Phytochemical Evaluation of some Anti-malarial Medicinal Plants used in the Dangbe West District of Ghana.

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Abstract: The ethanolic and aqueous extracts of six plants used to treat malaria by folklore in the Dange West district of Ghana were screened qualitatively for their photochemical constituents as well as evaluated for total flavonoids, phenolic contents and antioxidants capacities. Tannins, cardiac glycosides and saponnis were found to be present in most of the plant extracts studied. Alkaloids were absent in the extracts of *G. Sp* and *S. siamea* whereas *D. pinnata* and *V. amygdalina* lacked combined anthroquinones. Phlobatannins and anthroquinones were present in ethanol extracts of *S. alata* and aqueous extracts *S. siamea*. Phlobatannins were further present in ethanol extract of *G. sp* as antroquinones were detected in aqueous extract of *S. torvum*. Even though the highest phenolic content of 6.858 ± 0.495 % was measured in the ethanolic extract of *S. siamea* neither solvent seemed to perform better at extracting phenolic compounds from the different plants. Total antioxidant activity was measured based on the reduction of Mo (VI) to Mo (V) by the extracts and subsequent formation of green phosphate/Mo (V) complex at acid pH. Overall, ethanol extracts showed relatively high antioxidant concentrations, which were also demonstrated by correspondingly high flavonoid content. All the plants studied had remarkable ability to quench $DPPH^{\bullet}$. Extracts of *S, siamea*, which gave the highest total phenolic content, elicited the highest $DPPH^{\bullet}$ free radical scavenging activity as well as demonstrating high total antioxidant content. These findings suggest that the rich phytochemical content of the medicinal plants herein analyzed and their good antioxidant activity may be responsible for their popular and wide traditional use and may offer effective protection from free radicals leading to reduction in oxidant stress in malarial conditions. This probably validates the basis for the traditional use of these plants against for the ailments claimed.

[Achel Daniel Gyingiri, Asase Alex, Akwetey George Ashong, Nana Afua Kobi Adu-Bobi, Adaboro Mba Rudolph , Donkor Shadrack, Achoribo Elorm Seyram, Fianko Joseph Richmond. **Phytochemical Evaluation of some Anti-malarial Medicinal Plants used in the Dangbe West District of Ghana**. Report and Opinion 2011;3(4):1-7]. (ISSN: 1553-9873). http://www.sciencepub.net.

Keywords: Antioxidants, malaria, free-radicals, oxidant stress, phytochemical constituents.

1. Introduction:

Malaria disease is caused by protozoa of the genus plasmodium which are capable of invading the circulatory system from the salivary glands of infected female anopheles mosquitoes which bite in order to obtain a blood meal during the reproductive phase of their lives. The four species of protozoa able to pass on the malarial disease in humans are *Plasmodium vivax, P. malariae, P. ovale, and P. falciparum* (Gento *et al*, 2008, Guerinn et al, 2002; Phillips, 2001).

Malaria is one of the major treats to mankind, and given that the clinical utility of available antimalarial drugs such as chloroquine, sulphodoxine, pyremethamine, quinine and mefloquine have been compromised due to appearance of drug resistant *P. falciparum* strains (Achudeme, 2009; Karou *et al*, 2007; Hay *et al*, 2004; Guerin *et al*, 2002) development of new anti-malarials is imperative (Wright and Phillipson, 1990).

In the absence of effective vaccines, management of the disease has depended largely on chemotherapy and chemoprophylaxis. In developing countries where malaria is endemic even though little scientific data are available to assess the efficacy of herbal remedies, majority of the populace still depend on traditional medicine to combat the disease. Consequently it is important that medicinal plants with folkloric reputation for anti-malarial properties be investigated in order to establish their therapeutic utility as anti-malarials. An estimated 65 – 80% of the world's population living in the developing world depends essentially on plants for primary health care, because of the easy access and affordability of these plants compared to modern medicine (Calixo, 2000, Akerele

1993). Karou *et el*, 2007 and WHO 2003 report that 60% of the children with high fevers in Ghana, Mali, Nigeria and Zambia resort to herbal medicines as the first line of treatment for malaria.

