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EVALUATION OF PHYTOCHEMICALS AND ANTIOXIDANTS OF FOUR BOTANICALS WITH ANTIHYPERTENSIVE PROPERTIES.

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ABSTRACT

Evaluation of phytochemicals and antioxidants levels were conducted in the dry leaves of aqueous and methanolic extracts of *Vernonia amygdalina, Carica papaya, Persea americana* and *Cnidosculous aconitifolius.* Methanolic extract was found to contain the highest value for sterols, tannin, saponin, flavonoids, alkaloids, phenols, phlobatannins, anthraquinones, triterpenes and cardiac glycosides. However, oxalate and phytate were absent in both aqueous and methanolic extracts. The results also showed that leave extracts of these plants are good sources of antioxidants as revealed by the presence of these antioxidants in these plant but with highest values in the methanolic extract. Conclusively, the presence of some of these phytochemicals and antioxidants explained the hypotensive action of these plants in cardiovascular diseases.

KEYWORDS

Phytochemicals, antioxidants, extracts, aqueous, methanolic

INTRODUCTION

The importance of medicinal plants in orthodox medicine can not be overemphasized. Various diseases treated with medicinal plants include respiratory infection, diarrhea, fever, hypertension, obesity, diabetes mellitus among others.

Vernonia amygdalina

Vernonia amygdalina is a shrub in the family of *Asteraceae*. The leaves are green with characteristic odour and a bitter and sweet taste[1]. It is common in most West African and Central African countries. *Vernonia amygdalina* is well known as a medicinal plant with several uses attributed to it. It stimulates digestive system and helps in reduction of fever. It is also used in making beer in Nigeria and as a remedy against high blood pressure[2].

Cnidoscolous aconitifolius

Cnidoscolous aconitifolius also known as Chaya[3] is an arbrescent shrubs of 6cm in height with alternate palmate lobed leaves, milky sap and small flowers on dichotomous branched chymes[4]. The leaves are large and are usually 32cm long and 30cm wide. *Cnidoscolous aconitifolius* originated as a domesticated leafy green vegetable in the Maya region of Guatemele, Belize, Southeast Mexico during pre-Cambrian

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period[5]. It traditionally has been recommended for the treatment of various ailments such as diabetes, obesity, kidney stone, acne and eye problem[6]. Chaya leaves have been taken as a laxative, circulation stimulant, to improve digestion and diuretic[7], hence its involvement in the regulation of blood pressure.

Carica papaya

Carica papaya is a large, woody herb that can grow up to 12feet in height with a single cylindrical trunk with leaves jutting from the top of the trunk connected by a long stem. It is a native of the tropics of the Americans and originated from Mexico[8]. *Carica papaya* has been used as folk medicine with the following properties: laxative, analgesic, antibacterial, amebicide, cardiotonic and hypertensive[9].

Persea americana

Persea americana is a tree that may be erect, usually to 60 feet with a trunk of 30-60cm in diameter. The leaves of *Persea americana* are alternate, dark green and glossy on the upper surface and whitish on the underside. The leaves are also variable in shape, they may be elliptic, oval and ovate or obovate. *Persea americana* has been used for a variety of medical applications. The leaves extract has antibiotic activity and hypotensive effect[10]. The leaves decoction is taken as remedy for diarrhea, sore throat and heamorrage[11].

Various substances such as oxalate, saponin, phytate, alkaloids, phenolic compounds, basic metabolites, glycosides as well as secondary metabolites have been found to be present in plants used in treating various diseases[12,13,14]. These active substances are good precursors of drug in drug manufacturing[15].

Antioxidants have been found to be substances that when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevent oxidation of that substrate known as free radicals[16]. Oxidative stress is thought to contribute to the development of a wide range of diseases including Alzheimer' disease, Parkison's disease, diabetes and cardiovascular diseases. The role of oxidative stress in cardiovascular diseases is well understood. Here, Low Density Lipoprotein (LDL) oxidation appears to trigger the process of atherogenesis, which results in atherosclerosis and finally cardiovascular diseases. Antioxidants perform important roles in protecting the human body against damage induced by reactive free radicals produced a as result of oxidative stress[17].

The present study was carried out to evaluate the potential of these medicinal plants in terms of their phytochemicals and antioxidant compositions for their use in the treatment of cardiovascular diseases.

