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Bambusa vulgaris leaf extracts contain myriad of bioactive phytochemicals: a possible attestation of its medicinal relevance

Temidayo Ogunmoyole[1]

ABSTRACT

The use of medicinal plant as alternative and complementary therapy is fast gaining attention in recent times particularly in developing nations. However, little or no attention is placed on identifying their bioactive constituents in terms of structures in relation to therapeutic effect. The present study investigates the bioactive compounds as well as the antioxidant mechanisms of *B. vulgaris* leaf with a view to providing scientific explanation for its widespread usage in folk medicine. Total phenolic and flavonoid content of the aqueous, methanolic and ethanolic extract of *B. vulgaris* leaf was determined according to established protocols. Moreover, structure and relative abundance of its active principles were determined using gas chromatography- mass spectroscopy (GC-MS). *In vitro* antioxidant mechanisms such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide ($NO^{\cdot}$) radicals' scavenging as well as ferric reducing antioxidant potential (FRAP) were performed following established protocols. GC-MS chromatogram of methanolic extract of *B. vulgaris* leaf showed that it contains 19 bioactive constituents of varying abundance and retention times. Methanolic extract contained more phenolics and flavonoids than the other two extracts tested. Free radical scavenging and ferric reducing potential was higher in the methanolic extract than the other two extracts used in the study. In conclusion, methanol extracted more bioactive phytochemicals and therefore exhibited higher antioxidant property than water and ethanol. Hence, for optimal medicinal usage of *B. vulgaris* leaf, methanol should be adopted for its extraction.

Keywords: *B. vulgaris*, GC-MS, Phytochemicals, Extraction, Bioactive, Antioxidants, Medicinal plant.

INTRODUCTION

Since antiquity, plants have been utilized for food, clothing, shelter and more importantly medicine for the treatment of several diseases [1,2]. Till date, herbal medicine still forms a major bulk of healthcare delivery system where herbal products are compounded as drugs for the treatment of several ailments [3-5]. In recent times, complementary herbal medicine is fast gaining attraction particularly in developing nations where access and affordability to orthodox healthcare is restricted. Although, these herbal products have been reported to be therapeutically effective, there is a need to evaluate the bioactive constituents of these plants that are responsible for the observed effects. This effort has led to a marked boost in the global healthcare delivery system [6,7].

Medicinal plants are veritable raw materials for rational drug design [8]. Generally, synthetic drugs present with side effects, probably due to their unnatural origin. Perhaps, this explains why advocacy for the use of herbal products as drugs is increasing in recent times [9].

Medicinal plants are enriched with a cocktail of phytochemicals, primarily synthesized for their survival in their habitat. However, these phytochemicals, have been identified with profound therapeutic effects that can be exploited for the treatment of several diseases. These secondary metabolites can act additively or synergistically when extracted to bring about the desired therapy intended [10]. The process of drug synthesis begins with screening plant extracts for the presence of active principles such as polyphenols, flavonoids, terpenoids, cardiac glycosides, tannins, *Sutherland frutescens*, *Carpobrotus edulis*, *Crossyne guttata* and their isolated bioactive compounds/molecules are well known internationally for their potency [8-11]. saponins and alkaloids. These phytochemicals have been proven to exhibit biological activities such as antiulcer, antimalarial, anticancer, anti-inflammatory, antidiabetic among others [11-13].

B. vulgaris has been noted globally for its nutritional and medicinal potentials [14-16]. The first medicinal use of *B. vulgaris* started about ten millennia ago, where the plant was used to prepare a health-boosting tonic which has been acknowledged for its anti-aging and anti-stress potentials. Several *B. vulgaris*

based herbal preparations such as bamboo vinegar, bamboo silica, bamboo salt, bamboo extracts and bamboo charcoal are available for the treatment of various pathological disorders [17]. Leaves and shoot extract of *B. vulgaris* have been reported for exhibiting antioxidant [18]. anticancer [19]. antibacterial [20]. cardioprotective [21]. activities in several models.

Gas chromatography-mass spectroscopy (GC-MS) involves the combination of gas chromatography and mass spectrometer in the analytical determination of compounds present in a material such as crude extract of plants [22-25]. This analytical technique has been applied in the qualitative and quantitative estimation of compounds even at trace concentration [26-31].

Considering the potential usefulness of *B. vulgaris* in the treatment of diseases, it is pertinent to investigate its bioactive constituents as well as unravel its antioxidant mechanism of action. The present study therefore fills this knowledge gap.