Studies support that oxidative stress is one of the major reasons for the development of anaemia in malaria subjects (Nmorsi *et al*, 2007). Oxidation is essential to many living organisms for the production of energy to fuel biological processes and free radicals are produced during these normal and/or pathological cell metabolic processes. Furthermore, it has been established that the immune system of the body is activated by infections, including malaria, thereby causing the release of reactive oxygen species (ROS) which deplete the body of its limited supply of antioxidants thus leading to oxidant stress (Pabo´n *et al*, 2003; Postma *et al*, 1990). Others believe that malarial anaemia develops under oxidative stress (Kremsner *et al*., 2000; Clark and Hunt 1983) during which plasmodium parasites provoke the release of ROS by certain cells leading to haemoglobin breakdown. Additionally, it has been corroborated that depressed levels of plasma antioxidants in *Plasmodium falciparum* infected children have contributed to the high morbidity and mortality of malaria patients (Ayoola et al, 2008; Kulkarni, 2003).

Elevated levels of total antioxidant status in the body are thought to be vital in recovery from malaria for instance, Adelekan *et al.*, 1997 have shown that antioxidants provide protection against oxidative stress induced by malaria. On this basis, a phyto-medicine's ability in scavenging or suppressing free radicals, to some degree implies potential clinical applications to malaria but also to age related diseases and some cancers. Despite the role that these antioxidants seem to play in moderating malarial pathogenesis, data concerning antioxidant profiles of plants and herbs used to treat patients for malaria is lacking in Ghana. In malaria endemic areas such as Africa, often times feverish patients are made to consume aqueous or alcoholic decoctions and/or infusions of barks, roots and leaves of different plants either alone or in combination for relief from the clinical manifestation of malaria (Asase *et al* 2005; *ibid*, 2010).

In order to assign a scientific rationale for the continuous traditional usage of these plants to treat malaria and other ailments the present study analysed six (6) plants namely *Deinboulia pinnata*, *Greenayodendron sp*, *Senna alata*, *Senna siamea*, *Solanum torvum and Vernonia amygdalina* for their antioxidant, phytochemical and total phenolic profiles. These plants were selected based on leads supplied by traditional healers for the treatment of malaria following a report by Akwetey, 2009 and Asase *et al*, 2010 on a review of medicinal plants used in the Dangbe west district of Ghana for malaria treatment. Plants used were obtained from Dodowa, Ayikuma and Agomeda all falling within the Dangbe west district of Ghana whose geographical coordinates are latitude 5° 45' S and 6° 05' N and longitudes 0° 05' E and 0° 20' W and covering a total land area of 1442 Km^2 (Asase *et al* 2010). The Ghana Statistical Service, 2005 estimated the population of the district is to be 120,000. The main occupation of the people in the district is subsistence farming and small scale fishing. In view of the appallingly low level of education of the indigenes coupled with correspondingly low income levels, the first line of call in the event of an ailment is healing by medicinal plants (Akwetey, 2009).

2. Materials and Methods

2.1 Chemicals

Folin-Ciocalteu phenol reagent, gallic acid, 2,2-diphenyl-1-picrylhydrazyl hydrate ($DPPH^{\bullet}$), quercetin and Vitamin C were from Sigma. $AlCl_3$, , H_2SO4, Na_3PO_4, , Na_2CO_3, CH_3COOK, $K_2S_2O_8$ were from Merck (Darmstadt, Germany). Ethanol and methanol were from Jansen Chimica (Beerse, Belgium). Unless otherwise stated, all chemicals were of analytical grade.

2.2 Plant Material

Plants were collected from various parts of the Dangbe west district and confirmed by competent authorities at the Ghana Herbarium, University of Ghana.