MATERIALS AND METHODS

(i) ***Collection and preparation of the plant materials***

Fresh leaves of of *Vernonia amygdalina, Carica papaya, Persea americana* and *Cnidosculous aconitifolius* were collected separately from a farm in Ado-Ekiti, Ekiti State, Nigeria and were identified at the Herbarium of the Department of Plant Science, University of Ado-Ekiti, Nigeria. The leaves were washed with sterile distilled water to remove dust and air dried and ground into fine powder using a blender. The aqueous and methanolic extracts of the leaves of *Vernonia amygdalina, Carica papaya, Persea americana* and *Cnidosculous aconitifolius* were prepared by soaking 100g of dried powdered samples in 500ml of distilled water and methanol respectively.

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(ii) ***Analysis***

Quantitative determination of phytochemicals were carried out for phenolic[18], flavonoids[19], steroids[20], alkaloids[21], tannins[22], saponins[23], anthraquinones, triterpenes, phlobatannins and cardiac glycosides[24]. Evaluation of the concentration of non-enzymatic antioxidants were made for carotene[25], ascorbic acid[26] and α-tocopherol[27]. Activities of enzymatic antioxidants were done for catalase[28], Glutathione peroxidase[29] and superoxide dismutase[30].

RESULTS AND DISCUSSION.

Table 1

Phytochemical constituents (%) of methanolic extract of the leaves of the experimental medicinal plants.

Phytochemicals	***Vernonia amygdalina***	***Carica papaya***	***Cnidosculous aconitifolius***	***Persea americana***
Sterols	3.50± 0.37	1.22± 0.89	2.55± 0.75	3.71± 0.57
Tannin	1.55± 0.81	1.11 ± 0.12	1.50± 0.81	1.60± 0.38
Saponin	2.85± 0.39	1.80± 0.91	2.85± 0.39	2.99± 0.39
Flavonoids	0.17 ± 0.004	0.08± 0.001	0.35± 0.11	0.58± 0.09
Alkaloids	2.95± 0.40	1.35± 0.81	2.66± 0.43	2.75 ± 0.81
Phenol	1.95± 0.38	1.14 ± 0.22	1.80 ± 0.43	1.22± 0.52
Phlobatannin	1.50± 0.81	1.21± 0.07	1.31± 0.08	1.62 ± 0.02
Anthraquinones	0.08± 0.001	0.05± 0.001	0.06± 0.001	0.04 ± 0.001
Triterpenes	0.54 ± 0.02	0.32 ± 0.001	0.40 ± 0.001	0.43± 0.001
Cardiac glycosides	1.10 ± 0.03	0.91± 0.09	1.51± 0.31	1.00± 0.11
Oxalate	ND	ND	ND	ND
Phytate	ND	ND	ND	ND

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Table 2

Phytochemical constituents (%) of aqueous extract of the leaves of the experimental medicinal plants.

Phytochemicals	*Vernonia amygdalina*	*Carica papaya*	*Cnidosculous aconitifolius*	*Persea americana*
Sterols	2.10 ± 0.18	0.88 ± 0.11	0.003 ± 0.001	2.50 ± 1.12
Tannin	1.11 ± 0.21	0.71 ± 0.04	0.89± 0.12	1.50 ± 0.91
Saponin	1.80 ± 0.38	0.91 ± 0.13	2.00 ± 0.32	2.00± 0.92
Flavonoids	0.02 ± 0.001	0.02 ± 0.001	0.19± 0.001	0.50± 0.02
Alkaloids	1.72 ± 0.53	1.32 ± 0.48	2.01± 0.21	2.11± 0.82
Phenol	0.92 ± 0.08	0.32 ± 0.05	1.21± 0.31	1.31± 0.94
Phlobatannin	0.91 ± 0.08	0.51 ± 0.001	0.50 ± 0.01	1.11± 0.03
Anthraquinones	0.02 ± 0.001	0.01 ± 0.001	0.02± 0.001	0.12± 0.001
Triterpenes	0.02 ± 0.001	0.01± 0.001	0.05 ± 0.001	0.34± 0.11
Cardiac glycosides	0.23 ± 0.01	0.21± 0.001	0.21± 0.01	0.21± 0.01
Oxalate	ND	ND	ND	ND
Phytate	ND	ND	ND	ND

Table 3

Antioxidants in the methanolic extract of the experimental medicinal plants.

