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Original Research Article

Evaluation of the Phytochemical and Antioxidant Properties of Cold, Hot Water and Wine Extracts Produced from *Ficus capensis* Leaf

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ABSRTACT

Red wine consumption has increased tremendously due to the apparent health benefits and pleasure derived from red wines. In the quest to search for alternative sources of raw material for wine production, the phytochemical and antioxidant properties of the cold and hot water extracts of *Ficus capensis* leaf as well as the wine produced from hot water extract of *F. capensis* leaf were evaluated. The qualitative screening of phytochemical constituents in cold and hot water extracts of *F. capensis* leaf revealed the presence of flavonoids, tannins, phenols, steroids, carbohydrates, proteins, amino acids, alkaloids and phytate. The quantitative phytochemical screening of the cold and hot water extracts as well as the wine extracts of *F. capensis* using HPLC method revealed the presence of important bioactive ingredients with the concentration of the phenolic and flavonoid compounds of *F. capensis* wine significantly ($p<0.05$) higher than the cold and hot water extracts. The wine extract exhibited significantly ($p<0.05$) decrease in the antioxidant activity against DPPH, nitric oxide, total antioxidant capacity and FRAP compared to the cold and hot water extracts. Moderate consumption of *F. capensis* wine would help to protect the organism against oxidative damage to lipids, proteins, and nucleic acids caused by the phenols in the drink. Moderate consumption of *F. capensis* wine would help to protect the organism against oxidative damage to nucleic acids lipids, and proteins caused by the phenols in the drink.

Keywords: *Ficus capensis*, DPPH, Phytochemicals, Hot water extract, Cold water extract, wines, Antioxidants

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Introduction

Wines have been made from fruits, herbs, vegetables, and sometimes a combination of both fruits and vegetables for decades.[1, 2] Making wine from vegetables has enhanced people's exposure to the plant's beneficial nutrients, chemicals, and medicinal properties.[3] Most vegetables lack the necessary nutrients for yeast feeding, and water to form a stable drink naturally hence must is altered for local vegetable fermentation to produce their wines. Veggies with low quantity of fermentable sugars must be "chaptalized" in order for the resulting wine to have acceptable alcohol levels.[2] As a result, sucrose is added as the primary carbohydrate so that fruits with high acid levels (typically citric or malic acid) can hydrolyze sucrose into fermentable fructose and glucose sugars.[1] Some fruits and veggies have naturally high levels of acidity, making them unsuitable for making a tasty fruit wine. Therefore, the "must" is typically diluted with water to reduce the acidity to a more tolerable level prior to fermentation.[4] These wines produced from fruits and vegetables are named after the parent plant.[5] Fermentation is a bioengineering that involves feeding sugars and nutrients in solution to a required microorganisms, which reciprocates by producing carbon dioxide gas and alcohol.[6]

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As a result, fruits rich in acid (usually citric or malic acid) are added to the mix as the major carbohydrate, and the sucrose they contain is hydrolyzed into fermentable fructose and glucose sugars.[1] Some fruits and veggies have naturally high levels of acidity, making them unsuitable for making a tasty fruit wine. Therefore, the "must" is typically diluted with water to reduce the acidity to a more tolerable level prior to fermentation. At the moment, most of the wine production processes are relying on *Saccharomyces cerevisiae* strains that allow rapid and reliable fermentations, reduce the risk of sluggish or stuck fermentations and prevent microbial contaminations.[7] Yeast starter cultures that are deliberately chosen for the winemaking process based on scientifically proven features often complement and optimize the raw material quality and individual characteristics[4] of the alcohol, resulting in a more desirable outcome.[7,8] These musts and wines are fermented and aged, and their alcohol concentration typically ranges from 5 to 13%.[9]