2.3 Preparation of Ethanol Extract

The leaves of each plant were air dried and reduced to powder. Alcohol extracts was prepared by exhaustive percolation at room temperature of 100g of pulverized leaf material in 100ml 80% ethanol for 48 hours with occasional stirring. The mixture was subsequently filtered through non-absorbent cotton followed by removal of the solvent under reduced pressure in a Heidolph type VVI rotary evaporator (Normschiff Geratelbau Werthein, Fedral Republic of Germany). The concentrate was then lyophilized in an Eyela Freeze drier FDI (Tokyo Rikakikai Co. Ltd., Tokyo Japan). The freeze-dried fraction was weighed and stored in a desiccator over silica until required.

2.4 Aqueous extraction

40g of each dried pulverized plant material were extracted in 800ml of deionised water under reflux for 6hours. The refluxing method was employed to simulate the extraction procedures employed by our

traditional herbalists. The method of reflux was employed to eliminate occasional addition of water as pertains in traditional settings. The hot decoction was allowed to cool and filtered through a Watman number 1 filter paper, followed lyophilization to remove water in an Eyela Freeze drier FDI (Tokyo Rikakikai Co. Ltd., Tokyo Japan.

2.5 Phytochemical Screening

The ethanolic and aqueous plant extracts were evaluated for the presence of different phytochemicals using standard qualitative tests procedures (Trease and Evans, 1989; Harborne, 1998; Sofowora, 1993)

2.6 DPPH• radical scavenging activity

The DPPH• free radical scavenging activity by the different plant extracts was determined according to the method reported by Blois (1958) as modified by Brands and Williams (1995), and Yen and Chen (1995). Plant extracts were all diluted to a concentration of 1mg/ml and dilution factors noted. One hundred microlitres (100µl) of the plant extract in methanol and water were each mixed with 3900 µl of 0.004% DPPH• in methanol solution yielding 25µg/ml of extract in each reaction. 100µl pure methanol was used as the experimental control. After 20min of incubation at room temperature, the reduction in DPPH• free radical absorbance was measured at 517nm against the experimental control. The results were corrected for dilution and expressed in mg Gallic Acid Equivalent per 100g dry weight (mgGAE/100g dry wt). Each experiment was carried out in triplicate.

2.7 Determination of Total Antioxidant Activity:

This assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. Total antioxidant activities of the various plant extracts were determined according to the method of Prieto *et al. 1999*. Briefly 200µl of each sample was taken into 4,800µl of standard reagent containing 0.6 M Sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate. A calibration curve was made using 0.5, 1.0, 1.5, 3.0 and 5.0 mg/ml vitamin C standards prepared by serial dilution. The reaction mixtures were incubated at 95° C for 90min following which absorbance was measured at 695nm against a blank. The regression linear equation y = 3.8648x - 0.0825 was generated from which the Vitamin C equivalent for the various extracts were obtained. The antioxidant capacity was expressed as Vitamin C equivalent (mgVCE/100g dry wt).

2.8 Estimation of total polyphenol content.

Total polyphenol content in aqueous and alcoholic plant extracts was determined by the Folin-Ciocalteu colorimetric method as described elsewhere (Folin and Ciocalteu, 1927). Plant extracts (50µl) were mixed with 2.9ml dH_2O, 250µl of Folin-Ciocalteu reagent (diluted ten-fold) and 0.75 ml of 20% sodium carbonate solution. Absorbance readings of the resulting blue colour was taken at 765nm after incubating for 30 min at ambient temperature with a Shimadzu UV-Vis spectrophotometer (Kyoto, Japan). Quantification was done with respect to the standard curve of gallic acid, the curve yielded equation Y = 0.1871x – 0.1825 and $r^{2 =}$ 0.9988 (Y = absorbance and x= concentration in mg/ml). The results were corrected for dilution and expressed as mgGAE/100g dry wt and percentage by mass. All determinations were performed in triplicate.

