Journal of Applied Life Sciences International

Volume 26, Issue 5, Page 96-103, 2023; Article no.JALSI.108330
ISSN: 2394-1103

In-vitro Cytotoxicity of Extracts of Selected Malaria Medicinal Plants Used by Traditional Healers of Kericho East Sub-county, Kenya

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JALSI/2023/v26i5623

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/108330

Original Research Article

Received: 26/08/2023
Accepted: 31/10/2023
Published: 06/11/2023

ABSTRACT

Background: Malaria is a fatal disease which affects people of all ages; especially pregnant women, young children <5 years, and the elderly because of their weakened immune systems. The currently used anti-malarial drugs have been linked to a variety of negative side effects including the parasite resistance. Additionally, the costs associated with the conventional malaria management approach are arguably high, particularly for people living in low-income countries, highlighting the need for alternative and complementary approaches. Medicinal plants therefore are a viable alternative since they are arguably less expensive and easily accessible. However, there is limited information on safety and efficacy of the plants. This study was designed to investigate the cytotoxic activities of polar and non-polar crude extracts solvents of selected plants used by traditional healers in Kericho East Sub-County to treat malaria.
Materials and Methods: Plants studied included *Pittosporum viridiflorum* (stem barks), *Phytolacca dodecandra* (Leaves), and *Gardenia ternifolia* (roots barks). Plant parts selected were collected

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from Kericho East Sub-county; Kapsoit, Kaitui, and Fort-Ternan. Their crude extracts were obtained from hexane, dichloromethane (DCM), Methanol (MeOH), and 5% H2O/MeOH. In vitro cytotoxic effects and safety of the studied plants' extracts were identified using mammalian Vero E6 cells.
Results: Most of the plants tested yielded impressive cytotoxicity results, indicating that therapeutic doses could be achieved at safe concentrations. However, *P. viridiflorum* hexane, DCM, MeOH, and 5% H_2O/MeOH crude extracts were toxic to the cultured cells expressing the mean CC_{50} ± SE of 65.11±0.40, 25.63±0.23, 87.94 ±0.59 and 98.54±0.66 µg/ml, respectively.
Conclusion: *G. ternifolia* and *P. dodecandra* have offered hope in the treatment of malaria since their crude extracts have demonstrated no toxicity. The study found *P. viridiflorum* crude extracts to be toxic but there is the possibility of isolating safe nontoxic compound/s because they were less toxic at lower doses. This study therefore identified potential plants that could be used to develop novel anti-plasmodial agents.

Keywords: Anti-malarial drugs; anopheles mosquito; medicinal plants; malaria; cytotoxicity.

1. INTRODUCTION

Malaria is a potentially fatal disease caused by a protozoan parasite transmitted through the bites of the female *Anopheles* mosquito. It is estimated that over 241 million people worldwide are affected, with 627 000 deaths occurring each year [1]. Expectant mothers and children aged 0 to 5 years are the most vulnerable groups to malarial infections [2]. Malaria is frequently associated with poverty, but it is also a cause of poverty and a significant impediment to economic development. In the year 2021, the World Health Organization (WHO) forecasts over 241 million malaria cases, with Sub-Saharan Africa accounting for more than 95% of all cases. [1]. In the year 2017, 3,215,116 of *Plasmodium falciparum* cases were reported in Kenya [3]. Additionally, the World Health Organization [4] estimates that over 3.2 billion people in 91 countries are at high risk of contracting malaria. The high mortality rates of malaria subjects are of great concern in light of the rising statistics. Many studies have found that parasites are becoming more resistant to conventional anti-malarials [5,6]. Furthermore, the toxicities and adverse events associated with conventional antimalarial drugs render them ineffective in malaria treatment, necessitating the urgent need for alternative and complementary approaches [7, 8]. In addition, convectional treatment regimens face the challenge of high production costs, necessitating the search for less expensive, yet effective alternatives [9,10].

