Original Research Article

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

UNDER PEER REVIEW

ABSTACT:

Black currant*(Ribesnigrum* L. Grossulariceae) promotes good health. A lot of researches have been carried out on common fruits but little attention is given to indigenous fruits such as *Ribesnigrum* 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 studyis to compare the phytochemical composition, iron-chelating and the free radical scavenging activity of fresh *Ribesnigrum*and Nutraceutical C24/7.

Fleshy parts of fresh *Ribesnigrum* fruit was homogenized using an electrical blender and was macerated with 75ml of ethanol for 15 minutes and then filtered. The filtrate was condensed using a rotary evaporator and the extract was stored at 4^{o}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. The findings of the study shows that the ethanolic extract of *Ribesnigrum*contains a large amounts of flavonoids, saponins tannins, alkaloids and steroids compounds and exhibits iron chelating and high antioxidant (free radical scavenging) activity than nutraceutical-C24/7 caplets.

Keywords: *Ribesnigrum*; 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 (*Ribesnigrum* L. Grossulariceae) 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 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 essential 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

UNDER PEER REVIEW

Fresh *Ribesnigrum*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 *Ribesnigrum*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 75ml of ethanol for 15 minutes and then filtered through Whatman No 1 filter paper. The filtrate was condensed using rotary evaporator and the extract was stored at 4^{o}C.

Biochemical analysis:

The following biochemical assays were carried out on the C42/7 caplets and ethanolic extract of Ribesnigrum

2.2 Phytochemical Screening (Qualitative)

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

2.3 Test 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.

UNDER PEER REVIEW

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. Frosting which persisted on warning indicates the presence of saponins. The same procedure was repeated for the nutraceutical C24/7 caplet.

2.5 Test for Phenols

About 0.5g of the extract was added to 1% $Fecl_3$ solution. A deep bluish green precipitate indicates the presence of phenol. The same procedure was repeated for the nutraceutical C24/7 caplet.

2.6 Test for Steroids and Triterpenoids

Salkowski test: crude extract of Ribesnigrum was mixed with chloroform and a few drops of concentrated H_2SO_4 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 caplet.

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 caplet.

2.8 Test for Flavonoids

A small quantity of the extract was dissolved separately in diluted NaoH. A yellow solution that turns 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

UNDER PEER REVIEW

About 0.5ml of acetic anhydride was mixed with 1ml of the extract and a few drops of conc. H_2SO_4 was added. A reddish green precipitate indicates the presence of terpenoids. The same procedure was repeated for the nutraceutical C24/7 caplet.

2.10 Quantitative Phytochemical Analysis

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.

2.11Determination 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

2.12Determination 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.

UNDER PEER REVIEW

Calculation

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

2.13Determination 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^{o}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^{o}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

2.14Determination 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 $FeCl_3$ 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.

2.15Steroids

UNDER PEER REVIEW

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 tetra-methyl ammonium hydroxide. The mixture was allowed to react for 90min, absorbance of concentration was measured at 525nm, using ethanolicsitosterol as standard [16]. The same procedure was repeated for the nutraceutical C24/7 caplets.

2.16Free 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.

2.17Iron chelating activity

Iron chelating activity of the plant extract was determined according to the method described by Benzie and Strain, [19]. The reaction mixture containing 1.0ml of O-phenanthroline, 2.0ml of $FeCl_3$ 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 = test absorbance – control/test absorbance x 100.

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

2.18 Statistical analysis:

Data were expressed as mean ± 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.

UNDER PEER REVIEW

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.

3.0Results

Qualitative Phytochemical Assessment of Ethanolic Extract of Ribesnigrum and Nutraceutical C24/7

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

From the result, it was revealed that both *Ribesnigrum* 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 ribesnigrum extract while it is absent in C24/7 caplet.

Quantitative Phytochemical Assessment of Ethanolic Extract of Ribesnigrum and Nutraceutical C24/7.

The result presented in table (2) shows the approximate quantitative phytochemical contents of *Ribesnigrum* extract and the neutraceutical C24/7

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

UNDER PEER REVIEW

DPPH radical scavenging activity and % inhibition of standard vitamin C tablet, nutraceutical C24/7 caplet and ethanolic extract of *Ribesnigrum*

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

Iron Chelating Activity Assessment of Ethanolic Extract of *Ribesnigrum* and Nutraceutical C24/7

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

4.0DISCUSSION

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 *Ribesnigrum*, 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]. *Ribesnigrum* 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 content [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 condition 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 *Ribesnigrum*ethanolic extract and the nutraceutical C24/7 as well as their free radical and iron chelating properties.

