

Original Research Article

**COMPARISON OF PHYTOCHEMICAL, IRON CHELATING, AND FREE RADICAL
SCAVENGING ACTIVITY OF FRESH RIBES NIGRUM (BLACK CURRANT) AND
NUTRACEUTICAL C24/7**

ABSTRACT:

Background: Black currant (*Ribes nigrum* L. Grossulariaceae) promotes good health. A lot of researches have been carried out on common fruits but little attention is given to indigenous fruits such as *Ribes nigrum* which promotes good health with its high content of phytochemicals that influences its antioxidant activity in neutralizing free radicals as well as its iron chelating property the

Aim of this study: To compare the phytochemical composition, iron-chelating and the free radical scavenging activity of fresh *Ribes nigrum* and Nutraceutical C24/7.

Place and Duration of Study: Department of Biochemistry and Molecular Biology, Nasarawa State University, Keffi. Nasarawa State, Nigeria between June 2017 and January 2019

Methodology: Fleshy parts of fresh *Ribes nigrum* fruit was homogenized using an electrical blender and was macerated with 70% ethanol (Sigma Aldrich, 99.8%) for 72 hours and then filtered. The filtrate was condensed using a rotary evaporator and the extract was stored at 4°C. Preliminary phytochemical screening was carried out on the extract and the nutraceutical C24/7 caplets using standard procedures and the identified phytochemicals: saponins, tannins, alkaloids and steroids were quantitatively estimated. Iron chelating and total antioxidant activity assay was conducted using the DPPH radical scavenging and spectrophotometric methods respectively on the extract and the nutraceutical C24/7. Values were analyzed using One way analysis of Variance (ANOVA). Values of $P < 0.05$ were considered significant.

Result and Conclusion: The findings of this study show that the hydro-ethanol extract of *Ribes nigrum* contains a large amount of flavonoids, saponins tannins, alkaloids and steroids compounds and exhibits iron chelating and high antioxidant (free radical scavenging) activity compared to nutraceutical-C24/7 caplets.

Keywords: *Ribes nigrum*; Phytochemical; Nutraceutical

1.0 INTRODUCTION

The generation of free radicals in the body beyond its antioxidant capacity leads to oxidative stress and this seems to be the apparent fundamental mechanism underlying a number of disorders [1,2]. As a result of this, much attention is being focused on the use of antioxidants to inhibit and protect damage due to free radicals and reactive oxygen species. Antioxidants are substances known to protect the body from damage caused by reactive oxygen and nitrogen species induced oxidative stress.

Studies have shown that the consumption of fruits and vegetables is capable of inhibiting the damaging effect of free radicals in the body by acting as antioxidants [3].

Black currant (*Ribes nigrum* L. Grossulariaceae) is a perennial shrub commonly grown in various parts of the world with temperate climate. Its local name is lemu sunsu in Jos, Plateau State, Nigeria. It is a wild fruit that has a high content of ascorbic acid (Vitamin C) and other beneficial phenolic compounds with antioxidant properties that scavenge free radicals [4, 5, and 6]. The decoction of various part of the plant has been used in the treatment of myriad diseases of man. [7].

The term nutraceutical is derived from the words 'nutrition' and 'pharmaceutical'. Thus, it is a food or a part of food which exerts a curative or preventive effect on disease. These include various nutrients, dietary supplements, specially designed diets or herbal products. Nutraceuticals of both plant and animal origin hold great opportunities for food industries to bring out novel foods to cater for future needs [8, 9].

Scientific research has provided evidence regarding biologically active compounds and the underlying physiological mechanisms of nutraceuticals, highlighting the importance of nutraceuticals as complements to the conventional therapies and medications allowing dose reduction and decreasing the occurrence of adverse effects. Despite the undeniable progress in the field of nutraceuticals, there are still several issues that remain to be addressed. These include clinical evidence supporting in vitro claims, regulatory aspects and assurance of nutraceuticals' identity, quality and safety [10].

From the foregoing, it is **conceivable** to compare the medicinal benefits of these nutraceuticals with naturally occurring medicinal plants to ascertain their levels of contribution to the well-being of individuals as compared to the naturally occurring ones.