In Nigeria, red wine consumption has increased tremendously due to the apparent health benefits and pleasure derived from red wines.[10] European countries such as France, Spain, and Italy contribute for around 60% of overall imports to Nigeria, while the Republic of South Africa and America account for approximately 22% and 8% of the nation's total imports, respectively.[11,12] The rise in import costs for good-quality red wine, the rapid depreciation of the Nigerian currency, and competition for the use of these fruits between wineries and man as a result of wines being produced from veggies and fruit due to grape scarcity in the world's tropical regions today have necessitated the search for plants with physicochemical and antioxidant attributes for wine production.[13] *Ficus capensis*, often known as *Ficus sur*, is a member of the Moraceae family. The plant matures into a deciduous tree that can reach a height of 35 to 40 meters. It has extensive roots, branches, and large green leaves (115-131ft). Young leaves have a glabrous top surface and a hairy below surface.[14] They are spirally and alternately organized, with uneven margins and elliptical to ovate

forms. All year long, *F. capensis* bears fleshy fruits along the trunk and main branches, either in a single or branched raceme.[15] It is a well-known medicinal plant that has long been used to treat a variety of diseases and disorders, such as diabetes, liver disorders, diarrhea, inflammatory conditions, hemorrhoids, respiratory ailments, and urinary diseases. The fruits of *F. capensis* are used to cure dry cough, loss of voice, kidney and spleen disease, intestine astringency, leucorrhoea, a burning feeling, weariness, and urinary tract infection.[16] The fruit extract has antiulcer qualities, and the component -sitosterol present in the fruit has substantial hypoglycemic activity, which means it lowers blood glucose levels.[17] Ayurvedic practitioners employ the fruits of *F. capensis* to treat bleeding conditions such as nose hemorrhage, menorrhagia, and rectal bleeding.[18] The leaves and roots have been shown in Sudan and Nigeria to exhibit biologically beneficial drug-like qualities such as microbial inhibitory activity,[19] antiulcer activity,[20] antisickling activity,[21] antidiarrheal activity,[22] leukocyte mobilization, and immune-boosting action.[23,14]

Phytochemical characteristics of *F. capensis* leaf, root, and seed extracts have previously been documented in the literature.[24,14,16]

There has been no research on the phytochemical qualities of hot water and wine extracts created from *F. capensis* leaf, thus the current work explores the phytochemical and antioxidant properties of cold, hot, and wine extracts produced from *F. capensis* leaf.

Materials and Method

Fresh matured *F. capensis* leaf separated from stalks was collected from a local bush in 2019 in Abraka, Ethiope East Local Government Area of Delta State. It was identified and authenticated with a voucher specimen (UBH-f331) by a taxonomist Dr. H.A. Akinnibosun, at the Division of Botany, Faculty of Life Sciences, University of Benin, Nigeria.

Preparation of *F. capensis* extract/sample

Cold water sample

The plant tissue homogenization technique was applied for the preparation of cold water leaf samples of *F. capensis*. About 100 g *F. capensis* leaf was weighed, and crushed to a smooth paste using mortar and pestle. Before filtering, about one liter of deionized water was added and shaken vigorously at 36.8ºC for 5–10 minutes. The centrifugation of filtrate was done for sample clarification.[25]

Hot water sample

The decoction technique was applied for the preparation of the hot water leaf sample of *F. capensis*. This was achieved by heating 150g of the fresh leaf in 1litre of water for 15 - 20 minutes at 100 degrees Celsius and thereafter left to cool down.[25] Both sample extracts were appropriately labeled and stored in a refrigerator at 4 ^{0}C to avoid deterioration and wastage for future use.

Bioactive compounds of cold and hot water extracts of F. capensis determination

The qualitative phytochemical screening was done using standard principles to identify alkaloids,[26] amino acids, carbohydrates, phenolic compounds and tannins, phytosterol, cardiac glycoside, proteins (millon's test),[25] anthraquinones, anthocyanosides, flavonoids and saponins.[27]

Chromatographic analysis of phytochemical compounds in the cold, hot and wine extracts of F. capensis

The mobile phase is made up of 1% aqueous acetic acid solution (Solvent A) and acetonitrile (Solvent B), with a flow rate of 2ml/min. The sample injection volume was 5l, and the column temperature was set to 28 ^{0}C. To achieve gradient elution, the percentage of solvent B to solvent A was changed. The mobile phase composition was returned to its initial solvent B: solvent A: 10: 90 ratio in 55 minutes, and it was let to stand for another 10 minutes before another sample injection. The entire analysis time per sample was 65 minutes. The HPLC chromatograms were detected using a photo diode array UV detector at three different wavelengths: 272, 280, and 310 nm, depending on the chemicals investigated. Compounds were identified using retention time and spiking with standards under the same conditions. The integrated peak area was measured and the content was estimated to complete the sample measurement.