2.9 Determination of total flavonoid content

The aluminum chloride colorimetric method was used to evaluate total phenolic content as described by Woisky and Salatino 1998 using quercetin as reference standard. Ten milligrams of quercetin was dissolved in 50ml of 80% ethanol and then diluted to 6.25, 12.5, 25, 50 and 100µg/mL. The diluted standard solutions (0.5 mL) were separately mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415nm with a Shimadzu UV-160A spectrophotometer (Kyoto, Japan). The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. Similarly, 0.5 mL of ethanol and aqueous plant extracts were reacted with aluminum chloride for determination of flavonoid content as described above. A calibration linear regression curve with equation y = 0.0061x -0.005 was generated with r2 = 0.9984.

3. Results

Table 1. Phytochemicals in ethanolic and aqueous extract of antimalarial plants

	D. pinnata		*G. sp*		*S. alata*		*S. siamea*		*S. torvum*		*V. amygdalina*	
	Aq	**Et.**	**Aq.**	**Et**	**Aq.**	**Et**	**Aq.**	**Et**	**Aq.**	**Et**	**Aq.**	**Et.**
Alkaloids	+	-	-	-	+	-	-	-	+	+	+	-
Anthroquinones	-	-	-	-	-	+	+	+	+	-	-	-
Combined anthroquinones	-	-	+	-	+	-	+	-	-	+	-	-
Phlobatannins	-	-	-	+	-	+	+	+	-	-	-	-
Saponins	+	-	+	+	-	-	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+	+	+	+	-	+	-
Tannins	+	+	+	+	-	+	+	+	+	+	+	+

Aq. = Aqueous; Et = Ethanol; + = present; - = absent

Table 2. Total phenolic and antioxidant content of aqueous and ethanolic extracts of medicinal plants.

Scientific name	**Total phenolic content (mg GAE/100g dry wt)**		**Total phenolic content (% mass)**		**Total antioxidant (mgVEC/100g dry wt)**	
	Aqueous	**Ethanol**	**Aqueous**	**Ethanol**	**Aqueous**	**Ethanol**
Deinboulia pinnata Schum	0.357 ± 0.005	0.351 ± 0.019	0.313 ± 0.050	0.325 ± 0.019	232.09 ± 0.013	652.95 ± 0.019
Greenwendondron sp	1.800 ± 0.011	0.756 ± 0.007	1.66 ± 0.011	0.699 ± 0.0.007	766.32± 0.029	159.92± 0.021
Senna alata (L). Roxb	0.854 ± 0.006	2.882 ± 0.035	0.79 ± 0.016	2.66 ± 0.035	268.95± 0.018	1843.52± 0.036
Senna siamea (Lam)	3.383 ± 0.021	7.412 ± 0.445	3.126 ± 0.021	6.858 ± 0.495	567.67± 0.017	2027.77±0.111
Vernonia amygdalina Delile	1.115 ± 0.008	0.996 ± 0.010	1.031 ± 0.008	0.921 ± 0.010	561.45± 0.045	1815.36 ± 0.471
Solanum torvum Schtdl	1.233 ± 0.018	0.344 ± 0.079	1.145 ± 0.018	0.319 ± 0.0.079	244.56±0.031	266.63±0.026

Values (mean ±SD) are average of three samples of each medicinal plant material, analysed in triplicate. (DW= dry weight).

Table 3. DPPH free radical Scavenging capacities and flavonoids content of plants studied