2.0 Materials and Methods

Fresh *Ribes nigrum* was bought from terminus market located at Jos, Plateau State, Nigeria in June 2017 and identified at the Department of Plant Science and Biotechnology, Nasarawa State University, Keffi by the laboratory technologist. Nutraceutical C24/7 caplet is a product of Nature way products, Inc, Green Bay, U.S.A. The two samples (Nutraceuticals C24/7 and *Ribesnigrum* fruit) were analyzed at the Department of Advanced Chemistry Laboratory, Sheda Science and Technology Complex (SHESTCO), ABUJA.

2.1 Plant extraction

About 20g of fresh *Ribes nigrum* was washed under running water properly and the seeds were removed manually, and then the fleshy part of the fruit was homogenized using an electrical blender and macerated with 70% ethanol (Sigma Aldrich, 99.8%) for 72 hours and then filtered with Whatman No 1 filter paper. The filtrate was condensed using rotary evaporator and the extract was stored at 4°C.

Biochemical analysis:

The following biochemical assays were carried out on the C42/7 caplets and hydro-ethanol extract of *Ribes nigrum*

2.2 Phytochemical Screening (Qualitative)

This was carried out according to the standard methods described by Sofowora [11] and Trease and Evans [12].

2.3 Tests for Alkaloid

About 0.5g of the plant extract was stirred with 5ml of 1% aqueous HCL on water bath and then filtered, 1ml of the filtrate was taken individually into 3 test tubes. Mayer's, Wagner and Dragendroff reagents were added respectively. The formation of precipitate indicates the presence of alkaloids with Mayer's reagents, Wagner gives a reddish brown precipitate and

Dragendroff gives an orange brown. All of these indicate the presence of Alkaloids. The same procedure was repeated for nutraceutical C24/7 caplets.

2.4 Test for Saponins

About 1g of the extract was boiled with 5ml of distilled water and filtered; to the filtrate about 30 ml of distilled water was further added and shaken vigorously for about 6 minutes. Frothing which persisted on warning indicates the presence of saponins. The same procedure was repeated for the nutraceutical C24/7 caplets.

2.5 Test for Phenols

About 0.5g of the extract was added to 1% FeCl₃ solution. A deep bluish green precipitate indicates the presence of phenol. The same procedure was repeated for the nutraceutical C24/7 caplets.

2.6 Test for Steroids and Triterpenoids

Salkowski test: crude extract of *Ribes nigrum* was mixed with chloroform and a few drops of concentrated H₂SO₄ were added. The mixture was well shaken and allowed to stand for some time. The formation of red colour at the lower layer indicates the presence of steroids and formation of yellow coloured layer indicated the presence of triterpenoids. The same procedure was repeated for the nutraceutical C24/7 caplets.

2.7 Test for Tannins

A few drops of 1% HCl were added to 1ml of the extract and boiled. A reddish precipitation indicates the presence of tannin. The same procedure was repeated for the nutraceutical C24/7 caplets.

2.8 Test for Flavonoids

A small quantity of the extract was dissolved separately in diluted NaOH. A yellow solution that turned colourless on addition of concentrated HCl indicates the presence of flavonoids. The same procedure was repeated for the nutraceutical C24/7 caplets.

2.9 Test for Terpenoids

About 0.5ml of acetic anhydride was mixed with 1ml of the extract and a few drops of conc. H₂SO₄ was added. A reddish green precipitate indicates the presence of terpenoids. The same procedure was repeated for the nutraceutical C24/7 caplets.

2.10 Phytochemical Analysis (Quantitative)

After confirmation of the presence of these phytochemicals, Tannins, steroids, saponins, alkaloids and flavonoids were quantified based on the results from the qualitative phytochemical screening.

2.11 Determination of alkaloids

The alkaloids acid was determined using the procedure described by Obadoni and Ochuko, [11] About 5g of the plant extract was weighed into a 250 ml beaker, and 200mL of 20% acetic acid in ethanol was added, covered and allowed to stand for 4hrs. This was filtered and the extract was concentrated using a water bath to evaporate one-quarter of the original volume. Concentrated ammonium solution was added drop-wise to the extract until precipitation was completed. The entire solution was allowed to settle and the precipitate was collected by filtration, after which it was weighed. The same procedure was repeated for the nutraceutical C24/7 caplets.

Alkaloid (g/100g) = weight of untreated sample / weight of treated sample x100

2.12 Determination of flavonoids: About 5g of the plant extract was weighed in a 250 ml titration flask, and 100mL of 80% aqueous methanol was added at room temperature and shaken for 4hrs on an electric shaker. The entire solution was filtered with Whatman filter paper no. 42 and again, this process was repeated. The filtrate as a whole was later transferred into a crucible and evaporated to dryness over a water bath and weighed [14].