Calculation

Total and individual parameter specifications

The following are the total and individual standard concentrations of cold, hot, and clarified wine:

Parameter concentration (mg/g) = (Peak area)/(Standard peak area) x Standard concentration

Total phenols quantitative estimation[28] reducing sugar determination assay,[29] total flavonoids estimation,[30] reducing sugar determination assay,[29] alkaloids,[31] and phytate.[32]

Assay for antioxidants

The antioxidant activity and free radical scavenging of *F. capensis* cold, hot, and wine extracts were determined in vitro using the 1, 1-Diphenyl-1-picrylhydrazyl (DPPH) assay,[33] Nitric Oxide (NO),[34] FRAP Assay [35], and Total Antioxidant Capacity.[36]

Statistical investigation

Using SPSS version 22.0, the generated data were subjected to the student T test. At $p < 0.05$, the mean differences were judged significant.

Results and Discussion

Table 1 shows the qualitative phytochemical composition of cold and hot water extracts of *F. capensis* leaf. The presence of significant flavonoids, tannins, phenols, steroids, carotenoids, terpenes, alkaloids, and phytate was discovered in the cold and hot water extracts of *F. capensis* leaf (Table 1), (Figure 1), and (Figure 2). The study revealed that the hot water and wine extracts were high in phenol, flavonoids, reducing sugar, and alkaloid content. (Table 2). The wine had more phenol, alkaloids, and reducing sugars than flavonoid and phytate (Table 2). Flavonoids are water-soluble chemicals that diminish free radicals by quenching, up-regulating, or protecting antioxidant defenses, as well as chelating radical intermediate molecules.[37] The HPLC phytochemical constituents of cold and hot water extracts of *F. capensis* leaf (Figure 1- 4) was found to contain Quercitin > Kaemoferol > Geranyl acetone > Rutin > Alpha Caryophyll > Linalool > Orientin > Catechin > Epigallogatechin > Myricetin > Scytaline > Lupeol > Cetylbehenite > Luteolin, which are very important bioactive phytochemicals. In the cold water extract of *F. capensis*, the highest quantities were Quercitin (96.25 ppm) and Kaemoferol (31.74 ppm), while the lowest values were Luteolin (0.61 ppm) and Cetylbehenite (0.46 ppm). In the hot water extract of *Ficus capensis*, the greatest concentrations were Quercitin (98.44 ppm) and Kaemoferol (34.4 ppm), while the lowest values were Cetylbehenite (0.64 ppm) and Luteolin (0.61 ppm). The hot water extract's total phytochemical concentrations (21.25 ppm) were found to be greater than the cold water extract's total phytochemical concentrations (19.39 ppm). As a result, the presence of these essential phytochemicals in the water extracts of *F. capensis* highlights the leaf's potential nutritional and therapeutic value. Phytochemicals are bioactive substances found naturally in plants that have protective and disease-preventive characteristics, making them useful in medicine and pharmacology.[38,39,40,41,42] Alkaloids protect plants against herbivores, play metabolic roles in living organisms, and have pharmacological activities such as analgesic, antimicrobial, antibacterial, and antibiotic properties. Plant flavonoids and phenols have been shown to have antioxidant, free radical scavenging, anti-inflammatory, and anticarcinogenic effects.[40,41,42] plant-derived phenol molecules have been shown to contribute to the color, sensory, and antioxidant qualities of food.[42,43,44,45] Several methods have been proposed to explain the antioxidant activity of phenolic compounds, including reducing capacity, chain initiation prevention, binding of transition metal ion catalysts, peroxide breakdown, continuing hydrogen abstraction prevention, and radical scarvenging.[46,47,48,49] The findings confirm the work of [50,51,52,16] who discovered that *F. capensis* has high nutritional value and can be used as a neutraceutical as well as a possible source of natural antioxidants that may slow the progression of various oxidative-related disorders.

