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PHYTOCHEMICAL SCREENING AND IN-VITRO ANTIOXIDANT ACTIVITY ON *VERNONIA AMYGDALINA* (EWURO- BITTER LEAF)

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ABSTRACT: *Most of the plants exhibit a variety of phyto-pharmaceuticals, which have important applications in the fields of agriculture, human and veterinary medicine. This study was conducted to evaluate phytochemical screening and in-vitro antioxidant activity on Vernonia amygdalina (bitter leaf) at the Department of Biological Sciences, Federal Polytechnic, Ado Ekiti. It was revealed that the phytochemical constituents in Bitter leaf (Vernonia amygdalina) were alkaloids, saponin, tannins, terpenoids, flavonoids and cardiac glycosides in the extract. Anti-oxidant property results of the aqueous extract of Bitter leaf (Vernonia amygdalina) showed Total phenolic content was 120.16mg Gallic acid equivalent/g extract while the total flavonoid was 235.147mg Quercetin equivalent/g extract. The plant could be exploited as source of antioxidant additives and used for future project to evaluate the potentials of Bitter leaf (Vernonia amygdalina) as a strong medicinal plant in improving human health status.*
KEYWORDS: phytochemical, in vitro, *vernonia amygdalina*, anti-oxidant, Nigeria

INTRODUCTION

Most of the world's population depends on plant due to its medicinal values and scarcity (Hudaib *et al*., 2008). Medicinal plants have been used for the treatment of illness since ancient period (Gajalaksmi *et al*., 2012). Despite the immense technology advancement in modern medicine, 75% of African populations still rely on traditional medicinal plants for their daily healthcare needs. Medicinal plants are used in treatment of diseases either alone or in combination with other plants.

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are non-essential nutrients, meaning that they are not required by the human body for sustaining life. It is well-known that plants produce these chemicals to protect themselves but recent research demonstrates that they can also protect against diseases (Breslin, *et al*., 2017).
Vernonia amygdalina Del. (bitter leaf) is a medicinal plant and fresh bitter leaf of great importance in human diet because of the presence of vitamins and mineral salts (Sobukola, and

Dairo, 2007). It is a very important protective food and useful for the maintenance of health and prevention and treatment of various diseases.The plant (especially the leaf) has been found useful in the ethno-therapy of diabetes (Nwajo, 2005), asthma, headache (Akah, Okoli, and Nwafor, 2002), skin infections such as ringworm, rashes and eczema, schistosomiasis, malaria (Masaba, 2000), measles, diarrhea, tuberculosis, abdominal pain and intestine complaints as well as fevers, cough, induction of fertility in barren women and hyperlipidemia (Adaramoye *et al*., 2008).

Anthelmintic, antimalarial, anti-tumourigenic as well as bacteriostatic and bactericidal effect on some bacteria properties of *Vernonia amygdalina* extracts has been employed (Izevbigie *et al.,* 2004). Specifically, Nwajo (2005) reported the hypoglycaemic and hypo-lipidaemic effect of the leave extracts *in vivo*. Several studies carried out on this plant had suggested that it contains different bioactive compounds, including: flavonoids, saponins, alkaloids, tannins, phenolics, terpenes, steroidal glycosides, triterpenoids, and several types of sesquiterpene lactones (Erasto *et al.,* 2007; Farombi and Owoeye, 2011; Toyang and Verpoorte, 2013; Luo *et al.,* 2017).

MATERIALS AND METHODS

Study area
The study was carried out at the Department of Biological Sciences, Federal Polytechnic, Ado-Ekiti, Nigeria.

Plant Materials
Fresh Bitter leaves (*Vernonia amygdalina*) were collected from a private farm in Ado Ekiti Ado Ekiti Local Government Area of Ekiti State, Nigeria, in the month of December, 2018. Botanical Identification and authentication was carried out at the Department of Biological Sciences, The Federal Polytechnic, Ado Ekiti.

Chemicals
DPPH (2, 2-Diphenyl-1-Picryl Hydrazyl), a product of Sigma Pharmaceuticals, China, was obtained from Rovet Chemicals, Benin City, Nigeria. All other chemicals were of analytical grade and were obtained from British Drug Houses, (Poole, UK). The water used was glass distilled.

Preparation of Aqueous Extract of Bitter Leaf (*Vernonia amygdalina*)
The leaves were air-dried for 30 days at room temperature. The air-dried samples were ground to fine powder using a blender. A 500 g sample of the powdered material was soaked in 1200 ml of distilled water for 72 hours. This was filtered and was later freeze-dried to obtain the extract powder. The extract was kept in the freezer at 4 ^{0}C for further studies.

Phytochemical Screening
Phytochemical screening of the extract was carried out to identify the constituents, using standard phytochemical methods as described by Trease and Evans (1989) as well as Sofowora (1993). The screening involves detection of alkaloids, flavonoids, terpenoids, saponin, tannins, anthraquinones, and cardiac glycosides.

Data Analysis
All values are expressed as mean ± SD of triplicate results and ANOVA were used to analysis variance.

RESULTS

Presented in Table 1 were the phytochemical constituents of Bitter leaf (*Vernonia amygdalina*) aqueous extract which revealed the presence of alkaloids, saponin, tannins, terpenoids, flavonoids and cardiac glycosides in the extract.