From the result presented in Table1, It is obvious that *Ribesnigrum*ethanolic extracts and the nutraceutical C24/7 caplets are rich in the phytochemicals: Tannins, Steroids, Saponins, Phenols,

UNDER PEER REVIEW

Alkaloids and flavonoids. While triterpenoids is conspicuously absent in both. Terpenoids is present in *Ribesnigrum* extract but it is absent in the nutraceutical C24/7 caplets.

This confirms the therapeutical 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, antidiabeticetc [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 *Ribesnigrum*ethanolic 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 *Ribesnigrum* 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 potentials as medicinal plants [24].

Table 3 compared the DPPH radical scavenging and the percentage inhibition of*Ribesnigrum*ethanolic extract and the nutraceutical C24/7 with the standard antioxidant Vitamin C at varying concentrations of 2,4,6,8 and 10mg/ml respectively.

Free radicals are electron deficient and highly reactive of species oxygen 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 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 ethiopathogenesis of almost all disease 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 a never ending 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 *Ribesnigrum*ethanolic 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 *Ribesnigrum* is better able to scavenge free radicals compared to the nutraceutical C24/7. This is to attest to the medicinal potentials of *Ribesnigrum*. Decades of research in ethnomedicine has shown that medicinal plants with immense antioxidant

UNDER PEER REVIEW

potentials can be used ameliorate and /or treat almost all known disease of man [34];[35]; [36];[37]; [32]. These means that, there may be other important health benefits of *Ribesnigrum* yet to be discovered.

Table 4 shows the iron chelating ability of *Ribesnigrum*ethanolic extract and the nutraceutical C24/7 caplets. From the results, it is obvious that the iron chelating ability of *Ribesnigrum*ethanolic 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. Ethanolic extract of ribesnigrum 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 ribesnigrum. Plants with high flavonoid and phenol contents are used as antioxidants and as chelating agents [41].

5 Conclusion

From the foregoing, it is obvious that the health benefits of ribesnigrum far exceeds that of the nutraceutical C24/7 as evident in its higher levels of phytochemicals, antioxidant and iron chelating abilities. The reduced activities of the nutraceutical C24/7 may be due to pre-treatment these phytochemicals were exposed to in the process of converting them to Nutraceuticals

REFERNCES:

1 PisoschiAM,Pop A. 2015. The role of antioxidants in the chemistry of oxidative stress: a review. Eur. J. Med. Chem. 2015; 97: 55–74.

2 Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. Clin Chem. 2006; 52: 601-623.

3 Stanner SA, Hughes J, Kelly CN, Buttriss J. 2004. A review of the epidemiological evidence for the 'antioxidant hypothesis'. Public Health Nutr. 2004; 7: 407-422.

4 Tabart J, Kevers C, Evers D, Dommes J. 2011. Ascorbic Acid, Phenolic Acid, Flavonoid and Carotenoid profiles of selected extracts from Ribesnigrum. Journal of Agricultural and Food Chemistry. 2011; 59: 4763-70.

UNDER PEER REVIEW

5 Mattila PH, Hellstrom J, McDougall G. Polyphenol and vitamin C contents in European commercial blackcurrant juice products, Food Chemistry. 2011; 127(3): 1216–1223.

6 Karjalainen R, Anttonen M, Saviranta N, Stewart D, McDougall G, Hilz H, Mattila P, Törrönen R. A review on bioactive compounds in black currants (*Ribes*nigrum L.) and their potential health promoting properties. Actahorticulturae. 2008; 839: 301-307.

7 Hou L, Zhou B, Yang L, Liu ZL. 2004. Inhibition of free radical initiated peroxidation of human erythrocyte ghosts by flavonols and their glycosides, Organic and Biomolecular Chemistry. 2004; 2: 1419–1423.

8 Pandey M, Verma RK, Saraf SA. Nutraceuticals: new era of medicine and health. Asian J Pharm Clin Res. 2010; 3:11–15.

9 Kalra, EK. 2003. Nutraceutical- Definition and Introduction. AAPS Pharm Sci 2003; 5: 27-28.

10 Estevinho LM. Editorial – Special Issue 'Nutraceutical in Human Health and Diseases. International Journal of Molecular Sciences. 2018; 19:1-3.