Table 1: Qualitative Phytochemical Screening

Phytochemicals	Cold sample	Hot sample
Saponins	-	-
Phlobatannins	-	-
Cardiac gycosides	-	-
Flavonoids	+	+
Tannins	+	+
Phenols	+	+
Steroids	+	+
Terpenes	-	-
Carbohydates	+	+
Protein	+	+
Amino acid	+	+
Gum and mucilage	-	-
Alkaloid	+	+
Phytate	+	+

Key: +Present, -Absent

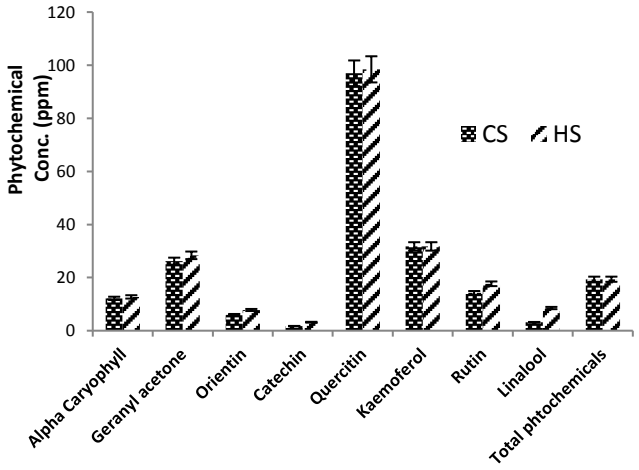

Figure 1: Phytochemical profile of cold and hot water leaf extract of *F. capensis* with concentration
CS – Cold sample; HS- Hot sample

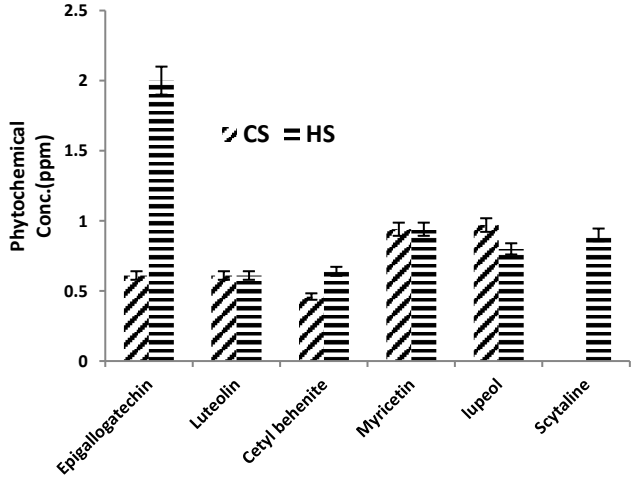

Figure 2: Phytochemical profile of cold and hot water leaf extract of *F. capensis* with concentration
CS – Cold sample; HS- Hot sample

The work is also consistent with[21,19,51,20,] Saponin was shown lacking in both extracts, as demonstrated by Otitoju's research.[53] In the ethanol extract of *F. capensis* leaves, Ezeigwe[16] found a significant percentage of flavonoids, alkaloids, tannin, saponin, phtate, oxalate, phenol, haemaglutinnin, and cardiac glycosides. Wilfred[54] reported the presence of these phytochemicals in abundance in *F. capensis* leaf extracts, whereas Chizoruo[55] found the presence of varied levels of flavonoids, terpenes, and steroids in *F. capensis* leaf extracts. Saponins, cardiac glycosides, flavonoids, and tannins were found in the aqueous extracts of *F. capensis* thumb by Ayinde and Owolabi [22], but alkaloids, cyanogenic glycosides, and anthrancene derivatives were not. The presence of phytochemical elements may differ depending on the climatic and soil makeup of the place where the plant was harvested.[55,56] Figure 5 - 6 depicts the phytochemical profile and HPLC chromatogram of *F. capensis* wine, which included more phytochemicals in the following order of concentration: Resveratrol, quercitin, trans-E-viniferin, gallic acid, chlorogenic acid, beta carotene, delphyridin, caffeic acid, lutein, epicatechin, petunidin, cyanidin, stigmasterol, catechin, beta sitosterol, and taxifolin are all examples of phytonutrients. Resveratol (11.96 ppm) had the highest concentration in the wine, whereas taxifolin (0.11 ppm) had the lowest. The overall concentration of phytochemicals is 29.69 ppm. The phenolic chemicals kaempferol, quercitin, and catechin are capable of chelating metallic ions.[46,47,48,49,52,53,54] These secondary bioactive metabolites play a significant function in red wine by acting as anti-alcohol agents.[46] The wine's higher phenolic content could be linked to the fermentation process's ability to convert conjugated forms of phenolic compounds to their free forms. This could possibly be due to the microbes producing proteolytic enzymes, which are responsible for hydrolyzing phenolic complexes into soluble - free phenols.[57]