For thousands of years, plants have played an important role in the treatment of malaria. Therefore, herbal medicine has been used throughout history, making it the oldest form of healthcare known to humanity. WHO endorses its use and considers it one of the most effective strategies for combating emerging diseases [3]. Plants have been found to contain powerful antimalarial compounds such as quinine and artemisinin. As a result, medicinal plants represent a vast reservoir from which powerful antimalarial drugs can be developed. It is regarded as one of the world's most reliable methods of achieving total health because it is less expensive than conventional medicine. Traditional medicine serves the primary healthcare needs of approximately 80% of the world's population [11]. Affordability, availability, and accessibility are the primary reasons for people's reliance on herbal medicine [12]. Medicinal plant use is also integrated into most African cultures, making it more acceptable than conventional medicine [13]. Because of the numerous bioactive compounds found in herbal drugs, increasing research data has demonstrated their potential [14]. These phytocompounds have been identified as potential leads for some currently used drugs, including anti-cancers, analgesics, and anti-malarials [15].

Despite the tremendous contribution and potential of medicinal plants, particularly in the treatment of malaria, no scientific data exists to validate the claimed antimalarial safety. Investigated medicinal plant parts and their constituents (*Pittosporum viridiflorum Sims. var. viridiflorum* (S.L) local name *Chepngororiot* (stem barks), *Phytolacca dodecandra* L Herit local name *Patkawet* (leaves) and *Gardenia ternifolia* Schum.&Thonn local name *Kipulwet* (roots barks) are majorly used by Kipsigis people in Kericho County, Kenya for the management of malaria [16]. Their unknown toxicities despite long history of use in anti-malarial and anti-plasmodial activities informed the current study.

2. MATERIALS AND METHODS

2.1 Plant Parts Collection

Plant parts (roots, barks for *Gardenia ternifolia* Schum.&Thonn, leaves for *Phytolacca dodecandra* L Herit, and stem barks for *Pittosporum viridiflorum Sims. var. viridiflorum*) were collected in Kericho County, specifically in the Kapsoit, Kaitui, and Fort-Ternan areas with the help of a traditional herbalist. The parts were then transported to the Kisii University laboratory for crude extract removal.

2.2 Crude Extractions

The collected plants parts were then air-dried for one week at room temperature in a well-aerated room before being grounded into coarse powder using Kisii University laboratory mill. Crude extracts were then prepared following the procedure stated by [17] with some few modifications. Briefly, 100 g powder of each plant part extract were macerated in 300 ml of each hexane, DCM, MeOH and 5% H_2O/MeOH methanol at room temperature for 72 hours. The extracts were then filtered using double-layer Whatman's number one filter papers and concentrated with a rotary evaporator using different boiling points ranging from 60–80°C for 5 hrs. Additionally, for 5% H_2O/MeOH methanol plant crude extracts, it required another process of mixing the solution with ethyl acetate to remove water. After 24 hours, the mixture was separated into two layers using a separating funnel. The upper layer contained all of the organic substances, while the lower layer was the aqua phase, which was heavier and carried the water components. Following the removal of the aqua phase, the organic phase was passed through a rotary evaporator to remove ethyl acetate, which was concentrated at temperatures ranging from 60^{o}C to 80^{o}C.

2.3 Cytotoxicity Studies

The growth-inhibition assay was performed on actively dividing sub-confluent Vero E6 cells (Kurokawa et al., 1995). Vero E6 cells were grown in 25 ml cell culture flasks incubated at 37^{o}C in 5% CO_2 in Eagle's minimum essential medium (MEM) (GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS). When the cells reached confluence, they were seeded with 5×104 cells/well in 24-well plates and incubated at 37^{o}C for 2 days. Positive and negative controls including chloroquine and untreated Vero E6 cells respectively were set up. The culture medium was replaced with fresh MEM containing test extracts at various concentrations, and the cells were incubated for another 2 days. Trypsinization was used to detach cells from each sample in triplicate wells, and the number of viable cells was determined using a tryphan blue exclusion test. To count viable cells, a haemocytometer was used. CC_{50} (concentration required to cause visible alterations in 50% of intact cells) was estimated using inhibition data plotted as dose-response curves using nonlinear regression analysis. Mean CC_{50} (X±SE) < 100 µg/mL was considered toxic. Additionally, at each drug concentration, the results were also recorded as optical density (OD) per well. The data was entered into Microsoft Excel 2016 and expressed as a percentage of the untreated controls using the formula described below [18].

$$Percentage\ cell\ growth\ inhibition\ = \left(\frac{A-B}{A}\right) \times\ 100$$

Where;
A = the OD of the untreated cells
B = the OD at each drug concentration

The Percentage Yield of the Crude Extract was also calculated using the formula below;

$$\text{Percentage Yield} = \frac{\text{weight of extracts obtained}}{\text{weight of powder used for extractions}} \times 100$$

2.4 Data Analysis

The statistical significance between the means of the data was analyzed using chi-square where P-value of less than 0.05 was considered statistically significant.

