European Journal of Medicinal Plants
1(2): 33-39, 2011

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Total Phenolic Contents and Lipid Peroxidation Potentials of Some Tropical Antimalarial Plants

H. O. T. Iyawe[1*] and M. C. Azih[2]

[1]Department of Biochemistry, Ambrose Alli University, P. M. B. 14, Ekpoma, Nigeria.
[2]Department of Chemistry, Ambrose Alli University, P. M. B. 14, Ekpoma, Nigeria.

Research Article

Received 9th January 2011
Accepted 11th January 2011
Online Ready 25th March 2011

ABSTRACT

In this investigation extracts of leaves and barks from five tropical antimalarial plants namely; *Magnifera indica*, *Anacardium occidentale*, *Azachiractha indica*, *Carica papaya Linn* and *Cymbopogm citrates* were studied *in vitro* for their total phenolics, total flavonoids and inhibition of lipid peroxidation abilities. Crude extracts from each plant material were obtained by maceration in ethanol and water respectively. The FolinCiocalteu procedure was used to assess the total phenolic concentrations of the extracts and results expressed as gallic acid equivalents (GAE). Total flavonoid contents in extracts were determined by the aluminium chloride colorimetric assay and expressed as quercetin equivalents (QAE). The percentage inhibition of lipid peroxidation was assayed by estimating the thiobarbituric acid-reactive substances (TBARS). The phenolic contents in water extracts of *Anacardium occidentale* leaves was 452.57 ± 8.08mg/gGAE and that of bark was recorded as 267.15 ± 6.06mg/gGAE. The ethanolic and water extracts of *Azachiractha indica* bark were found to be 310.71 ± 7.07mg/gGAE and 390.64 ± 6.97mg/gGAE respectively. The extracts of *Magnifera indica* leaves had the highest flavonoid content of 139.08 ± 0.77mg/100gQAE in ethanol and 69.55 ± 0.39 mg/100gQAE in water. The least values observed were 21.19 ± 0.64 mg/100gQAE for water extract of *Anacardium occidentale* leaves and 30.73 ± 0.26 mg/100gQAE for ethanolic extract of *Anacardium occidentale* bark. Inhibition of lipid peroxidation in liver and kidney were observed as 15.92 ± 3.01% and 17.10 ± 3.48% in ethanolic extracts of *Anacardium occidentale* bark and leaves respectively while it was 30.67 ± 0.47% for *Carica papaya Linn*. The water extract of *Azachiractha indica* bark inhibited liver lipid peroxidation by 8.70 ± 0.32% while that of *Anacardium occidentale* bark inhibited kidney lipid peroxidation by 11.78 ± 1.08%. These results suggest a need for further examination of the water extract of *Anacardium occidentale* bark as this part of the plant appears to be critical in the phytotherapy of malaria infection.

Keywords: Antimalarial plants, phenolics, flavonoids, lipid peroxidation;

**Corresponding author: Email: iyawehanson@yahoo.com*

1. INTRODUCTION

Africa is a continent endowed with an enormous wealth of plant resources, where over five thousand distinct plant species are known to occur. In this region phytotherapy plays a vital role in the management and treatment of infections and a World Health Organisation (WHO) report put the population of Africans using traditional plants and herbs to overcome their health problems at over 80% (Bum et al., 2010). The role of medicinal plants in disease prevention or control has been attributed chiefly to the antioxidant properties of their constituents, usually associated with a wide range of amphipathic molecules broadly termed polyphenolic compounds (Demiray et al., 2009).

Phenolic compounds are diverse plant secondary metabolites comprised of aromatic rings, bearing one or more hydroxyl substituents and range from simple phenolic molecules like phenol to highly polymerized compounds often referred to as polyphenols. Plant polyphenols are known to have multifunctional properties such as ability to act as reducing agents, and singlet oxygen quenchers, in addition to their hydrogen donating properties. The largest and most important group of polyphenols are the flavonoids and their derivatives with their capacity to act as antioxidants protecting the body against reactive oxygen species [ROS] (Aberoumand and Deokule, 2008; Michalak, 2006).