11 Sofowora, E.A. Medicinal plants and traditional medicine in Africa Spectrum Books ltd, Ibadan Nigeria, 1993; 134-155.

12 Trease GE, Evans WC. A Textbook of Pharmacognosy. 13th Edition. BailliereTindall, London, UK. 1992; 79-92.

13 Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria. Global J. Pure Appl. Sci. 2001; 8: 203-208.

14 Boham BA, Kocipai-Abuyazan. Flavonoids and condensed tannins from the leaves of Vaccinumraticulatum and Vaccinumcalycinium. Pacific Sci. 1994; 48: 458-463.

15 Van Buren JP, Robinson WB. Formation of Complexes between Protein and Tannic Acid, Journal of Agric Food Chemistry. 1981; 17:772 – 777.

16 Harborne JB. Phytochemical methods:A guide to modern techniques of plant analysis. Chapman and Hall Ltd, London. 193; 279.

UNDER PEER REVIEW

17 Brand-Williams W, Cuvelier M, Berset C. Use of free radical method to evaluate antioxidant activity, LWT-Food Science and Technology. 1995; 28 (1): 25-30.

18 Ayoola GA, Sofidiya T, Odukoya, O, Coker HAB. Phytochemical screening and free radical scavenging activity of some Nigerian medicinal plants. J. Pharm. Sci. & Pharm. Pract. 2006; 8: 133-136.

19 Benzie S. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power: The FRAP assay. Anal Biochem. 1996;239(1):70-76.

20 Egharevba RKA, Ikhetus MI. 2008. Ethno-Medicinal uses of Plants in the Treatment of Skin Diseases in Ovia North East, Edo State Nigeria. Res. J. Agricult. Biological Sci. 2008; 4(1): 58-64.

21 Wang LF, Chen JY, Xie HH, Ju XR, Liu RH. 2013. Phytochemical Profiles and antioxidant activity of adlay varieties. J. Agric. Food Chem. 2013;61: 5103–5113.

22 Prakash D, Kumar N. Cost Effective Natural Antioxidants. In: RR Watson, JK Gerald and VR Preedy (eds) Nutrients, Dietary Supplements and Nutriceuticals. Humana Press, Springer, USA. 2011; 163-188.

23 Prakash D, Gupta C, Sharma G. Importance of Phytochemicals in Nutraceuticals. Journal of Chinese Medicine Research and Development (JCMRD). 2012;1 (3): 70-78.

24 Li L, Hai J, Li Z. 2014. Resveratrol modulates autophagy and NF-κB activity in murine model for treating non-alcoholic fatty liver disease. Food ChemToxicol. 2014; 63: 166–73.

25 Starkov AA. 2008. The role of mitochondria in reactive oxygen species metabolism and signaling. Annal. New York Acad. Sci. 2008; 1147: 37-52.

26 Marnett LJ.Oxyradicals and DNA damage. Carcinogenesis. 2000; 21:361–70.

27 Stadtman ER. 2000. Protein oxidation in aging and age-related diseases. Ann. New York Acad. Sci, 2000; 928: 22-38.

UNDER PEER REVIEW

28 Ellidag HY, Eren E, Aydın O, Akgol E, Yalcınkaya S, Sezer C. Ischemia Modified Albumin Levels and Oxidative Stress in Patients with Bladder Cancer. Asian Pacific J Cancer Prev. 2013; 14(5): 2759–63.

29 Olivieri S Conti A, Iannaccone S, Cannistraci CV, Campanella A, Barbariga M. 2011. Ceruloplasmin oxidation, a feature of Parkinson's disease CSF, inhibits ferroxidase activity and promotes cellular iron retention. J. Neurosci.31. 2011; 18568–18577. doi: 10.1523/JNEUROSCI.3768-11.

30 Pan XD, Zhu YG, Lin N, Zhan J, Ye QY, Huang HP, Chen XC. Microglial phagocytosis induced by fibrillar β-amyloid is attenuated by oligomeric β-amyloid: implications for Alzheimer's disease. MolNeurodegener. 2011; 6(45): 1–17.

31 Bashan N, Kovsan J, Kachko I, Ovadia H, Rudich A. Positive and negative regulation of insulin signaling by reactive oxygen and nitrogen species. Physiological Reviews. 2009; 89: 27-71

32 Sung J, Lee J. Antioxidant and antiproliferative activities of grape seeds from different cultivars. Food Sci. Biotechnol. 2010: 19, 321–326.

33 Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI, Bahorun T. 2005. Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. Mutat. Res. 2005;579: 200–213

34 Kim HJ, Cha BY, Park IS, Lim JS, Woo JT, Kim JS. Dehydroglyasperin C, a component of liquorice, attenuates proliferation and migration induced by platelet-derived growth factor in human arterial smooth muscle cells. Br. J. Nutr. 2013; 110: 391–400.