3. RESULTS

3.1 Crude Extracts Percentage Yield

Following extraction, the respective extracts' percentage yields were calculated. The results revealed that, in general, high percentage yields were recorded in 5% H2O/MeOH of all plants parts followed by MeOH, Hexane and lastly DCM (Table 1).

Table 1. Plants parts and the percentage yield

Plant name	Plant parts used	Extraction solvent	% Yield
Gardenia ternifolia Schum.&Thonn.	Roots bark	Hexane	2.43
		Dichloromethane (DCM)	0.53
		Methanol (MeOH)	3.65
		5% H_2O/MeOH	6.84
Pittosporum viridiflorum Sims. var. viridiflorum (S.L)	Bark	Hexane	2.92
		Dichloromethane (DCM)	0.94
		Methanol (MeOH)	4.01
		5% H_2O/MeOH	6.03
Phytolaca dodecandra L Herit	Leaves	Hexane	2.36
		Dichloromethane (DCM)	0.42
		Methanol (MeOH)	2.94
		5% H_2O/MeOH	7.97

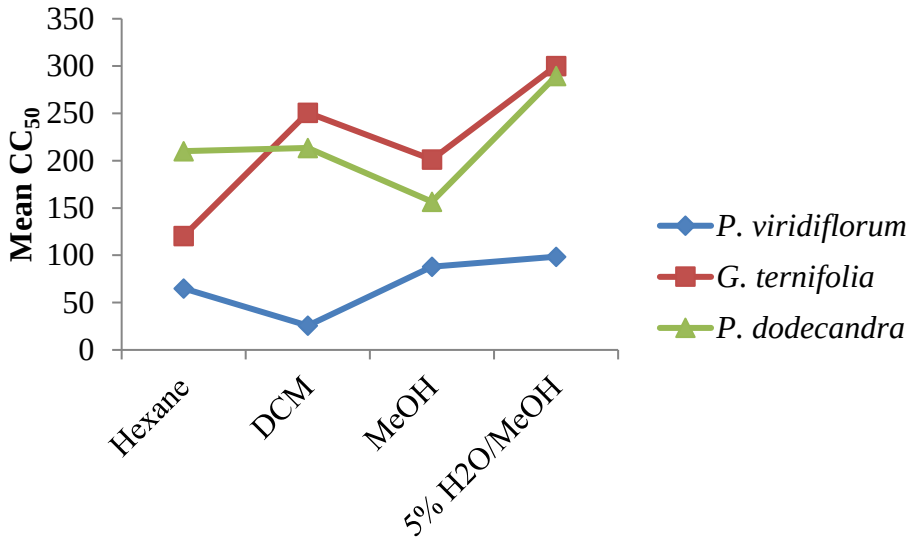

Fig. 1. Mean CC_{50} of *P.viridiflorum*, *G. ternifolia* and *P. dodecandra* crude extracts

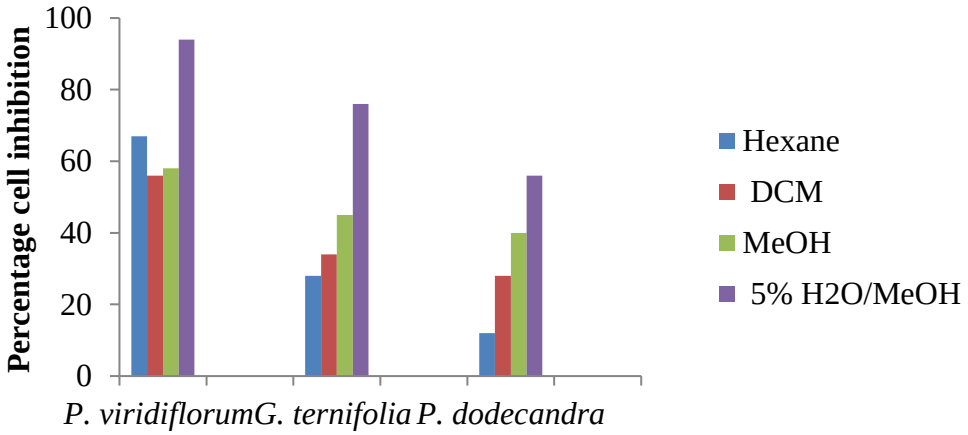

Fig. 2. Percentage cell inhibition of *P.viridiflorum*, *G. ternifolia* and *P. dodecandra* crude extracts