ROS from both endogenous and exogenous sources are involved in the aetiology of diverse human diseases (Kiselova et al., 2005) including malaria (Potter et al., 2005) and consumption of plant materials are reported to be associated with reduced incidence of these pathologies (Kiselova et al., 2005).

A large number of medicinal plants have been shown to have beneficial therapeutic potentials in the treatment of malaria infections (Ayoola et al., 2008).

Popular among these plants are *Magnifera indica, Anacardium occidentale, Azachiractha indica, Carica papaya Linn* and *Cymbopogm citrates*. Qualitative phytochemical screening of some antimalarial plants have shown the presence of flavonoids, terpenoids, saponins, tannins and reducing sugars (Ayoola et al., 2008). Quantitative data on the phytochemicals of many antimalarial plants used as treatment for malaria infection in the tropics are rare in literature and there is a dire need for such scientific information. It is on the strength of this rationale that we decided to conduct this study, determining the lipid peroxidation potentials, total phenolics and flavonoid contents of some tropical antimalarial plants

2. MATERIALS AND METHODS

2.1 COLLECTION AND IDENTIFICATION OF PLANT MATERIALS

Fresh leaves and the stem barks of *Anacardium occidentale, Azachiractha indica,* and *Magnifera indica* and fresh leaves only of *Carica papaya Linn* and *Cymbopogm citrates* were collected from the premises of Ambrose Alli University. The plants were identified and authenticated in Botany Department of the University.

2.2 EXTRACTION OF PLANT MATERIALS

The plant materials (leaves and the stem barks) were air-dried at 80°C with a size 2 air-circulation Gallenkamp Hotbox oven for one week, after which the samples were pulverized with a type 14-580S Glen Creston hammer miller to pass through a 40mm sieve. The crude ethanol extracts were prepared by maceration of 5g each of the dry powdered plant materials in 100mL of ethanol

at room temperature over night. The extracts were filtered after 24h; through a Whatman No. 1 filter paper under laid with cotton wool. Water crude extracts were obtained through same processes except that 10g of each sample was macerated with 200mL of distilled water. The extracts prepared were preserved in a refrigerator for further use.

2.3 REAGENTS AND INSTRUMENTS

Folin-Ciocalteu reagent and ascorbic acid were from Merck (Darmstadt, Germany). Gallic acid and quercetin standards were obtained from Sigma–Aldrich Quimica (Alcobendas, Spain). All other reagents used were of analytical grade. UV spectra were recorded with Shimadzu 210A double beam spectrophotometer.

2.4 ANIMALS

Three inbred adult wistar albino rats (150 –200 g) of either sex were obtained from the University animal house. The animals were initially kept in cages at 28 ± 2°C, with relative humidity 45-55% under 12h light and dark cycles. The animals were acclimatized to the laboratory conditions for a week before use. They were fed with standard animal feed and water *ad libitum*.

Liver and kidney homogenates used for lipid peroxidation assay were obtained from the animals after anaesthesia with chloroform. The tissues were separately homogenised with mortar and pestle in 1M phosphate buffer and the crude homogenates were immediately used for assay.

2.5 DETERMINATION OF TOTAL PHENOLICS

Total phenolic contents in the extracts were determined by the Folin-Ciocalteu reagent method described by Demiray et al., (2009). Briefly, an aliquot of the extracts was mixed with 5 mL Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 mL (75g/L) of sodium carbonate. The tubes were vortexed for few seconds and allowed to stand for 30 min at 26°C for color development. Absorbance of samples and standard were measured at 765 nm using Shimadzu 210A double beam spectrophotometer. Total phenolic content was expressed as mg/g gallic acid equivalent using the following equation based on the calibration curve: $y = 0.007x$ and $R2 = 0.995$, where x was the absorbance and y was the tannic acid equivalent (mg/g).

2.6 DETERMINATION OF TOTAL FLAVONOIDS

Total flavonoid contents in standards and samples were estimated by the aluminium chloride colorimetric assay method earlier reported by Krishna et al., (2010). Briefly, a volume of 0.5 ml of 2% $AlCl_3$ ethanol solution was added to 0.5 ml of sample solution. After one hour at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoids. Total flavonoid contents were calculated as quercetin (mg/100g) using the following equation based on the calibration curve: $y= 0.055x$ and $R2 = 0.999$, where x was the absorbance and was the quercetin equivalent (mg/100g).