The same procedure was repeated for the nutraceutical C24/7 caplets.

Calculation

Flavonoids (g/100g) = weight of untreated sample / weight of treated sample x100.

2.13 Determination of saponins

About 5g of the plant extract was weighed, and dispersed in 100ml of 20% ethanol. The suspension was heated over a shot water bath for 4hr with continuous stirring at about 55°C. The filtrate and residue were re-extracted with another 100ml of 20 % ethanol. The combined extracts were reduced to 40mls over water bath at about 90°C. The concentrate was transferred into a 250ml separating funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and about 30ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated over a water bath. After evaporation, the samples were dried in the oven to a constant weight [13]. The same procedure was repeated for the nutraceutical C24/7 caplets.

The saponin content was calculated as:

Saponin (g/100g) = weight untreated sample / weight of treated sample x100

2.14 Determination of tannins

The level of tannin in the plants was determined using the method of Van-Burden and Robinson [15]. 5g of the plant extract was weighed into a 50ml plastic bottle. 50ml of distilled water was added and shaken for 1hr on a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filtrate was pipetted out into a test tube and then mixed with 2ml of 0.1 M FeCl₃ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance of concentration was measured at 420 nm within 10 min using spectrophotometer. The same procedure was repeated for the nutraceutical C24/7 caplets.

2.15 Steroids

About 1g of the plant extract was weighed into a conical flask and 20ml of ethanolic sodium hydroxide was added followed by 1ml of tetrazolium in methanolic hydroxide and 1ml of tetramethyl ammonium hydroxide. The mixture was allowed to react for 90min, absorbance of concentration was measured at 525nm, using ethanolic sitosterol as standard [16]. The same procedure was repeated for the nutraceutical C24/7 caplets.

2.16 Free radical scavenging activity

The total antioxidant activity assay was conducted using the DPPH free radical scavenging method as described by Brands-Williams *et al.*, [17] and modified by Ayoola *et al.*, [18]. Using UV spectrophotometer, the free radical scavenging activities of the extracts was assessed at 517nm. The same procedure was repeated for the nutraceutical C24/7 caplets.

2.17 Iron chelating activity

Iron chelating activity of the plant extracts was determined according to the method described by Benzie and Strain, [19]. The reaction mixture containing 1.0ml of O-phenanthroline, 2.0ml of FeCl₃ and 2.0ml of the plant extract at various concentrations ranging from 2ml to 10 ml in a final volume of 5.0ml was incubated for 10mins at room temperature. The absorbance was recorded at 510nm. Ascorbic acid was added to the reaction mixture instead of the extract and the absorbance obtained was taken as equivalent to 100% reduction of all ferric irons. The experiment was performed in triplicate.

The percentage iron chelating activity of the black currant extract and the standard compound ascorbic acid was calculated as follows.

Percentage iron chelating activity = $\frac{\text{test absorbance} - \text{control}}{\text{test absorbance}} \times 100$.

The same procedure was repeated for the nutraceutical C24/7 caplets.

2.18 Statistical analysis:

Data were expressed as mean \pm standard deviation and significant differences were determined by student's t-test and ANOVA at 95% confidence level using SPSS soft-ware version 19.0. Student's *t*-test and ANOVA was carried out to test any significant differences between their means. Values of $P < 0.05$ were considered statistically significant.

3.0 Results

Qualitative Phytochemical Analysis of hydro-ethanol of *Ribes nigrum* and Nutraceutical C24/7

The result presented in Table (1) shows the different phytochemicals present and absent in *Ribes nigrum* and nutraceutical C24/7 caplets.

From the result, it was revealed that both *Ribes nigrum* extract and Nutraceutical C24/7 caplet both contains tannins, steroids, saponins, phenols, alkaloids and flavonoids in appreciable quantities. However, triterpenoids are absent in both, terpenoids is present in the *Ribes nigrum* extract while it is absent in C24/7 caplets.

Quantitative Phytochemical Analysis of hydro-ethanolic Extract of *Ribes nigrum* and Nutraceutical C24/7.

The result presented in table (2) shows the approximate quantitative phytochemical contents of *Ribes nigrum* extract and the nutraceutical C24/7 caplets.