Table 1*: The Phytochemical Constituents of Bitter leaf (*Vernonia amygdalina*) aqueous extract

Phytochemical Constituents	Results
Alkaloid	Present
Saponin	Present
Tannins	Present
Phlobatannin	Present
Anthraquinones	Absent
Glycosides	Present
Flavonoids	Present

In vitro Antioxidant properties
Table 2 showed the antioxidant property results of the aqueous extract of Bitter leaf (*Vernonia amygdalina*). Total phenolic content was 120.16mg Gallic acid equivalent/g extract while the total flavonoid was 235.147mg Quercetin equivalent/g extract.

Table 2: In vitro Antioxidant indices of Bitter leaf (*Vernonia amygdalina*) aqueous extract

Property	Level
Total Phenol	120.16mg Gallic acid equivalent/g extract
Total flavonoid	235.147mg Quercetin equivalent/g extract

DPPH Radical Scavenging Activity of aqueous extract of Bitter leaf (*Vernonia amygdalina*)

The result of the DPPH radical scavenging activity of aqueous extract of Bitter leaf (*Vernonia amygdalina*) was presented in **Table 3**. The percent of DPPH radical scavenged by aqueous extract of Bitter leaf (*Vernonia amygdalina*) and standard quercetin increased with increasing concentrations. The highest concentration of the extract 400µg/ml had the highest scavenging activity (85.42 ± 0.25) which compares favorably with the standard (96.50 ± 0.20).

Table 3: DPPH Radical Scavanging Activity of Bitter leaf (*Vernonia amygdalina*) aqueous extract

Concentration Standard (µg/ml)	(*Vernonia amygdalina*) extract (% scavenging activity)	Quercetin (% scavenging activity)
0	0	0
10	22.49 ± 0.18	33.17 ± 0.15
25	36.26 ± 0.23	46.43 ± 0.20
50	52.01 ± 0.24	59.05 ± 0.19
100	61.23 ± 0.24	73.65 ± 0.26
200	81.38 ± 0.14	93.17 ± 0.17
400	85.42 ± 0.25	96.50 ± 0.20

Values are expressed as mean ± standard deviation (n=3).

Metal chelating Activity of aqueous extract of Bitter leaf (*Vernonia amygdalina*)

The result of metal chelating activity of **aqueous extract of Bitter leaf (*Vernonia amygdalina*)** was presented in **Table 4**. The metal chelating activity of aqueous extract of Bitter leaf (*Vernonia amygdalina*) was concentration-dependent and the highest activity was obtained for the highest concentration (250µg/ml). The metal chelating effect of the extract compared well with the standard at similar concentrations.

Table 4: Metal chelating activity of Bitter leaf (*Vernonia amygdalina*) aqueous extract

Concentration (µg/ml)	Extract (% chelating activity)	EDTA (% chelating activity)
0	0	0
50	32.71±0.12	36.90±0.14
100	57.41±0.23	64.41±0.15
150	63.28±0.21	74.31±0.20
200	70.84±0.26	76.61±0.22
250	88.05±0.19	93.84±0.17

Values are expressed as mean ± standard deviation (n=3).

DISCUSSION

The qualitative phytochemical screening (Table 1) showed that all the seven (7) phytochemical classes tested for were present in all the extract except Anthraquinones. The six phytochemical classes present include alkaloids, tannins, flavonoids, saponin, Phlobatannin and phenols while

Anthraquinones were absent. The quantitative determination (Table 2) shows **that in In vitro Antioxidant indices of bitter leaf (*Vernonia amygdalina*) the quantity of total phenol and flavonoid were substantial.** Alkaloids have been reported to be good in reducing headache and fever due to their analgesic and antibacterial properties (Wadood *et al.*, 2013, Yadav and Agarwala, 2011).

Tannins were found in ***Vernonia amygdalina*** extracts which can be useful in the treatment of cancers. The high concentration is also in consonance with its ability as a good anticancer agent. Flavonoids were also detected in the aqueous extract and which also has some concentration in the quantitative analysis. Thus, the aqueous extract would be good in modifying the body's reaction to allergies, virus and carcinogens. Phenols have been known to be good anti-aging, anti -carcinogen, good in cardiovascular protection etc. (Yadav and Agarwala, 2011). Saponin were also detected in the aqueous extract and this would be a good anti-feeding agent and the aqueous extract would have good detergent properties (Chaieb, 2010). The presence of alkaloids, flavonoids, tannins, saponin, Phlobatannin, and phenolic in the extracts of *V. amygdalina* may explain the reason for its antimicrobial actions since the antimicrobial properties of most of these phyto-constituents have previously been documented (Taleb-contini *et al.*, 2003; Mandalari *et al.*, 2007; Nenaah, 2013; Jin *et al.*, 2017). Sooner than expect, broad-spectrum drug which might be able to cure various human ailments could be developed from *V. amygdalina*. Further work should be carried out in order to isolate the active compounds for further antimicrobial, pharmacological and clinical testing.

CONCLUSION AND RECOMMENDATION

So from the present study, it had been concluded that aqueous extract of Bitter leaf (*Vernonia amygdalina*) possess significant amount of phytochemicals and exhibited significant total flavonoid and phenolic content and also showed high radical scavenging activity. The plant could be exploited as source of antioxidant additives. It is hoped that this report would serve as a basis of information for future project to be embarked on in order to evaluate the potentials of Bitter leaf (*Vernonia amygdalina*) as a strong medicinal plant in improving human health status.

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