The most effective wine antioxidants include resveratol, anthocyanins, and catechins.[58,59] The levels of resveratrol, quercitin, and gallic acid in the wine were higher than the levels of other phytochemicals. Resveratrol has been found to play a role in cardiovascular disease prevention.[59] Quercetin has been shown to have health advantages in a variety of chronic conditions, including neurological disorders, diabetes, cardiovascular disease, and cancer.[60,61] It can also prevent the oxidation of glutathione (GSH), which aids in the prevention of neurotoxicity caused by oxidative stress.[62,63] It has been discovered to protect the brain from cytotoxicity caused by H_2O_2.[63] Trans-E-viniferin reduces reactive oxygen species while increasing gluthione (GSH) levels.[64,65] Gallic acid (3,4,5-trihydroxybenzoic acid) is a naturally occurring antioxidant that may protect healthy people by preventing apoptosis.[47,50] It is widely acknowledged that chlorogenic acid (CGA) offers various health benefits as an anti-hypertension drug.[66] Chlorogenic and caffeic acids, two of the most prominent hydroxycinnamic acids, have also been linked to a reduction in oxidative and inflammatory stress. Beta-carotene is a provitamin A that can be transformed into vitamin A (retinol), which plays important roles in the control of physiological activities in animal bodies.[67] The discovered anthocyanin profiles are delphinidin > petunidin > cyanidin. Anthocyanin is a powerful antioxidant and anti-inflammatory substance that can reduce the risk of acquiring diseases such as atherosclerosis. It promotes longevity and reduces oxidative stress.[68,69,70] Caffeic acid has numerous therapeutic effects, including antioxidant, immunomodulatory, antibacterial, neuroprotective, antianxiety, antiproliferative, and anti-inflammatory qualities.[71,72] Caffeic acid and chlorogenic acid have been demonstrated to inhibit key enzymes associated with hypertension and to protect the heart from prooxidant-induced oxidative damage in rats.[73] Lutein is a carotenoid that has anti-oxidative and anti-inflammatory qualities that protect against alcohol-induced liver injury[68,74,75] ameliorate or even prevent age-related macular disease, which is the primary cause of blindness and vision impairment. Steroids and phytosterols have been shown to increase nitrogen retention in osteoporosis and wasting disease mice.[38] As a rich source of bioactive components and powerful antioxidants, *F. capensis* wine will guard against alcohol damage while also providing various health benefits such as anti-diabetic, anti-aging, anti-microbial, and chronic disease protection. However, the amount drank should be limited because excessive alcohol use can have negative economic, medical, and societal implications.[44,59,69]

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Table 2: Quantitative Phytochemical and reducing sugar screening of the cold, hot water and wine extracts of *F. capensis* leaves using spectrophotometer

Phytochemicals	Cold sample	Hot sample	Clarified wine
Phenols (mg/gGAE)	0.03 ± 0.000a	0.04 ± 0.000ac	0.042 ± 0.001c
Flavonoids (mg/gGAE)	0.15 ± 0.010b	0.45 ± 0.080ac	0.074 ± 0.004d
Alkaloid (mg/gCAE)	0.17 ± 0.010a	0.22 ± 0.020ac	0.472 ± 0.006d
Phytate (mg/g)	0.37 ± 0.010b	0.14 ± 0.020bc	0.050 ± 0.001d
Reducing Sugars (mg/gGluE)	0.36 ± 0.010b	1.42 ± 0.010ac	1.528 ± 0.016d

Values are means ± standard deviations of triplicate determinations. Values not sharing common superscripts on the same column differ significantly ($p<0.05$). The mean differeneces were considered significant at $p < 0.05$. GAE= Gallic acid equivalent, CAE = Catechin equivalent, GluE = Glucose equivalent.