2.7 DETERMINATION OF INHIBITION OF LIPID PEROXIDATION

Lipid peroxidation was induced with ferrous sulphate and the degree of lipid peroxidation was assayed by estimating the thiobarbituric acid-reactive substances (TBARS) while inhibition of lipid peroxidation was assessed in the presence of sample extracts as described by Ilavarasan et al., (2005).

2.8 STATISTICAL ANALYSIS

Data are expressed as Mean ± SD. Student's t test was used for statistical comparison between ethanolic and aqueous extract and p-values less than 0.05 were considered significant.

3. RESULTS AND DISCUSSION

The total phenolic contents (Table 1) determined in water were found to be higher ($p<0.05$) in comparison to that of ethanolic extracts with the exception of *Anacardium occidentale* and *Cymbopogm citrates* barks.

Table 1. Total phenolic contents (mg/gGAE) in some antimalarial plants

Name of plant	Parts	Ethanolic extract.	Water extract	Eth. /Wat. Ratio
Anacardium occidentale	Leaves	58.57±0.81a	452.57±8.08b	0.13
	Bark	268.57±2.02a	267.15±6.06a	1.01
Magnifera indica	Leaves	65.00±1.01a	157.72±3.23b	0.41
	Bark	90.72±1.01a	112.58±2.43b	0.81
Azachiractha indica	Leaves	18.43±1.01a	68.00±1.22b	0.27
	Bark	310.71±7.07a	390.64±6.97b	0.80
Cymbopogm citrates	Leaves	28.30±0.70a	32.57±1.69a	0.87
Carica papaya Linn	Leaves	21.80±0.85a	51.43±1.61b	0.42

Data are presented as Mean ± SD of triplicate determinations (n=3).
Values in same row with different letters are significantly different (P<0.05).

Total flavonoid contents (Table 2) in samples were observed to be generally higher ($p<0.05$) in ethanol in comparison with water extracts with *Anacardium occidentale* being an exception.

Inhibitions of lipid peroxidation (Table 3) by aqueous and ethanolic extracts were observed to be lower in magnitude in comparison with standard ascorbic acid. However, significant differences ($p<0.05$) were noted between the ability of the extracts of *Magnifera indica* and *Azachiractha indica* barks as well as between *Magnifera indica* and *Carica papaya* leaves to inhibit lipid peroxidation in liver and kidney tissues. On the other hand, water extracts of *Anacardium occidentale* bark and leaves as well as the extracts from *Magnifera indica* and *Azachiractha indica* leaves were different ($p<0.05$) in their degree of lipid peroxidation prevention in liver and kidney tissues.

Natural phenolics exert their beneficial health effects mainly through their antioxidant activity by decreasing oxygen concentration, intercepting singlet oxygen, preventing 1st-chain initiation by scavenging initial radicals such as hydroxyl radicals, binding metal ion catalysts, decomposing primary products of oxidation to non radical species, and breaking chains to prevent continued hydrogen abstraction from substances (Xu and Chang 2007).

Table 2. Total flavonoid contents (mg/100gQE) in some antimalarial plant

Name of plant	Parts	Ethanolic extract.	Water extract	Eth. /Wat. Ratio
Anacardium occidentale	Leaves	76.54±0.77a	21.19±0.64b	3.61
	Bark	30.73±0.26a	87.37±0.90b	0.35
Magnifera indica	Leaves	139.08±0.77a	69.55±0.39b	2.00
	Bark	108.17±0.78a	53.60±1.08b	2.02
Azachiractha indica	Leaves	50.18±0.52a	27.28±3.60b	1.84
	Bark	39.09±2.83a	27.36±0.13b	1.43
Cymbopogm citrates	Leaves	83.45±0.26a	49.09±0.25b	1.70
Carica papaya Linn	Leaves	106.36±0.77a	53.19±0.39b	2.00

Data are presented as Mean ± SD of triplicate determinations (n=3).
Values in same row with different letters are significantly different (P<0.05).