35 Prahalathan P,Saravanakumar M, Raja B. 2012. The flavonoid morin restores blood pressure and lipid metabolism in DOCA-salt hypertensive rats. Redox Rep. 2012; 17: 167–175.

36 Barrajón-Catalán E, Fernández-Arroyo S, Saura D, Guillén E, FernándezGutiérrez A, Segura-Carretero A, Micol V. Cistaceae aqueousextracts containing ellagitannins show antioxidant and antimicrobial capacity, and cytotoxic activity against human cancer cells. Food and Chemical Toxicology. 2010;48: 2273-2282.

37 Cho AS, Jeon SM, Kim MJ, Yeo J, Seo KI, Choi MS, Lee MK. Chlorogenic acid exhibits anti-obesity property and improves lipid metabolism in high-fat diet-induced-obese mice. Food Chem. Toxicol. 2010; 48: 937–943.

UNDER PEER REVIEW

38 Eruvbetine D. Canine nutrition and health. A paper presented at the seminar organised by kensington pharmaceuticals nig. Ltd, lagos on august 21, 2003

39 Jarup L. 2003. Hazards of heavy metal contamination. Br Med Bull. 2003; 68:167-82.

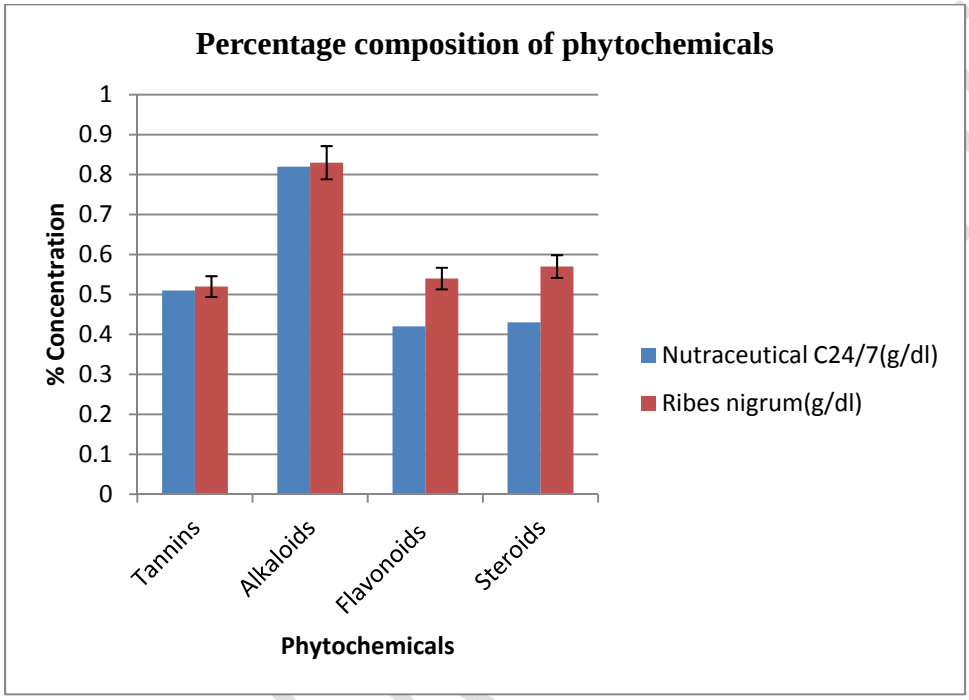
40 Flora SJS, Chouhan S, Kannan GM, Mittal M, Swarnakar H. Combined administration of taurine and monoisoamyl DMSA protects arsenic induced oxidative injury in rats. Oxidat. Med. Cell. Long. 2008; 1: 39–45.

41 41. Ebrahimzadeh M A, Pourmorad F, Bekhradnia A R. Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran. African journal of Biotechnology. 2008d;7(18): 3188-3192.

