., Dias, K. S., Silva, A. B. L., Mello, I. C. M., Carvalho, A. C. S., Cavalcanti, S. C. H., Antoniolli, Â. R., & Marçal, R. M. (2007). Antinociceptive effect and acute toxicity of the Hyptis suaveolens leaves aqueous extract on mice. Fitoterapia, 78(5), https://doi.org/10.1016/j.fitote.2007.01.006

25. Satish, V., Ravichandrian, V. D., Gavani, U., & Paarakh, P. M. (2010). Antimicrobial studies on the extracts of Cocculus hirsutus Linn. and Hyptis suaveolens Poit. Indian Journal of Natural Products and Resources, 1(1).

26. Shaikat, M. Z. H., Hossain, M. T., & Azam, M. G. (2012). Phytochemical screening and antidiarrheal activity of Hyptis suaveolens. International Journal of Applied Research in Natural Products, 5(2).
27. Sharma, A., Singh, H. P., Batish, D. R., & Kohli, R. K. (2019). Chemical profiling, cytotoxicity and phytotoxicity of foliar volatiles of Hyptis suaveolens. Ecotoxicology and Environmental Safety, 171. https://doi.org/10.1016/j.ecoenv.2018.12.091

28. Shenoy, C., Patil, M. B., & Kumar, R. (2009). Wound healing activity of Hyptis suaveolens (L.) Poit (Lamiaceae). International Journal of PharmTech Research, 1(3).

29. Singh, V., Shrivastava, G., Shukla, S., Shukla, A., & Pandey, V. (2011). Mosquito repellent activity of essential oils of Hyptis suaveolens. Journal of Pharmacy Research, 4(8).

30. Vera-Arzave, C., Antonio, L. C., Arrieta, J., Cruz-Hernández, G., Velázquez-Méndez, A. M., Reyes-Ramírez, A., & Sánchez-Mendoza, M. E. (2012). Gastroprotection of suaveolol, isolated from Hyptis suaveolens, against ethanol-induced gastric lesions in wistar rats: Role of prostaglandins, nitric oxide and sulfhydryls. Molecules, 17(8). https://doi.org/10.3390/molecules17088917

31. WHO. (2005). Guidelines for laboratory and field testing of mosquito larvicides. World Health Organization.

32. Zygler, A., Slomińska, M., & Namieśnik, J. (2012). Soxhlet extraction and new developments such as soxtec. In Comprehensive Sampling and Sample Preparation (Vol. 2). https://doi.org/10.1016/B978-0-12-381373-2.00037-5.