MATERIALS AND METHODS

Preparation of plant materials

Leaves of *B. vulgaris* were harvested fresh within the Ekiti State University, Ado Ekiti campus and authenticated at Department of Plant Science. The leaves were dried under a shade and ground to powder in a blender. Extraction of the powdered leaves was done with 80% methanol for 3 days. The filtrate was decanted, extraction solvent evaporated and the crude extract obtained weighed and kept airtight. Ethical approval with certificate number, ORD/AD/EAC/19/0083, was obtained from the Office of Research and Development (ORD), Ekiti State University, Ado Ekiti.

Chemicals

Phosphate buffer saline (PBS), 2,2-diphenyl-1-picrylhydrazyl (DPPH.) radical, phosphoric acid, ferric chloride, potassium dihydrogen phosphate (KH_2PO_4) were purchased from Fluke Chemicals, Sodium nitroprusside (SNP), sodium nitrite, trichloroacetic acid, ferrous sulphate, sodium carbonate, sulfanilamide, potassium hydroxide (KOH), naphthyl ethylenediamine dihydrochloride, perchloric acid, ethylenediaminetetraacetic acid (EDTA), aluminum trichloride and Folin-Ciocalteu reagent (FCR) were all purchased from Sigma Chemical Co. (St. Louis, MO).

Estimation of total phenolic content

Total phenolic content of *B. vulgaris* leaf was estimated according to the method of Singleton *et al* [32]. Graded amount of extract containing 200, 400 and 800 mg/ml were added separately to distilled water in labelled test-tubes. Briefly, 2.5 ml of Folin - Ciocalteu reagent and 2 ml of sodium carbonate (7.5% w/v) were measured and added to the test labelled test tubes and incubated for 40 min at 45°C. Absorbance of the resulting solution was monitored at 765 nm, while the total phenolic content was estimated as gallic acid equivalent (GAE).

Estimation of total flavonoid content

The method of Mead *et al* [33] was followed in the estimation of the total flavonoid content in *B. vulgaris* leaf extracts. Appropriate vole of each extract that contained 200, 400 and 800 mg/ml was added to 0.1 ml of aluminum trichloride, acidified with of 0.1 ml acetic acid, and made up to 5 ml with distilled water and incubated at 25°C for 40 min. Absorbance of solution was read at 415 nm against a blank containing all reaction constituents except the extracts. Total flavonoid in the *B. vulgaris* extracts were then estimated as quercetin equivalent (QE).

Nitric oxide radical ($NO^{\cdot}$) scavenging activity

NO radical scavenging activity of *B. vulgaris* leaf extracts was measured by the method of Mar cocci *et al* [34]. Appropriate volume of extracts containing 200, 400 and 800 mg/ml was measured and added to 5 mM sodium nitroprusside in phosphate buffer saline and incubated for 3 h at 25°C to release $NO^{\cdot}$ radical which reacts with oxygen to produce nitrite ion. One milliliter of the incubation mixture is withdrawn every 30 min and added 1 ml of Griess reagent. Absorbance of the colored product formed was then read at 546 nm. Amount of nitrite generated was obtained by interpolation from sodium nitrite curve and used for the estimation of NO scavenging activity of the extracts.

DPPH radical scavenging ability

The ability of *B. vulgaris* leaf extracts to scavenge DPPH radicals in vitro was determined according to the method of Awash *et al* [35]. Fifty microliters (50 µl) each *B. vulgaris* leaf extracts were added separately to 1.0 ml of DPPH (0.4 mM) solution in labelled test tubes, made up to 5 ml with methanol, vortexed for 60 s and incubated for 20 min at 25°C in the dark. Absorbance of the solution was measured at 517 nm against a blank which contains DPPH and methanol only without the extracts. Amount of DPPH radical scavenged (%) relative to the blank was estimated.

Ferric Reducing property

The method of Pulido *et al* [36]. was followed in the estimation of the ferric reducing potentials of the various extracts of *B. vulgaris*. Volumes of extract equivalent to 200, 400 and 800 mg/ml was measured and mixed with 200 mM sodium phosphate buffer pH 6.6 and 1% potassium ferrocyanide (w/v) and incubated for 20 min at 50°C. Ten percent TCA (w/v) was added and centrifuged for 10 min at 650 rpm. One milliliter (1 ml) of the supernatant obtained was mixed with equal volume of distilled water and 1% ferric chloride (w/v). Absorbance was then read at 700 nm against the blank which contains other reaction components except the extracts.