	Family	**Growth form**	**Part used**	**% DPPH scavenging activity**		**Flavonoid content (mg QE/100g dry wt)y**	
				Aqueous	**Ethanol**	*Aqueous*	*Ethanol*
Dienbolia pinnata	sapindaceae	tree	leaves	6.140 ± 0.071	7.767 ± 0.077	7.03 ± 0.31	62.36 ± 3.01
Greenwendron sp	Annonaceae	tree	leaves	34.923 ± 0.002	14.213 ± 0.287	16.85 ±1.90	14.89 ± 1.65
Sena alata	Fabcaeae	shrub	leaves	11.726 ± 0.071	29.289 ± 0.079	9.64 ± 0.57	56.86 ± 0.68
Senna siamea	Fabaceae	tree	R. bark	76.751 ± 0.001	87.767 ± 0.081	0.48 ± 0.17	2.14 ± 0.35
V. amygdalina	Asteraceae	shrub	leaves	30.601 ± 0.072	31.320 ± 0.074	11.19 ±0.37	28.56 ±0.71
Solanum torvum	Solanaceae	shrub	fruit	42.386 ± 0.080	24.822 ± 0.086	8.46± 0.28	1.05 ± 0.19

4. Discussion

Photochemicals in the aqueous and ethanolic extracts determined for the different medicinal plant materials have been evaluated and presented in table 1. From the data presented both *G. Sp* and *S.siamia* tested negative for alkaloids, however, for the others, only *S. torvum* registered alkaloids in both extracts. All other ethanolic extracts tested negative for alkaloids suggesting that the alkaloids were better extracted in the water milieu. Generally, most of the plants lacked anthroquinones which were detected only in both extracts of *S. siamea* and the aqueous extract of *S. torvum*. Combined anthroquinones were detected only in the ethanolic extracts of *G. Sp*, and *S. alata* and the aqueous extracts of *S. siamea*. As anticipated, tannins were detected in all extracts with the exception of the aqueous extracts of *S. alata*. Similarly, cardiac glycosides were detected in all the plant extracts examined except for the ethanolic extracts of *S. torvum* and *V. amygdalina*. The only plant that completely lacked saponins in both extracts was *S. alata*, this group of compounds were also not detected in the ethanol extract of *D. pinnata*. Three of the plant extracts (*G. Sp*, *S alata* and both extracts of *S. siamea)* tested positive for phlobatannins.

Table 2 displays the total polyphenol and antioxidant contents evaluated in the plant extracts on dry weight basis. Total phenolic content (TPC) has been expressed in mg gallic acid equivalent/100mg dry wt (mg GAE/100g dry wt) and percentage mass of total dry matter (% mass). It is quite clear that neither solvent performed better in extracting phenolic

compounds from the plants. While phenolic compounds were evenly extracted in both solvents of *D pinnata* and *V. amygdalina*, water extracted 2.4 and 3.5 times more phenolic components from *G. sp* and *S. torvum* respectively than did ethanol. However ethanol performed better in extracting the polyphenols in *S. alata* and *S. Siamea* (both belong to the family fabaceae) at a rate of 3.37 and 2.20 times respectively. TPC of the ethanolic extracts ranged from 0.325 to 6.858 % by mass while that of water ranged between 0.313 and 3.3132%. Phenolics are known to be the largest group of phytochemicals and have been touted as accounting for most of the antioxidant activity of plants or plant products. Earlier studies (Philipson and Wright, 1990; Christensen and Kharazmi, 2001) have showed that alkaloids, terpenes and flavonoids exert some anti-plasmodial activity. These compounds, except for terepenoids (which were not assayed), were found to be present in the extracts studied and may be responsible for the responses elicited in this study and also contribute some anti-plasmodial activity when taken as medication by users.

In this study it was found that ethanol generally extracted more flavonoids than did water with the exception of *solanum torvum* where flavonoids extracted by water were significantly greater than that extracted by ethanol (table 3). However, the higher amounts of flavonoids in ethanolic extracts were associated with a decrease in the amount of total phenolics and *vice versa* (data not shown). Levels of flavonoids correlated significantly with $DPPH^{\bullet}$ free radical scavenging activity and the flavonoids could probably be contributing a significant amount of the scavenging activity observed.