As shown in the table 2 below, there was no significant ($P > 0.05$) increase in the level of tannin of the extract compared to the nutraceutical caplets.

Similarly, in the value of alkaloids, there was no significant increase ($P > 0.05$) in the extract compared to the nutraceutical caplets.

However, there was a significant ($P < 0.05$) increase in the flavonoids content of the extract compared to the nutraceutical caplets was observed.

Also, there was a significant ($P < 0.05$) increase in the steroids content of *Ribes nigrum* extract compared to the nutraceutical caplets.

DPPH radical scavenging activity and % inhibition of standard vitamin C tablet, nutraceutical C24/7 caplet and hydro-ethanol extract of *Ribes nigrum*

From the result presented in table 3, it is obvious that the free radical scavenging activities of *Ribes nigrum* extract at varying concentrations is higher than that of nutraceutical C24/7 caplets as compared to the standard antioxidant Vitamin C.

Iron Chelating Activity of hydro-ethanol extract of *Ribes nigrum* and Nutraceutical C24/7

From the result presented above, it is obvious the iron chelating potentials of *Ribes nigrum* extract is significantly ($P < 0.05$) higher than that observed for the Nutraceutical caplets C24/7 at all concentrations.

4.0 DISCUSSION

Medicinal plants have made their mark in ethnomedicine. They have been used in different preparations for the treatment of myriad disease conditions not limited to cancer, diabetics, hypertension etc. to mention but a few [20], one of such medicinal plant is *Ribes nigrum*, a common fruit plant, and it is a common shrub, belonging to the family grossulariceae. It is commonly grown in various parts of the world of temperate climate. Its fruits are tasteful and are rich sources of vitamin C and other compounds such as pectins, fibres, micro and macronutrients with immense health benefits [5]. *Ribes nigrum* is also replete with several beneficial flavonoids and polyphenols which have been reported to possess antioxidant, antiviral, anti-carcinogenic and anti-nociceptive effects [7].

Nutraceutical is a recent trend in ethnomedicine, since researches became aware of the fact that the therapeutical/beneficial potentials of medicinal plants are tied to their phytochemical contents [21]. They thought it desirable to combine these phytochemicals in order to get the most of their benefits without the interference of other plant materials. This has led to the explosion of nutraceuticals, many of which have been used for the prevention, treatment and /or prophylaxis of myriad disease conditions of man [22].

There is therefore a debate about the efficacy and safety of medicinal plants itself in comparison to nutraceuticals, which are derived from combination of phytochemicals and other supplements. It is against this background that this study is poised to compare the phytochemical composition (qualitative and quantitative) of *Ribes nigrum* hydro-ethanol extract and the nutraceutical C24/7 as well as their free radical and iron chelating properties.

From the result presented in Table 1, It is obvious that *Ribes nigrum* hydro-ethanol extracts and the nutraceutical C24/7 caplets are rich in the phytochemicals: Tannins, Steroids, Saponins, Phenols, Alkaloids and flavonoids. While triterpenoids is conspicuously absent in both. Terpenoids is present in *Ribes nigrum* extract but it is absent in the nutraceutical C24/7 caplets.

This confirms the therapeutic potentials of the plant extract and the nutraceutical caplets. Since their efficacy is tied to their content of phytochemicals [21]. They are both rich in saponins, flavonoids and alkaloids- phytochemicals which decades of researches in Phytochemistry have revealed their medicinal and therapeutic potentials as antioxidants, anti-diabetic etc. [23].

These phytochemicals were quantified using standard procedures and the results presented in table 2 shows the percentage composition of Tannins, Alkaloids, flavonoids and steroids in both the *Ribes nigrum* hydro-ethanol extract and the nutraceutical C24/7 caplets. From the result, it is obvious that the levels of these phytochemicals are significantly ($P < 0.05$) higher than those in the nutraceutical caplets. This means that *Ribes nigrum* extract contains more of these phytochemicals and as such may have more medicinal potentials compared to the nutraceutical C24/7. The higher the levels of these phytochemicals in these plants, the better their potential use for medicinal purposes [24].

Table 3 compared the DPPH radical scavenging and the percentage inhibition of *Ribes nigrum* hydro-ethanol extract and the nutraceutical C24/7 with the standard antioxidant Vitamin C at various concentrations of 2,4,6,8 and 10mg/ml respectively.