GC-MS Analysis

Gas chromatography mass spectroscopy of methanolic extract of *B. vulgaris* leaves was performed according to manufacturer's instructions. Identification of volatile compounds (VOCs) was done using the National Institute of Standards and Technology (NIST) reference library. Mass spectra of the compounds were obtained by electron ionization at 70 eV, using a spectral range of m/z 30–1000. Each compound was estimated from standard curves calculated from three serial dilutions of analytical standards. All determinations were replicated thrice for each assay and the results are presented as mean of the determinations.

Statistical analysis

Values are expressed as mean ± standard error in mean (SEM) of an experiment performed in triplicate and were analyzed using appropriate analysis of variance (ANOVA) followed by Duncan's multiple range test. Significant difference was set at $p = 0.05$.

RESULTS

Total phenolics

Amount of phenolics in the methanol, ethanol and water extracts of *B. vulgaris* leaf is as presented in Figure 1. It revealed that methanolic extract contained the highest phenolics of the three extracts investigated. Total phenolic content was in the order methanol>ethanol>water.

Total flavonoids

Figure 2 reveals the total flavonoid content in the extracts of *B. vulgaris* extracts investigated. It shows that methanolic extract contained higher flavonoid than ethanol and aqueous extracts.

Nitric oxide radical scavenging

As presented in Figure 3, methanolic extract of *B. vulgaris* leaf demonstrated the most potent ability in scavenging nitric oxide radicals when compared to ethanolic and aqueous extracts.

DPPH radical scavenging ability

The result of DPPH radical scavenging ability of *B. vulgaris* is as presented in Figure 4. It shows that methanolic extract demonstrated the most effective DPPH radical scavenging property of all the extracts tested.

Ferric reducing antioxidant potential (FRAP)

The potential of *B. vulgaris* extracts to reduce ferric ions is as presented in Figure 5. It indicates that methanolic extract showed the highest ferric reducing potential of the three extracts tested.

GC-MS analysis

GC-MS chromatogram (Figure 6) of methanolic extract of *B. vulgaris* leaf showed the presence of 19 bioactive compounds (Table 1). Terpinen-4-ol and phytol has the highest peaks and retention times respectively while 3-hexyn-2-ol and furfural has the lowest peaks and retention times respectively (Table 6).

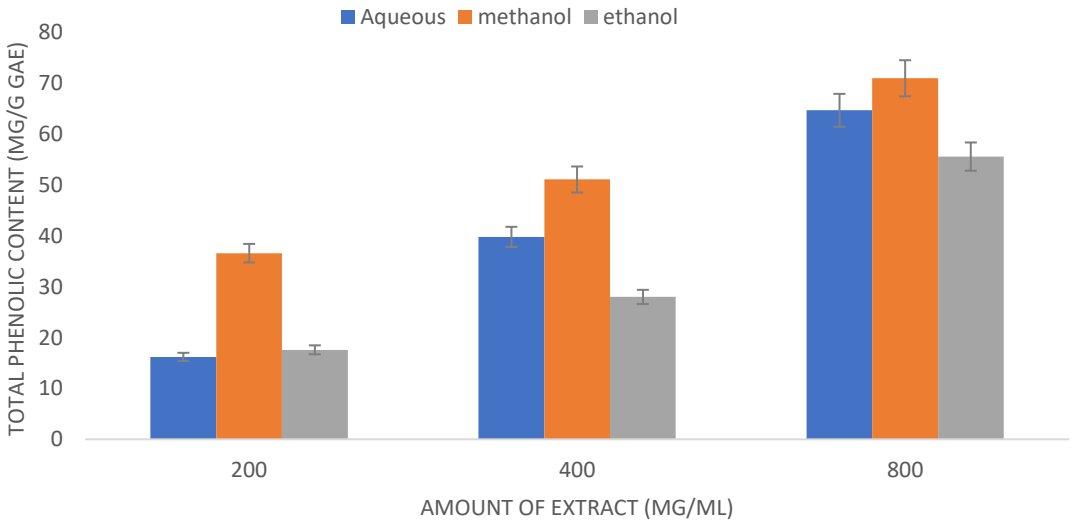


Figure 1: Total Phenolic Content of *B. vulgaris* leaf extracts. Each data point indicates the mean ± SEM of an experiment performed thrice. GAE indicates, gallic acid equivalent