3.2 Cytotoxicities of Plants Crude Extracts

P. viridiflorum crude extracts showed no toxicity in lower doses (10 µg/ml <) while in high dosage (>10 µg/ml <) it showed the maximum cell harmfulness when exposed to Vero E6 cells expressing the mean CC_{50} ± SE of 65.11±0.40, 25.63±0.23, 87.94 ±0.59 and 98.54±0.66 µg/mL Hexane, DCM, MeOH and 5% H_2O/MeOH, respectively (Fig 1).

3.3 Vero E6 Cells Inhibition

P. viridiflorum crude extracts had a high percentage of Vero E6 cells inhibited, with 5% H_2O/MeOH showing the highest percentage of inhibition across all concentrations (Fig 2).

3.4 Associations between Vero E6 Cells Inhibition and Plants Crude Extract Concentration

A statistical significant association between the Vero E6 cells inhibition and all plants crude extracts seeded in different concentrations was observed (χ^2 = 19.91; d.f = 5, P < 0.001), (χ^2 = 74.16; d.f = 5, P < 0.001), (χ^2 = 96.22; d.f = 5, P < 0.001), (χ^2 = 84.33; d.f = 5, P < 0.001), (χ^2 = 27.56; d.f = 5, P < 0.001), (χ^2 = 67.35; d.f = 5, P < 0.001) in Hexane, DCM, MeOH and 5% H_2O/MeOH respectively.

4. DISCUSSION

P. viridiflorum crude extracts of Hexane, DCM, MeOH and 5% H_2O/MeOH showed toxicity in high dosage in the present study despite the plant parts being is used for malaria and fevers in Kericho with IC_{50} of 3–10µg/ml [16], East Africa [19], and its DCM crude extracts of the whole plant showing an in vitro antimalarial activities in South Africa with IC_{50} of 3 µg/ml [20]. Additionally, *P. lanatum*, a related species of *P. viridiflorum* plant, had leaves that were toxic to brine shrimp (LC50, 27.4 µg/ml) [21]. It is unclear whether the inhibition is the result of a specific antiplasmodial action or general cytotoxicity, but an in vitro test in the present study with a lower dose less than 10µg/ml showed no toxicity, hence indicating that the toxic compounds may not be the same as the active constituents. The present study was in agreement with the study of [22] which stated that methanol and water extracts of *P. viridiflorum* had the highest level of cell cytotoxicity on Vero E6 cells in high dosage, with CC_{50} values of 18.08 and 69.21 µg/ml respectively. Furthermore, in the same study [22], mice treated with a 100 mg/kg methanol extract of *P. viridiflorum* died within 24 hours. The present study showed that *P. viridiflorum* 5% H_2O/MeOH was less toxic than DCM, MeOH and Hexane extracts. This means the polar component of *P. viridiflorum* plant is less toxic than the non-polar component. This suggests that traditional herbalist practitioners who prepare plant parts by boiling them in meat soup (water plus fat in the meat) have less toxicity when using the plant as an antimalarial agent than other traditional herbalist practitioners who ferment the plant parts with honey, resulting in the addition of ethanol, an organic (non-polar) solvent. The current study supported the findings of [22], which found that the water extract was less toxic than the methanol extract. The current study contradicted the findings of [23], which found that the stem bark extracts from this plant had no cytotoxicity or brine shrimp lethality. These findings appear to validate their traditional use as antimalarials [24]. In order to continue working on this plant, since the plant have been proven to have antiplasmodial activity, bioactivity guided isolation will allow the separation of active molecules from toxic constituents.