UNDER PEER REVIEW

FIGURES:

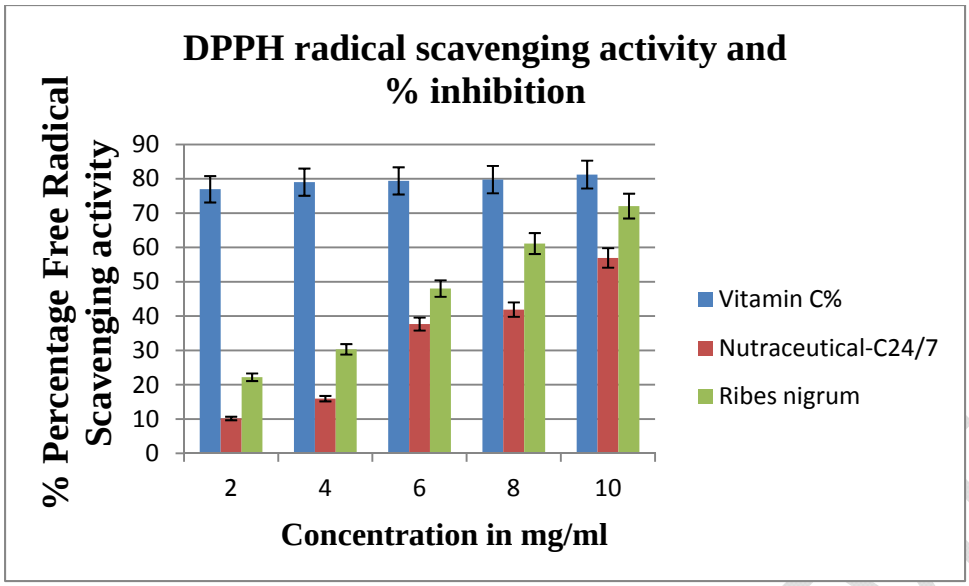
Fig 1: Percentage composition of phytochemicals



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

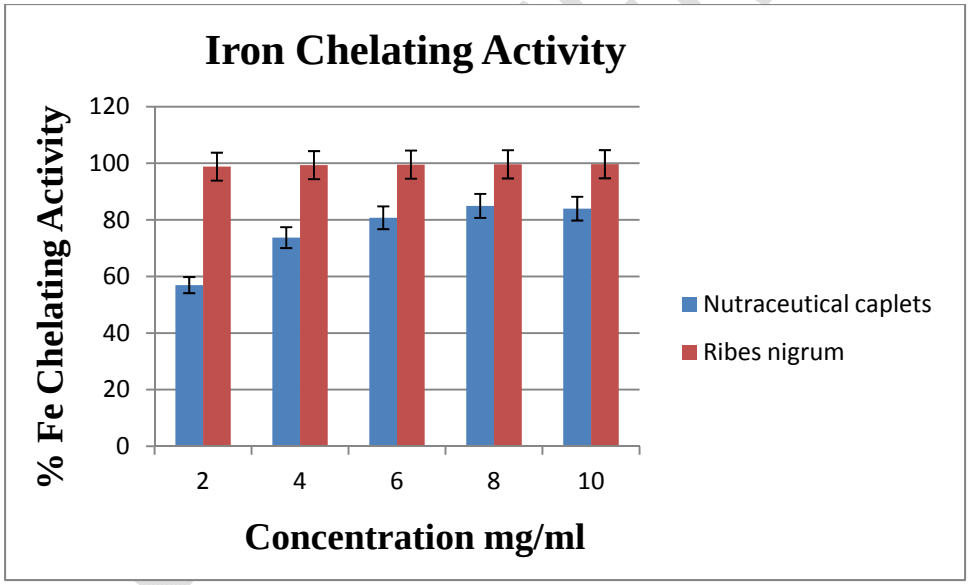
Fig 2: DPPH free radical scavenging and % inhibition

UNDER REVIEW



The figure above shows the percentage free radical scavenging activity of *Ribesnigrum* 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 *Ribesnigrum* extract and the nutraceutical C24/7 caplets.

TABLES:

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

Phytochemicals	Ribesnigrum	Neutracetical-C24/7
Tannins	+	+
Steroid	+	+
Triterpenoids	_	-
Saponins	+	+
Phenols	+	+
Alkaloids	+	+
Terpenoids	+	-
Flavonoids	+	+

KEY: (+) Present and (-) Absent

Table 2: Percentageconcentration (mean± SD) and wavelength of quantified phytochemicals

Phytochemical	% Concentration(mean± SD)		Wavelength(nm)
	Nutracetical C24/79(g/dl)	*Ribesnigrum*(g/dl)	
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 $P<0.05$

UNDER PEER REVIEW

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	***Ribesnigrum***
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 Ribesnigrum and the nutraceutical C24/7 caplets.

% Fe Chelating Activity		
Concentration (mg/ml)	**Nutraceutical caplets**	**R*ibesnigrum***
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 $P<0.05$.

UNDER PEER REVIEW