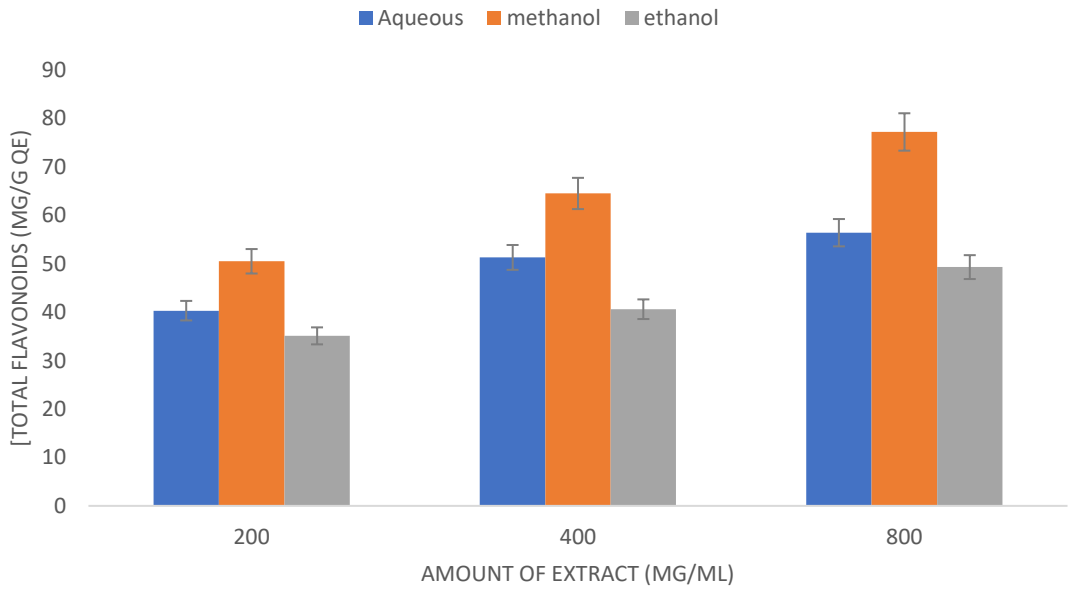


Figure 2: Total Flavonoids Content of *B. vulgaris* leaf extracts. Each data point indicates the mean ± SEM of an experiment performed thrice. QE- quercetin equivalent