Free radicals are electron deficient and highly reactive oxygen species and nitrogen that are capable of independent existence. They are ubiquitous in the cells of living organisms as some are produced in vivo as by-products of metabolism [25]. There normally exist a balance in the levels of free radical production and the antioxidant defense system (both enzymatic and non-enzymatic). The enzymatic aspect includes Catalase, Superoxide Dismutase and Peroxidase etc. while the non-enzymatic component involves the use of endogenous molecules such as Glutathione and Vitamin E. However, an imbalance in the levels of free radical production and the antioxidant system favoring increased free radical production will lead to oxidative stress which if not abated will almost always lead to oxidative damage [26,27].

Increased production of free radicals has been implicated in the etiopathogenesis of almost all diseases of man (Ellidag *et al.*, [28]; Olivieri *et al.*, [29]; Pan *et al.*, [30]; Bashan *et al.*, [31], this has led to a constant and unending screening of diverse medicinal plants for free radical scavenging and antioxidant potentials. It is thought that free radical scavenging can reduce if not totally eradicate the severity of these disease conditions [32, 33].

From the result presented in the table above Table 3, the free radical scavenging abilities of *Ribes nigrum* hydro-ethanol extract is significantly ($p < 0.05$) higher than that of the nutraceutical C24/7 caplets at all concentrations and it is closer to the values recorded for the standard antioxidant vitamin C. This shows that the extract of *Ribes nigrum* is better able to scavenge free

radicals compared to the nutraceutical C24/7. This is to attest to the medicinal potentials of *Ribes nigrum*. Decades of research in ethnomedicine has shown that medicinal plants with immense antioxidant potentials can be used to ameliorate and /or treat almost all known disease of man [34]; [35]; [36]; [37]; [32]. These mean that, there may be other important health benefits of *Ribes nigrum* yet to be discovered.

Table 4 shows the iron chelating ability of *Ribes nigrum* hydro-ethanol extract and the nutraceutical C24/7 caplets. From the results, it is obvious that the iron chelating ability of *Ribes nigrum* hydro-ethanol extract is significantly ($P < 0.05$) higher than that of the nutraceutical C24/7 caplets at all concentrations. Minerals within normal levels and concentrations in human bodies play essential roles in body metabolism and the maintenance of a healthy state [38]. However, in levels and concentration higher and above normal, they can cause toxicity with far reaching adverse effects [39] hence; the use of chelating agent is an optimal method to reduce metal toxicity in organisms [40].

The case of thalassemia which can be caused by iron overdose readily comes to mind [41]. It is therefore desirable to have a medicinal plant with antioxidant and iron chelating abilities. Hydro-ethanol extract of *Ribes nigrum* possess immense iron chelating activities at all concentrations compared to the nutraceutical C24/7 caplets ($P < 0.05$).

This is not surprising given the high flavonoid content of *Ribes nigrum*. Plants with high flavonoid and phenol contents are used as antioxidants and as chelating agents [41].

5 Conclusion

Black currant (*Ribes nigrum* L. Grossulariaceae) is a perennial shrub commonly grown in various parts of the world with temperate climate. It is a wild fruit that has a high content of ascorbic acid (Vitamin C) and other beneficial phenolic compounds.

This research was undertaken to compare the phytochemical (qualitative and quantitative), iron chelating, and free radical scavenging activity of fresh *Ribes nigrum* (black currant) and nutraceutical C24/7.

From the result of the experiment, it is obvious that the health benefit of *Ribes nigrum* far exceeds that of the nutraceutical C24/7 as evident in its higher levels of phytochemicals, antioxidant and iron chelating abilities. The lowered values recorded for the nutraceutical C24/7 may be due to the pre-treatments these phytochemicals were exposed to in the process of converting them to nutraceuticals.