Table 3. Inhibition (%) of tissue lipid peroxidation by some antimalarial plants

Name of plant	Parts	Ethanolic extract.		Water extract	
		Liver	Kidney	Liver	Kidney
Control	-	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
Anacardium occidentale	Leaves	5.12±0.88a	5.26±1.06a	2.93±0.11a	4.99±0.98b
	Bark	15.92±3.01a	9.61±2.00a	3.20±1.08a	11.79±1.08b
Magnifera indica	Leaves	5.34±0.10a	17.10±3.48b	5.05±0.81a	3.76±0.52a
	Bark	3.43±0.64a	0.81±0.16b	2.59±0.23a	4.29±0.22b
Azachiractha indica	Leaves	10.75±2.35a	6.89±1.44a	5.05±0.81a	1.68±0.33b
	Bark	8.69±0.56a	2.56±0.21b	8.70±0.32a	5.45±0.72b
Cymbopogm citrates	Leaves	2.69±0.38a	4.48±0.56a	5.00±0.42a	2.86±0.64a
Carica papaya Linn	Leaves	4.97±0.34a	30.67±0.47b	5.66±1.55a	7.36±0.63a

Data are presented as Mean ± SD of triplicate determinations (n=3).
Values in same row with different letters are significantly different (P<0.05).

The total phenolics contents in barks and leaves of samples as shown in Table 1 indicate that the phenolics in these plants are more hydrophilic and therefore better extracted with water as seen in the ethanol/water ratio. The aqueous extracts of leaves and bark of *Anacardium occidentale* and *bark of Azachiractha indica* stood out as having the highest values of total phenolic contents. The high contents of total phenolics in aqueous extracts of leaves and bark of *Anacardium occidentale* and bark of *Azachiractha indica* may explain the preference of water concoction of *Anacardium occidentale* and bark of *Azachiractha indica* in treating malarial infection. In cases of severe parasite infections, hot water extractions of these plant parts are preferred and the reason

may probably be due to greater extraction of total phenolics from the plants. Phenolic compounds are recognised as a class of antioxidant agents which act as free radical terminators (Doss, et al., 2010).

Apart from the water extract from the bark of *Anacardium occidentale* that had a high total flavonoid content, all other parts of the plants under study had better ethanolic extraction of total flavonoids. According to Pietta, (2000), flavonoids represent the most common and widely distributed group of plant phenolics with major flavonoid classes that include anthocyanidins, chalcones, flavanols, flavanones, flavones, flavonol, and isoflavones. Each of the major class may be monomeric, dimeric, oligomeric or polymeric (Pietta, 2000). This study restricts to the quantifications of total flavonoids in plant sample under investigation and there is hence a need for further investigation to know the class of flavonoid and types present in each plant sample.

The ethanolic extracts from *Carica papaya* and *Anacardium occidentale* leaves showed high degrees of inhibition of lipid peroxidation in kidney tissues. In the same vein, ethanolic extracts from *Anacardium occidentale* bark and *Magnifera indica* leaves inhibited lipid peroxidation in liver tissues. These observations on the levels of lipid peroxidation by the different plant parts used in this study are novel. The uniqueness of *Carica papaya* and *Anacardium occidentale* leaves in inhibiting lipid peroxidation in kidney tissues may be given to a particular flavonoid class that may be dominant in the ethanolic extracts of these plant parts, as flavonoids are known to exist in different classes (Pietta, 2000). Since the mechanisms of action of flavonoids are through scavenging or chelating processes (Doss, et al., 2010), these plant parts are therefore valuable in maintaining the membrane integrity of these organs.

The aqueous extract of *Anacardium occidentale* and *Azachiractha indica* barks had the highest degrees of lipid peroxidation in kidney and liver tissues. In most of malaria endemic areas, the aqueous extracts of these plants and not the ethanolic extracts are most often used to treat malaria infection.

4. CONCLUSION

Arising from the observations recorded in this study, we draw the conclusion that the preferred use of aqueous crude extract over ethanolic type are for safety and convenience, as ethanolic intake may have negative implications on the already stressed organs.

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