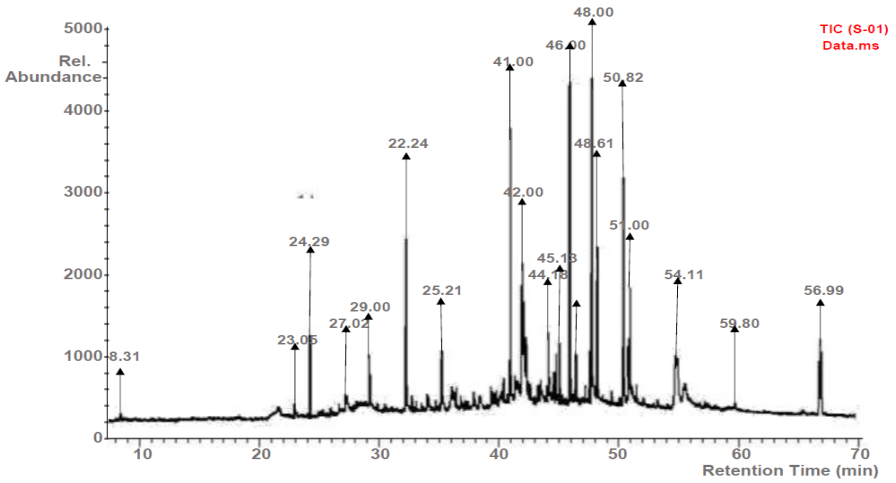


Figure 3: GC-MS Chromatogram of methanolic extract of *B. vulgaris* leaf

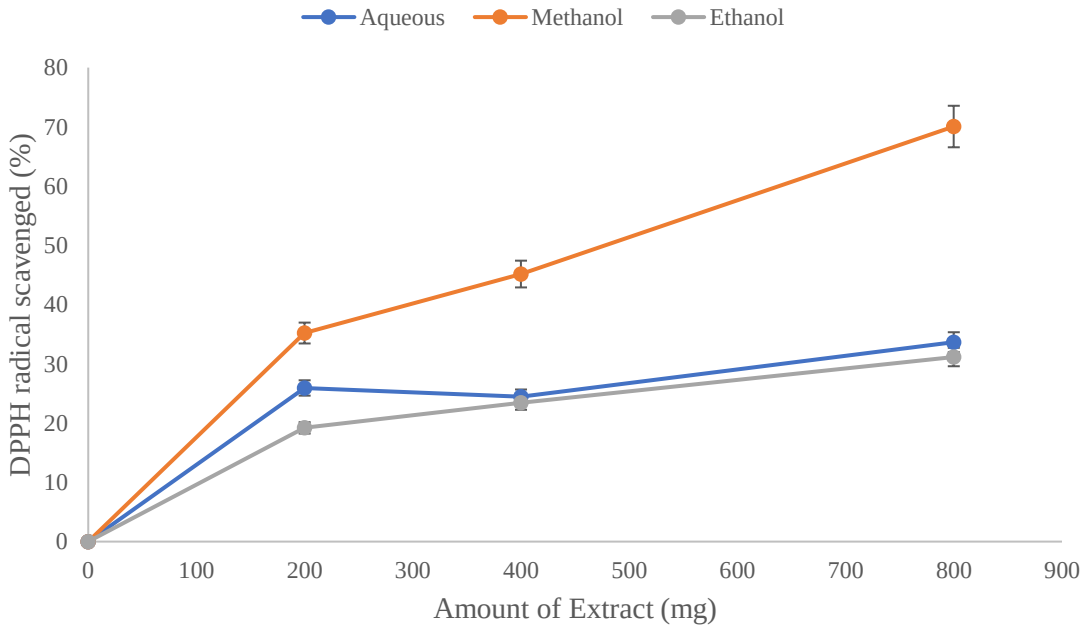


Figure 4: DPPH radical scavenging ability of *B. vulgaris* extracts in vitro. Each data point indicates the mean ± SEM of an experiment performed thrice

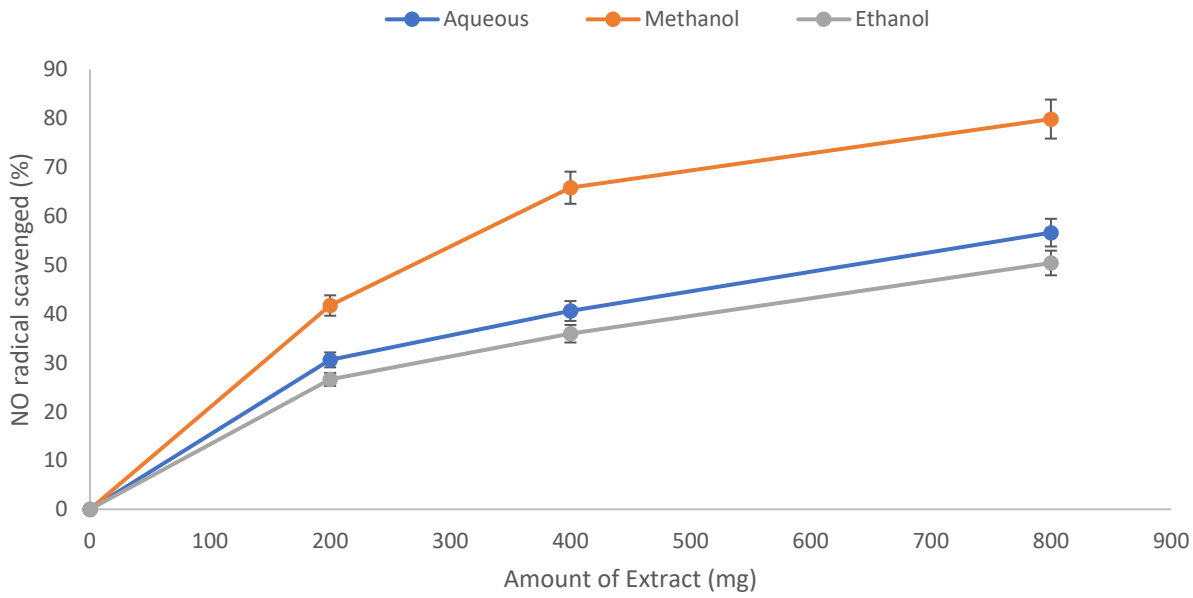


Figure 5: Nitric oxide (NO) radical scavenging ability of *B. vulgaris* leaf extracts in vitro. Each data point indicates the mean ± SEM of an experiment performed thrice