In the current study, *G. ternifolia* and *P. dodecandra* Hexane, DCM, MeOH, and 5% H2O/MeOH crude extract plant roots bark showed no signs of toxicity, similar to the study of [25], which found that in an acute oral toxicity test, a hydroalcoholic stem bark extract of *G. ternifolia* did not cause any observable damage in the studied mice at 2,000 mg/kg. Previous research have shown that if the LD_{50} value of a test chemical is three times greater than the minimum effective dose, the extract is considered a good candidate for further investigation [26] & [27]. Therefore, *G. ternifolia* and *P. dodecandra* have offered hope in the treatment of malaria since their crude extracts of Hexane, DCM, MeOH, and 5% H2O/MeOH roots bark have demo**n**strated antiplasmodial activity similar to chloroquine and mefloquine in the study of (15) with no toxicity. The study of [28] disagreed with the present study because it assessed the cytotoxicity activities of a 20.0% aqueous ethanol extract of *G. ternifolia* roots using the brine shrimp lethality test with an LC_{50} value of 54.5g/ml. These differences might be attributed to location and time in which the plants were collected as well as the different physiographic factors which influence plant phytochemicals.

5. CONCLUSION

P. viridiflorum extracts were toxic in Vero E6 cells at the test dose, but there is the possibility of isolating safe nontoxic compound/s because they were less toxic at lower doses. Similar to this study, (22) and (20) reported different levels of toxicities in samples of the same plant species collected in different regions, emphasizing the importance of georeference, implying that the location and, most likely, time of plant collection may influence the amount and composition of the toxic components. It is unknown whether such factors influenced the phytochemical composition of the *P. viridiflorum, G. ternifolia* and *P. dodecandra* plants, which has been linked to present or lack of cytotoxicity in East and South Africa. Hence, it would be of interest to isolate active constituents for identification, antiplasmodial evaluation, and further toxicological screening, where caution should be exercised if these plants are to be promoted as medicinal plants for malaria treatment.

ETHICAL APPROVAL

Ethical clearance to conduct this research was granted by Kenya Medical Research Institute (KEMRI) ethical review committee and permitted by the National Commission for Science Technology and Innovation (NACOSTI). The research was conducted in accordance with the established SERU guidelines.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World Health Organization. The World Malaria Report; 2021.
2. World Health Organization. The World Malaria Report;2016.
3. Waiganjo B, Moriasi G, Onyancha J, Elias N, Muregi F. Antiplasmodial and cytotoxic activities of extracts of selected medicinal plants used to treat malaria in Embu County, Kenya. Journal of Parasitology Research; 2020.
4. World Health Organization. The World Malaria Report; 2018.
5. Ferreira PE, Culleton R, Gil JP, Meshnick SR. Artemisinin resistance in Plasmodium falciparum: what is it really?. Trends in parasitology. 2013;29(7):318-320.
6. Bunney PE, Zink AN, Holm AA, Billington CJ, Kotz CM. Orexin activation counteracts decreases in nonexercise activity thermogenesis (NEAT) caused by high-fat diet. Physiology & behavior. 2017;176; 139-148.
7. Bitta MA, Kariuki SM, Mwita C, Gwer S, Mwai L, Newton CR. Antimalarial drugs and the prevalence of mental and neurological manifestations: a systematic review and meta-analysis. Wellcome open research. 2017:2.
8. Luo Y, Che MJ, Liu C, Liu HG, Fu XW, Hou YP. Toxicity and related mechanisms of dihydroartemisinin on porcine oocyte maturation in vitro. Toxicology and applied pharmacology. 2018;341:8-15.
9. Waiganjo B, Moriasi G, Onyancha J, Elias N, Muregi F. Antiplasmodial and cytotoxic activities of extracts of selected medicinal plants used to treat malaria in Embu county, Kenya. Journal of Parasitology Research. 2020 Jul 7;2020.
10. Christopher R, Msonga A, Hoppe HC, Boyom FF. Ethanol Extracts from Selected Tanzanian Medicinal Plants Selectively Inhibit Plasmodium falciparum Growth In Vitro. Tanzania Journal of Science. 2023 Mar 31;49(1):41-7.
11. World Health Organization. The World Malaria Report; 2015.
12. Olowokudejo JD, Kadiri AB, Travih VA. An ethnobotanical survey of herbal markets and medicinal plants in Lagos State of Nigeria; 2008.
13. Musa SM, Fathelrhman EA, Elsheikh AE, Lubna AA, Abdel LEM, Sakina MY. Ethnobotanical study of medicinal plants in the Blue Nile State, South-eastern Sudan. Journal of Medicinal Plants Research, 2011;5(17), 4287-4297.
14. Enechi OC, Amah CC, Okagu IU, Ononiwu CP, Azidiegwu VC, Ugwuoke EO, Ndukwe EE. Methanol extracts of Fagara zanthoxyloides leaves possess antimalarial effects and normalizes haematological and biochemical status of Plasmodium berghei-passaged mice. Pharmaceutical Biology. 2019;57(1):577-585.
15. Klayman DL. Qinghaosu (artemisinin): an antimalarial drug from China. Science, 1985;228(4703):1049-1055.
16. Chepchumba PB, Nyanchongi B, Masai R. Ethnobotanical survey of medicinal plants used for treatment of malaria by Kipsigis people in Kericho County, Kenya. J. Pharm. Biol. Sci, 2018;13:24-30.