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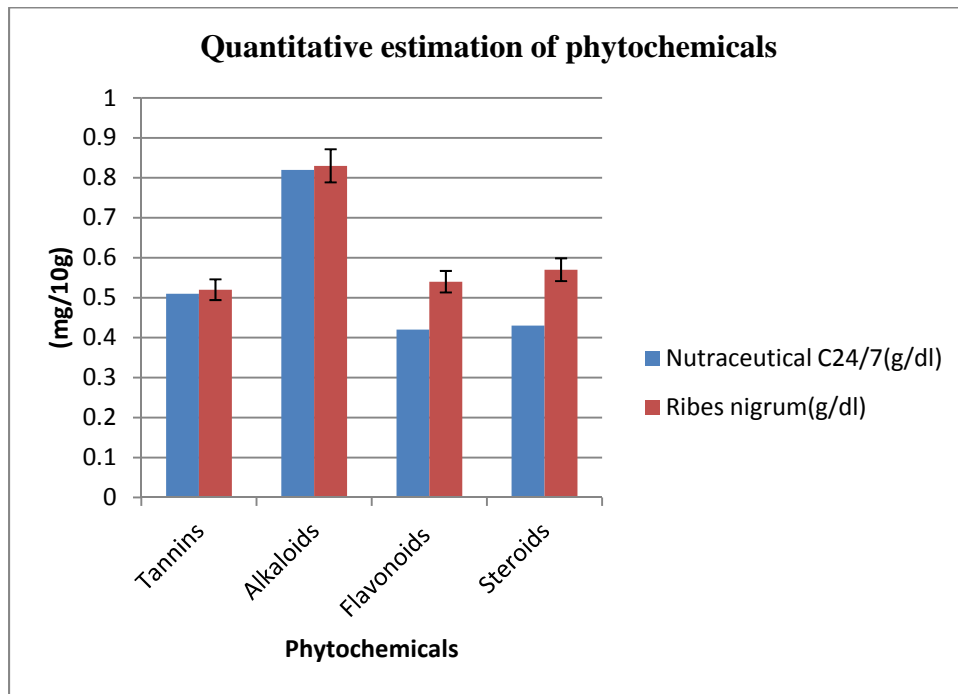
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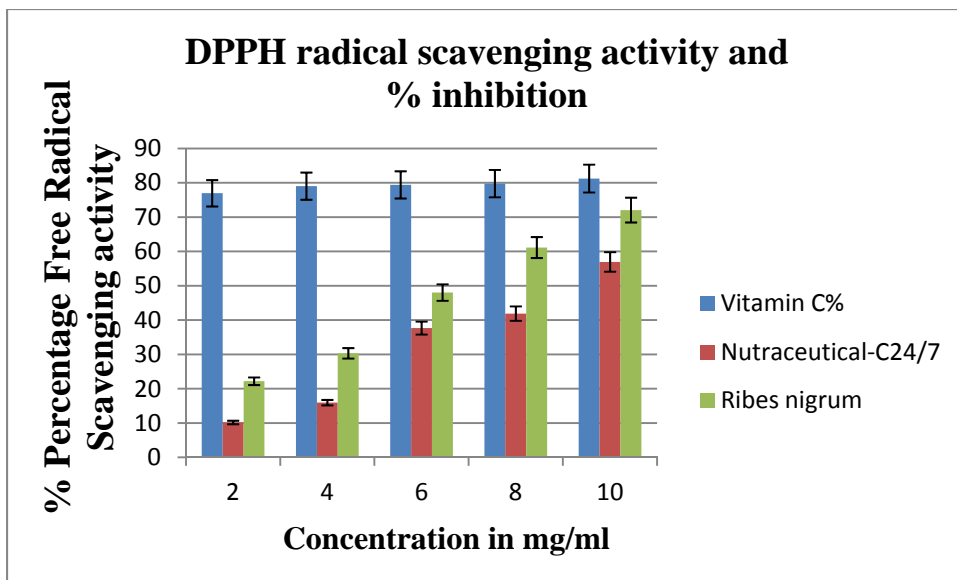
FIGURES:

Fig 1: Percentage composition of phytochemicals



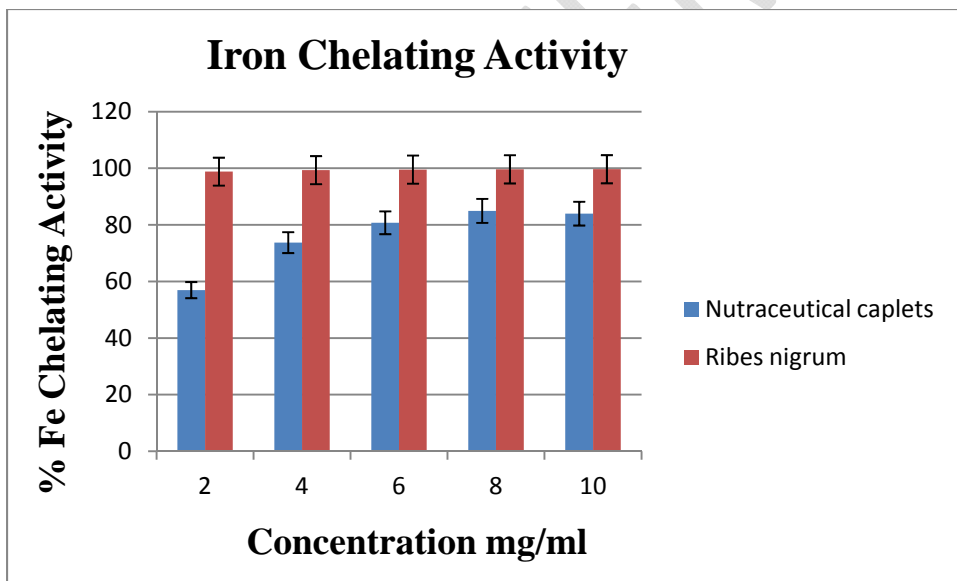
The figure above shows the percentage composition of the phytochemicals in *Ribes nigrum* extract and nutraceutical C24/7 caplets.

Fig 2: DPPH free radical scavenging and % inhibition



The figure above shows the percentage free radical scavenging activity of *Ribes nigrum* extract and the nutraceutical C24/7 caplets compared to the standard antioxidant Vitamin C

Fig 3: Iron chelating activity



The figure above shows the percentage iron chelating activities of *Ribes nigrum* extract and the nutraceutical C24/7 caplets.

TABLES:

Table 1: Phytochemical composition of ethanolic extract of Nutraceutical caplet and *Ribes nigrum*

Phytochemicals	<u>Ribesnigrum</u>	Neutraceutical-C24/7
Tannins	+	+
Steroid	+	+
Triterpenoids	-	-
Saponins	+	+
Phenols	+	+
Alkaloids	+	+
Terpenoids	+	-
Flavonoids	+	+

KEY: (+) Present and (-) Absent

Table 2: Quantitative estimation of phytochemicals and wavelength of quantification

Phytochemical	Composition of phytochemicals		Wavelength(nm)
	Nutraceutical C24/7(mg/10g)	<i>Ribes nigrum</i> (mg/10g)	
Tannins	0.51±0.05 ^a	0.52±0.50 ^a	395
Alkaloids	0.82±0.02 ^b	0.83±0.05 ^b	562
Flavonoids	0.42±0.05 ^a	0.54±0.50 ^c	490
Steroids	0.43±0.05 ^b	0.57±0.15 ^d	505

Values are presented as mean ± SD, where (n=3). Values bearing different superscripts (a-c) in the same row are significantly different at P<0.05

Table 3: Shows the free radical scavenging activities of the *Ribesnigrum* extract and Nutraceutical C24/7 as compared to the standard antioxidant Vitamin C

Concentration (mg/ml)	Vitamin C%	Nutraceutical-C24/7	<i>Ribes nigrum</i>
2	76.96±0.86 ^a	10.19±1.17 ^b	22.20±0.22 ^c
4	79.03±0.07 ^a	15.96±0.08 ^b	30.33±0.09 ^c
6	79.40±0.31 ^a	37.69±0.44 ^b	48.03±0.16 ^c
8	79.77±0.24 ^a	41.90±0.53 ^b	61.15±0.80 ^c
10	81.24±0.11 ^a	56.96±0.23 ^b	72.06±0.11 ^c

Values are presented as mean ± SD, where (n=3) values bearing different superscripts (a-c) in the same row are significantly different at P<0.05.

Table 4: Shows the iron chelating activities of ethanolic extract of *Ribes nigrum* and the nutraceutical C24/7 caplets.

% Fe Chelating Activity		
Concentration (mg/ml)	Nutraceutical caplets	<i>Ribesnigrum</i>
2	56.96±0.02 ^a	98.81±0.01 ^b
4	73.75±0.03 ^c	99.35±0.04 ^d
6	80.75±0.09 ^d	99.54±0.06 ^e
8	84.95±0.10 ^e	99.64±0.06 ^d
10	83.98±0.10 ^b	99.68±0.06 ^e

Values are presented as mean ± SD, where (n=3) values bearing different superscripts (a-e) in the same row are significantly different at P<0.05.