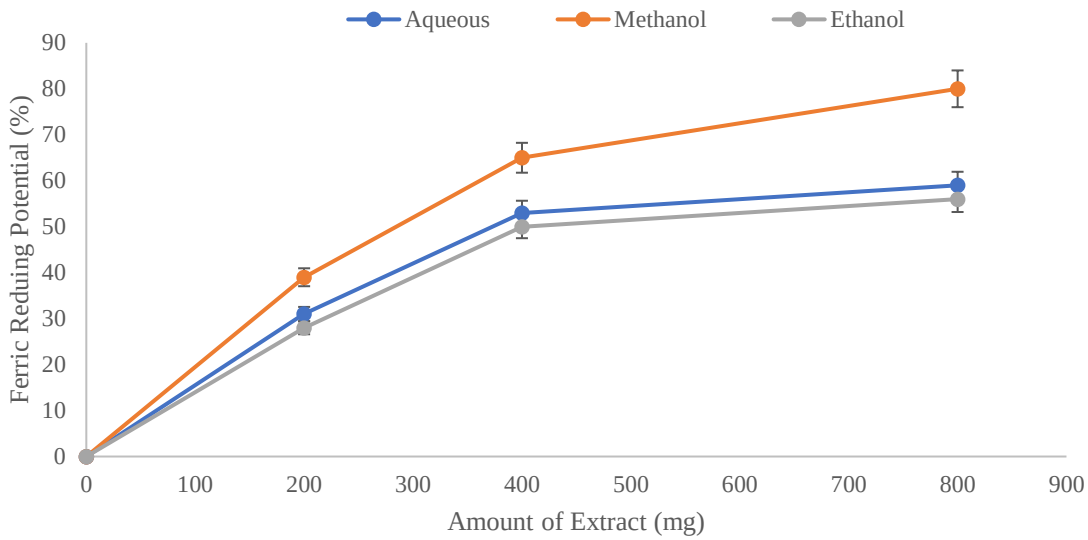


Figure 6: Ferric reducing Potential of *B. vulgaris* extracts *in vitro*. Each data point indicates the mean ± SEM of an experiment performed thrice

DISCUSSION

Medicinal plants have been identified with avalanche of secondary metabolites primarily synthesized for their adaptation and continuous survival in their habitat. These compounds (phytochemicals) have been noted for various medicinal purposes, hence, their continuous exploitation as raw materials for rational drug design. The potency and abundance of these phytochemicals depends largely depends of the solvents used for their extraction [37-39]. Depending on polarity, phytochemicals exhibit varying degree of solubility in polar and non-polar solvents [40,41]. In specific term, an active ingredient of a medicinal plants may be poorly or totally not extracted if the appropriate solvent that required for optimal solubility of the targeted phytochemicals is not employed for its extraction [42]. In the present study, a differential solubility of the phenolics in the leaf of *B. vulgaris* was observed. Total phenolic content was highest in the methanolic extract of the plant (Figure 1). This suggests that the class of phenols present in the leaf extract of the plant were more soluble in methanol than either of ethanol and water. This is in agreement with previous reports [43-45]. In terms of polarity, water is the most polar of the three solvents employed for extraction. The fact that total phenolic content was highest in the methanolic extract suggests that the type of phenolics present in the leaf of the plant exhibits optimal solubility in methanol. This is in agreement with the reports of Iloki-Assanga *et al*. [46]. where the order of phenolic content was methanol>water>ethanol, as observed in the present study. Previous reports on many medicinal plants such as *Rosmarinus officinalis* [47], *Thymus vulgaris* [48], *Marsupium globosum* [49], *Salvia officinalis*, *S. verbenaca*, *S. Egyptiac* and *S. argentea* [50]. established a linear relationship between polyphenolics and antioxidant potentials of medicinal plants. Hence, the use of these compounds as potent antioxidants with diverse applications in medicine. This is responsible for the ability of these compounds to act as free radical-scavengers and antioxidants.

Total flavonoids content was highest in the methanolic extract of *B. vulgaris* leaf (Figure 2). This suggests that the flavonoids present in the leaf exhibits intermediate solubility between water and ethanol indicating the importance of solvent polarity in the extraction of active principles from medicinal plants. This is in agreement with the report of Ogunmoyole *et al* [51]. Although, the two other solvents (ethanol and water) extracts show significant level of flavonoids indicating that certain flavonoids present in the leaf are soluble in ethanol and water respectively. Perhaps, flavonoids exhibit different physical and chemical properties to allow for their differential extraction in different solvents. Medicinal relevance of a phytochemical is not only a function of its abundance but also its therapeutic efficacy.