Total antioxidant capacity was measured by the phosphomolybdenum method and expressed in mg/100g dry wt (table 2). This phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with maximal absorption at 695 nm (Prieto *et al*, 1999). In general, total antioxidant activity was higher in the ethanolic extracts than its aqueous counterparts. With the exception of *G. sp* where the water extracted 766.32 ± 0.029 mg/100g dry wt of total antioxidants which exceeded that of the ethanolic extract of 159.92 ± 0.021 mg/100g dry wt ($P < 0.05$) all ethanolic extracts gave higher total antioxidants than their aqueous counterparts. The highest total antioxidant content of 2027.77 ± 0.111 mg/100g dry wt and 1843.52 ± 0.038 mg/100g dry material was recorded for the two plants belonging to the Fabacea family *(S. Siamea* and *S.alata)*. This family may hold promise as a reservoir for antioxidants and may warrant further investigation. *V. amydalina* a plant well known to have anti-cancer and antitumor properties (Khalafalla *et al*, 2009; Izevbigie, 2003) also exhibited a high total antioxidant content of 1815.36 ± 0.471 mg/100g dry wt. the ethanol extracts of *D. pinnata* had almost a third of total antioxidants compared with these three plants. These observations may be explained by the fact that alcohol extracts a wide range of components when used as a solvent than water which limits itself to the highly hydrophilic compounds (Harbone 1998). Feedback from ethanolic extracts will be a combination of both hydrophyllic and lipophyllic antioxidants present in the plants and might account for the higher antioxidant properties seen. Even though *S. torvum* a member of the Solonacea family had the lowest total antioxidant content in both aqueous and ethanolic media all the plants evaluated had appreciable amount of antioxidant capacity.

Regarding their $DPPH^{\bullet}$ scavenging activities the results are displayed in table 3. All undiluted extracts exhibited remarkable antioxidant activities thus all were diluted to a working concentration of 1mg/ml, assayed for $DPPH^{\bullet}$ free radical activity and compared with gallic acid. $DPPH^{\bullet}$ quenching activity ranged from a low of 6.2% to 87%. Both extracts of *Senna siamea* gave the highest quenching response of 87.767 ± 0.081 % and 76.751 ± 001% for ethanolic and aqueous extracts respectively. Again *V. amygdalina* and *D. pinnata* had almost the same effects from both extracts which is in agreement with the trend observed for total phenolics and flavonoids. Aqueous extracts of *G. sp* and *Solanum torvum* possessed higher free radical scavenging properties than their ethanolic counterparts by more than two fold. Given that these are only crude extracts, their ability to scavenge free radicals is very significant necessitating future scrutiny for specific lead molecules.

5. Conclusions:

Herbs exhibit their effects owing to a variety of constituents some of which have been qualitatively determined in the present study which also revealed that extracts from these plants are rich in antioxidants. The occurrence of phenolic compounds, flavonoids and the phytochemicals evaluated might be responsible for the high antioxidant capacities in these medicinal plants. From our results it is likely that a combination of the various plants may have higher antioxidant activity than individual components which may explain why some of them are formulated (Asase et al, 2005). These facts may justify the medicinal use of the plant for the treatment of malaria by minimizing antioxidant stress or contain some compounds toxic to the malaria parasite. However, further *in vivo* work is necessary to ascertain the clinical utility and safety of extracts from the plant and to determine appropriate concentration for therapy so as to safeguard the health of the teeming mass of traditional users who do not typically take these

factors into consideration. Our *in vitro* antioxidant studies provide sound scientific footing to enhance confidence on the traditional claims that some of these medicinal plants could be novel antioxidant reservoirs and formulae for malaria and many diseases associated with free radicals generation. We conclude that all six plants studied namely *Deinboulia pinnata*, *Greenwendondron sp*, *Senna siamea*, *Senna alata*, *Vernonia amygdalina*, and solanum torvum may have a definite role to play to reduce oxidant stress in malaria subjects.

6. Acknowledgement:

We are grateful to the Head of the Nutrition Research Centre, Mr. David Bansa as well as Ms Akushika Diaba and Mr. Christian Edward Brown for their invaluable assistance during this study.

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4/11/2011