Table 3: Free radical and *In-vitro* antioxidant activity of cold, hot water and wine extract of *F. capensis* leaves

Antioxidant parameter	DPPH (%inhibition)	FRAP (%inhibition)	NO (%inhibition)	TAC (mg/gGAE)
Cold sample	79.02 ± 2.94^{a}	1.60 ± 0.02^{b}	75.33 ± 0.33^{a}	0.09 ± 0.00^{b}
Hot sample	87.1 ± 0.92^{b}	2.22 ± 0.03^{a}	77.6 ± 1.73^{a}	0.24 ± 0.00^{a}
Wine sample	30.03 ± 15.57^{c}	0.02 ± 0.01^{c}	55.41 ± 4.09^{b}	0.27 ± 0.01^{a}

Values are means ± standard deviation of triplicate determinations. Values not sharing common superscripts on the same column differ significantly ($p<0.05$). DPPH = 1,1- diphenyl -2-picryl hydrazl, FRAP = Ferric reducing antioxidant power, NO (%inhibition) = Nitric Oxide and TAC (mg/gGAE) = Total antioxidant capacity.

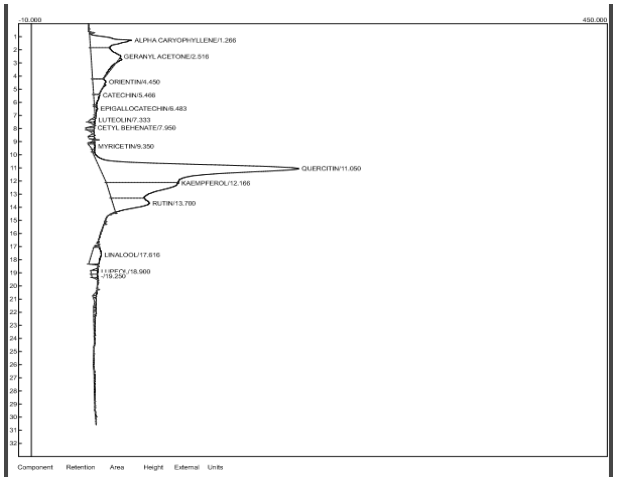

Figure 3: Chromatogram of phytochemical composition of cold water leaf extract of *Ficus capensis*

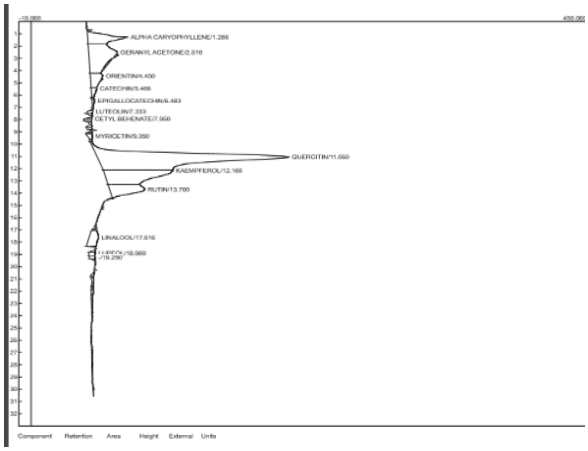

Figure 4: Chromatogram of phytochemical composition of hot water leaf extract of *F. capensis*

F. capensis hot and cold water extracts demonstrated antioxidant efficacy against DPPH, nitric oxide, total antioxidant capacity, and FRAP activities. (Table 3). The DPPH radical assay is a rapid, dependable, and reproducible method for evaluating antioxidant free radical scavenging activity. DPPH is a stable nitrogen-centered radical that readily accepts an electron or a hydrogen radical to form a stable diamagnetic molecule.[64,76,77] The DPPH test is an important procedure in determining a substance's antioxidant capacity. [60,77,78] The FRAP test is another frequently used approach for determining a phytochemical compound's ability to give electrons that decrease oxidized intermediates.[77] Doris and Rosemary[79] discovered that increasing the concentration of extract increased the antioxidant activity of F. capensis leaves. According to Seun *et al*.,[47,] radical scavenging and Fe^{2+} - chelating capacities are two probable mechanisms through which *F. capensis* leaves extract could be used to treat erectile dysfunction. Invitro antioxidant activity of *F. capensis* was also reported by Ehimwenma and Osarieme.[80]