Analysis of *B. vulgaris* leaf extract on gas chromatography column (Figure 3) showed the presence of nineteen bioactive compounds (Table 1), with different antioxidant properties. It must be mentioned that the medicinal relevance of a phytochemical is not only a function of its abundance but also of its efficacy. Phytol has been suggested to possess anticancer and antimicrobial potential [52,53]. Perhaps, phytol reacts with free radicals via protonation causing the radicals to attain configurational stability thereby becoming less reactive [54,55]. This might possibly explain the contribution of phytol to the free radical scavenging ability of *B. vulgaris* leaf extract observed in the present study.

Sesquiterpenes represent a group of phytochemicals (secondary metabolites) with multiple medicinal potentials including antihypertensive, analgesic [56]. anti-inflammatory [57]. antibiotics (Pavlov, 1956), sedative [58]. hair growth inducer [59]. platelet-activating factor (PAF) antagonist [60]. and anticarcinogenic activities [61]. Cedrol, a typical member of the sesquiterpenes family with anticancer and anti-inflammatory effects was found in the extract of *B. vulgaris*. Hence, the earlier reports of anticancer and anti-inflammatory effect of *B. vulgaris* leaf extract can be partly attributed to cedrol [62-65].

Megastigmatrienone was also found in the methanolic extract of *B. vulgaris*. This phytochemical has been linked with blackberries' aroma [66]. Octadecanoic acid with reported antitumor, antibacterial and antifungal properties was found in the methanolic extract of *B. vulgaris* leaf. This might suggest that a component of the medicinal effect of the plant might be due to the presence of octadecanoic acid [67]. n- hexadecenoic acid which has been associated with antioxidant, nematocidal, hypocholesterolemia, pesticidal, hemolytic, antipsychotic and antiandrogenic potentials was also found in the methanolic extract of the plant. This suggest that n- hexadecenoic acid contributes largely to the overall medicinal effect of the plant [67]. All other bioactive constituents of *B. vulgaris* leaf identified by GC-MS in the present study have been suggested to exhibit various antioxidant properties that are directly linked to the medicinal effects of the plant [68].

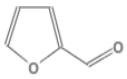
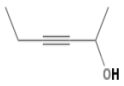
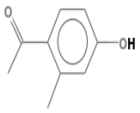
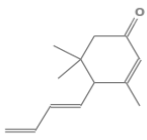
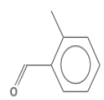
In the present study, extracts of *B. vulgaris* leaf exhibited marked radical scavenging effect against DPPH (Figure 4). This effect can be traced to the phenolic and flavonoids present in the extract. Phenols and flavonoids have been suggested as active free radical scavengers in vitro [68]. In terms of mechanism, phenols and flavonoids donate their free protons to DPPH radical which is usually unstable, thereby attaining stability in its conformation. Usually, the purple color of DPPH changes to golden yellow on interaction with antioxidants such

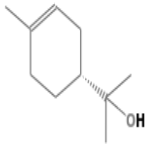
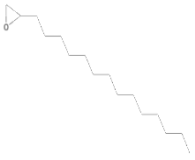
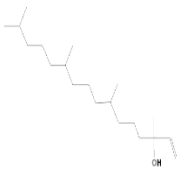
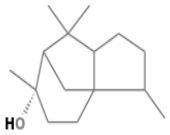
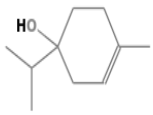
as phenols and flavonoids [69]. The fact that all extracts of *B. vulgaris* leaf scavenged DPPH radical although to varying degrees suggest that they all contain phenols and flavonoids in different concentrations producing different results. However, methanolic extract showed the highest radical scavenging effect suggesting that it extracted more phytochemicals than other solvents as revealed in the total phenolic and flavonoid content of the extracts.