Phytochemicals	*Vernonia amygdalina*	*Carica papaya*	*Cnidosculous aconitifolius*	*Persea americana*
Carotenes (mg/100g)	*46.32 ±0.11*	*34.91±11.22*	*50.02 ±13.18*	*85.23± 16.76*
Ascorbic acid (mg/100g)	*11.87 ±5.22*	*11.05 ± 3.11*	*14.38 ± 5.22*	*19.20 ±4.33*
α-tocopherol (µmol/l)	*18.38± 7.12*	*15.22 ± 5.18*	*14.11 ± 6.02*	*20.34 ±5.38*
Glutathione peroxidase (µg/l)	*15.90± 0.38*	*11.49 ± 0.15*	*10.32 ± 0.12*	*18.50± 7.66*
Superoxide dismutase (µg/l)	*20.22± 5.08*	*14.21± 4.32*	*18.11 ± 6.11*	*22.50 ±12.18*
Catalase (µg/mg)	*15.11 ±5.28*	*10.31 ± 4.82*	*16.18 ± 8.22*	*21.50 ±10.22*

Table 4

Antioxidants in the aqueous extract of the experimental medicinal plants.

Phytochemicals	*Vernonia amygdalina*	*Carica papaya*	*Cnidosculous aconitifolius*	*Persea Americana*
Carotenes (mg/100g)	25.22 ± 5.34	30.21 ± 10.22	33.20 ± 12.11	21.11± 5.22
Ascorbic acid (mg/100g)	5.22 ± 2.50	4.21± 1.38	7.22± 2.50	6.28± 2.11
α-tocopherol (µmol/l)	10.11± 3.82	5.21± 2.00	4.11± 1.38	5.24± 2.18
Glutathione peroxidase (µg/l)	9.20± 2.80	4.21± 1.81	3.22 ± 1.08	3.10 ± 1.11
Superoxide dismutase (µg/l)	9.55± 4.22	3.34 ± 1.03	6.34 2.11	11.50 ±2.82
Catalase (µg/mg)	7.52 ± 4.33	4.38± 1.81	4.50 ± 1.39	10.34 ± 3.50

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The quantitative phytochemical analysis of the dry leaf aqueous and methanolic extracts of *Vernonia amygdalina, Carica papaya, Persea americana* and *Cnidosculous aconitifolius* revealed the presence of some potent bioactive compounds (Tables 1 and 2). However, the concentrations of these phytochemicals are higher in methanolic extracts than in aqueous extracts. The presence of sterols in the leave extracts of these plants helps in lowering plasma cholesterol and therefore preventing LDL oxidation which occurs in hypertension. Hence, the use of the leaves of these plants in the management of hypertension. Similar observation has been reported[14].

The results obtained for antioxidants (Tables 3 and 4) followed similar trend as for that of phytochemicals (Tables 1 and 2). Antioxidants were found to be more abundant in methanolic extracts (Table 3) than the aqueous extracts (Table 4). The concentration of α-tocopherol was found to be relatively high in both extracts, however, *Pearsea americana* has the highest value for α- tocopherol. (20.34 $\pm$ 5.38 μmol/l). This suggests that elevated levels of α-tocopherol may be one of the factors responsible for the antihypertensive effects of these plants. α-tocopherol protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction that occurs in cardiovascular diseases. Ascorbic acid is present at high concentrations in all the extracts of the plants. Ascorbic acid is a reducing agent and therefore can reduce, and thereby neutralize, reactive oxygen species such as hydrogen peroxide, hence the use of these plants as potent hypotensive agents.

The activities of the enzymatic antioxidants (glutathione peroxidase, catalase and superoxide dismutase) were also found to be high in both aqueous and methanolic extracts of the plants. This also confirms the involvement of these plants in the management of cardiovascular diseases. Cells are protected against oxidative stress by interacting network of antioxidant enzymes. For instance, SOD catalyses the breakdown of the superoxide anion into oxygen and hydrogen peroxide. Catalase helps in removing hydrogen peroxide from the circulation.

From the above results, the concentrations of phytochemicals, non enzymatic antioxidants (carotenes, ascorbic acid and α-tocopherol) and the activities of enzymatic antioxidants (glutathione peroxidase, catalase and catalase) were high in methanolic extracts of the plants indicating that these compounds are best extracted for their maximum activity in methanol. The presence of phytochemicals and antioxidants in the leaves of the plants contributes to their medicinal value as antihypertensive in the treatment and management of cardiovascular diseases and thus they can be potential sources of useful drugs.

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