The antioxidant activity of the wine extract against DPPH, nitric oxide, total antioxidant capacity, and FRAP was reduced. (Table 3). This could occur as a result of the wine's aging. Jan et al.[81] discovered that the antioxidant activity of several fruit extracts decreases with age. The antioxidant capacity of wine is thought to be highly associated with the type of phenolic compounds found in wine. This demonstrates one of the most important roles of phenols in antioxidant activity.[40,42,44,47,48,49] Antioxidant activity is now regarded as one of the most important features of red wines, and it is linked to the presence of polyphenols such as flavonoids, phenolic acids, stilbenes, coumarins, lignoids, and so on.[44,45,49,73,82,83] Moderate consumption of *F. capensis* wine would help to neutralize free radicals, protecting the organism from oxidative damage to lipids, proteins, and nucleic acids caused by the phenols in the drink.[51,81]

Conclusion

This finding implies that phytochemical compounds discovered in the cold water extract of *F. capensis* leaf were also found in the hot water extract. Furthermore, *F. capensis* wine prepared from hot water extract using *Saccharomyces cerevisae* includes bioactive ingredients that, when consumed in moderation, will prevent cardiovascular disorders as well as act as a preventative agent against alcohol damage.

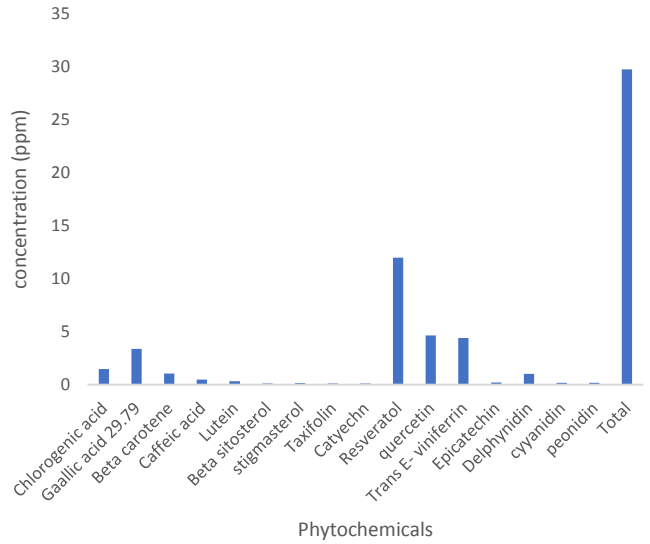

Figure 5: Phytochemical profile of *F. capensis* wine

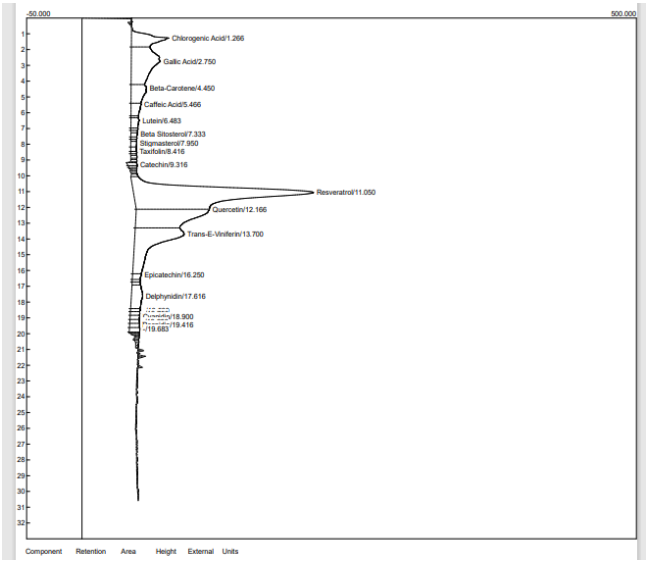

Figure 6: Chromatogram of phytochemical composition of *F. capensis* wine

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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