The antioxidant potency of an agent is routinely measured in the laboratory by its ability to scavenge NO radical in vitro [70]. In terms of reactivity, nitrite radicals are deleterious when in contact with critical macromolecules such as carbohydrates, lipids, proteins and nucleic acids [71]. Under normal physiological condition, NO is a potent neurotransmitter and vasodilator [72]. However, any disturbance in the fragile balance between antioxidant and free radicals that tends to favor free radicals in the physiological system could trigger inflammatory reactions, arthritis and ulcerative colitis due to increased level of NO radical. NO turn lethal on interaction with oxygen centered radicals such as superoxide radicals forming a very toxic proximities radical ($ONOO^-$) [71]. Medicinal relevance of phytoconstituents is often determined by their ability to prevent the formation of this radical in the physiological system [72,73]. Antioxidants compete with oxygen for interaction with nitric oxide radical thereby preventing the formation of NO scavenge nitric oxide radicals by competing with oxygen, thereby inhibiting the production of proximities radicals [74]. Extracts of *B. vulgaris* leaf used in the present study showed significant NO radical scavenging potential (Figure 5). However, methanolic extract was the most potent suggesting that specific phytoconstituents present in the methanolic extract prevented the interaction of nitric oxide with oxygen thereby blocking the formation of proximities radicals. These phytoconstituents have been suggested as phenolic and flavonoids [75].

The reductive capacity of medicinal plants on transition metals is a routine diagnostic test of their antioxidative potentials. During oxidative stress, protons that are sustain the electronic stability of critical macromolecules are abstracted leading to their oxidation. Hence, agents that can reduce these oxidized species via reduction are often classified as antioxidants, while the extent to which the reduction is achieved typifies their antioxidant strength. In the present study, methanolic extract of *B. vulgaris* exhibited stronger reductive capacity in comparison to ethanolic and aqueous extracts. This effect is traceable to the amount and potency of the phytochemical extracted (Figure 6). Perhaps, it might suggest that one of the mechanisms of antioxidant actions of the phytochemicals extracted is via reduction.

Table 1: Bioactive Compounds found in methanolic extract of *B. vulgaris* leaf on GC-MS analysis

Peak #	RT	Compound Detected	Mol. Formula	MW	Peak Area%	Comp % wt.	m/z	Structures
1	8.31	Furfural	$C_5H_4O_2$	96	1.05	0.37	35,95, 96	
2	23.05	3-hexyn-2-ol	$C_6H_{10}O$	98	1.20	1.09	43, 67, 88	
3	24.29	2-Undecanone	$C_{11}H_{22}O$	170	3.83	2.05	43, 58, 170	
4	27.02	4-Hydroxy-2-methylacetophenone	$C_9H_{10}O_2$	150	1.46	1.00	77, 107, 150	
5	29.00	Megastigmatrienone	$C_{13}H_{18}O$	190	2.37	1.93	91, 133, 190	
6	22.24	Octadecane	$C_{18}H_{38}$	254	7.19	5.32	42, 57, 254	
7	25.21	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254	2.99	1.81	55, 69, 254	
8	41.00	Benzaldehyde,2-methyl-	C_8H_8O	120	11.38	14.42	91, 119, 120	
9	42.00	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	7.25	8.34	67, 79, 278	

10	44.18	1-Hexadecanol	$C_{16}H_{34}O$	242	4.19	3.67	55, 69, 242	
11	45.13	n-Hexadecenoic acid	$C_{16}H_{32}O_2$	256	4.08	3.98	43, 73, 256	
12	46.00	Oleic acid	$C_{18}H_{34}O_2$	282	13.11	16.03	51, 65, 282	
13	48.00	α-Terpineol	$C_{10}H_{18}O$	154	13.17	15.82	59, 93, 154	
14	48.61	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	7.25	5.77	43, 58, 268	
15	50.82	Oxirane, tetradecyl-	$C_{16}H_{32}O$	240	9.61	10.03	42, 82, 240	
16	51.00	Iso-phytol	$C_{20}H_{40}O$	296	3.61	3.07	43, 71, 296	
17	54.11	Cedrol	$C_{15}H_{26}O$	222	3.02	2.71	95, 150, 222	
18	59.80	Terpinen-4-ol	$C_{10}H_{18}O$	154	0.90	0.15	71, 93, 154	
19	56.99	Phytol	$C_{20}H_{40}O$	296	2.40	2.00	43, 71, 296	

CONCLUSION

Methanolic extract of *B. vulgaris* leaf houses a wide array of phytochemicals as revealed on the GC-MS chromatogram. These phytochemicals were responsible for the in vitro antioxidant effects of the plant. Methanol appeared the best solvent for optimal extraction of phytochemicals from the leaf of the plant. Although water and ethanol are also good alternatives. The type and relative abundance of the various phytochemicals present in the crude extract of the plant could justify the continuous use of the plant in folkloric medicine for the management of diseases.

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Conflict of Interest

None declared.

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