17. Bwogo PC. In vitro anti-plasmodial activity of crude extracts of Gardenia ternifolia, Pittosporum viridiflorum and phytolaca dodecandra used for treatment of malaria in Kericho county, Kenya. International Journal of Progressive Sciences and Technologies. 2020;23(1):212-223.
18. Fatemeh K, Khosro P. *In vitro* cytotoxic activity of aqueous root extract of Althea kurdica against endothelial human bone marrow cells (line k562) and human lymphocytes. Bull Env Pharmacol Life Sci. 2013;*2*(6):23-29.
19. Gakunju DM, Mberu EK, Dossaji SF, Gray AI, Waigh RD, Waterman PG, Watkins WM. Potent antimalarial activity of the alkaloid nitidine, isolated from a Kenyan herbal remedy. Antimicrobial Agents and Chemotherapy. 1995;39(12):2606-2609.
20. Clarkson C, Maharaj VJ, Crouch NR, Grace OM, Pillay P, Matsabisa MG, Folb PI. In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa. Journal of ethnopharmacology. 2004;*92*(2-3):177-191.
21. Wanyoik GN, Chhabra SC, Lang'at-Thoruwa CC, Omar SA. Brine shrimp toxicity and antiplasmodial activity of five Kenyan medicinal plants. Journal of ethnopharmacology. 2004;90(1):129-133.
22. Muthaura CN, Rukunga GM, Chhabra SC, Omar SA, Guantai AN, Gathirwa JW, Njagi ENM. Antimalarial activity of some plants traditionally used in Meru district of Kenya. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2007;21(9):860-867.
23. Cepleanu F, Hamburger MO, Sordat B, Msonthi JD, Gupta MP, Saadou M, Hostettmann, K. Screening of tropical medicinal plants for molluscicidal, larvicidal, fungicidal and cytotoxic activities and brine shrimp toxicity. International Journal of Pharmacognosy, 1994;32(3): 294-307.
24. Gessler PE, Moore ID, McKenzie NJ, Ryan PJ. Soil-landscape modelling and spatial prediction of soil attributes. International journal of geographical information systems. 1995;9(4):421-432.
25. Nureye D, Sano M, Fekadu M, Duguma T, Tekalign E. Antiplasmodial activity of the crude extract and solvent fractions of stem barks of Gardenia ternifolia in plasmodium berghei-infected mice. Evidence-Based Complementary and Alternative Medicine; 2021.
26. Dikasso D, Makonnen E, Debella A, Abebe D, Urga K, Makonnen W, Guta M. Anti-malarial activity of withania somnifera L. Dunal extracts in mice. Ethiopian Medical Journal, 2006;44(3): 279-285.
27. Madara A, Tijani A, Nandi E. Anti-plamodial activity of ethanolic root bark extract of Piliostigma thonningii schum.(Caesalpiniacea) in mice infected with P. berghei NK 65. Report and Opinion. 2012;4(4):62-67.
28. Moshi MJ, Cosam JC, Mbwambo ZH, Kapingu M, Nkunya MH. Testing beyond ethnomedical claims: brine shrimp lethality of some Tanzanian plants. Pharmaceutical Biology. 2004;42(7):547-551.

APPENDIX

Appendix I: Photos of *Gardenia ternifolia* Schum.&Thonn., *Pittosporum viridiflorum Sims. var. viridiflorum* (S.L) and *Phytolaca dodecandra* L Herit

***Gardenia ternifolia* Schum.&Thonn.**

***Pittosporum viridiflorum Sims. var. viridiflorum* (S.L)**

***Phytolaca dodecandra* L Herit**

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