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Physicochemical, Antimicrobial, Lethality and *In Vitro* Antioxidant Profiles of Johnu Tisane: A Coffee (*Coffea Arabica*) Leaf Decoction Formula

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Abstract

Coffee leaves-based tea has relevance in ethno-medications due to its rich phyto-constituents-related diverse pharmacologic activities. Jonhu tisane, a typical *Coffea arabica* leaf decoction has no supporting scientific basis amidst reported location, processing and specie-related variations. This investigated physicochemical, antimicrobial, lethality and *in-vitro* antioxidant profiles of Johnu tisane by acceptable methods. Results recorded moisture (92.26 %), ash (0.65 %), unsaponified matter (1.46 mg/100 g), free fatty acid (0.56 mgKOH/g), acid value (1.12 mgKOH/g), potential hydrogen (6.85), lethal concentration (1000 ppm) and concentration-dependent antimicrobial activity. Anti-oxidation results revealed total antioxidant capacity, ferric reducing antioxidant power, nitric oxide, hydrogen peroxide and 2, 2-diphynyl-1-picrylhydrazyl scavenging activities increased concentration-dependently compared to standard. Thus, Johnu tisane demonstrated low minerals and keeping quality; requisite physicochemical mix for consumption and bioactivity; high safety margin; antimicrobial potency; and requisite anti-oxidation capacity for *in-vivo* antioxidant role. These provided scientific support for its ethno-medicinal uses. They underscored the need to elucidate its pharmacologically active compounds; mechanistic roles in animal models; and the impact of ash to moisture mix variation on potential hydrogen, microbial and antimicrobial activities in relation to shelf life, bioactivity and *in vivo* anti-oxidative roles for novel insights on preserving sample quality, safety, bioactivity and *in-vivo* anti-oxidative outcomes.

Keywords: *In-vivo* anti-oxidative outcomes and roles; Johnu tisane; Requisite physicochemical mix; Requisite anti-oxidant capacity; Rich phyto-constituents-related diverse pharmacologic activities.

Abbreviations: ANOVA = analysis of variance; DMRT = Duncan Multiple Range Test; DPPH = 2, 2-diphynyl-1-picrylhydrazyl; FRAP = ferric reducing antioxidant power; H_2O_2 = hydrogen peroxide; IZD = inhibition zone diameter; NO = nitric oxide; pH = potential hydrogen; NOSA = Nitric oxide scavenging activity; TAC = total antioxidant content.

INTRODUCTION

Coffee leaf tea has been used in varied ethnomedications against diverse diseases over the years notably in the coffee plant growing countries (Campa and Petitvallet, 2017; Chen et al., 2019). The relevance of tea prepared from coffee leaves in varied ethno-medications was attributed to its antioxidant-rich phyto-constituents and the attendant antioxidant, antimicrobial, antiobesity, antiinflammatory, antihypertensive and other pharmacologic activities. These led to growing interests in its application in functional foods, pharmafoods, nutraceutical and ethno-medicines (Upadhyay and Mohan Rao, 2013). Identification of novel antimicrobials from plant parts is fundamental to new drug discovery (Obasi et al., 2011) since microbial pathogens cause diseases with high morbidity and mortality (GBD 2019 Antimicrobial Resistance Collaborators, 2022). Oxidative stress has been implicated in the pathophysiology of many disorders (Vona et al., 2021). And, antioxidants (secondary metabolites in living organism that are abundant in fruits and leafy vegetables) slow or prevent oxidative stress known to be a fundamental phenomenon in diverse diseased states (Neha et al., 2019; Singh et al., 2020). Dietary antioxidants, mostly obtained from fruits and leafy vegetables consumption, have been associated with a great balance between free radicals and antioxidant

status, which helps to minimize oxidative stress (Kumar et al., 2016).The genus Coffea belongs to the family Rubiaceae with up to 124 species, among which, *Coffea canephora*, Coffea robusta and *Coffea arabica* are prevalently used (Flore et al., 2023). Jonhu tisane is a decoction formula prepared from *Coffea arabica* leaf and locally marketed and used as herbal tea for the management of varied ailments, including rheumatoid arthritis, obesity and inflammation of the lower limb during pregnancy. Till date, there is no scientific basis supporting these medicinal claims for Johnu tisane amidst the attendant variations due to processing methods, species and location on phyto-constinuents and bioactivities of tea prepared from coffee leaves and other plant food sources (Egbuonu and Nzewi, 2016; Chen et al., 2019; Monteiro et al., 2020).

Therefore, this study investigated some physicochemical, antimicrobial, lethality and *in vitro* antioxidant profiles of Johnu tisane - a decoction formula made from *Coffea arabica* leaves. Physicochemical properties indicate the quality, safety, palatability, and storability; antimicrobial properties indicate the potency against microbial pathogens, and lethality property indicates the safety margin when consumed while *in vitro* antioxidant properties indicate the probable *in vivo* antioxidant potential in combating oxidative stress that underlies every pathology. Oxidative stress has been implicated in the pathophysiology of many disorders (Vona et al., 2021). Diseases caused by microbial pathogens present with high morbidity and mortality hence are of significant public health concerns (GBD 2019 Antimicrobial Resistance Collaborators, 2022).Therefore, this study determined the 1. Physicochemical profile (moisture, Ash, acid value, free fatty acid, unsaponified matter, and potential hydrogen (pH) values); 2. Lethality property through the brine shrimp lethality test; 3. Antimicrobial activity against selected fungi (*Tricophyton (T.), Shigella dysenteriae* (*S. dysenteriae*)) and bacteria (*Tricophyton (T.), Shigella dysenteriae* (*S. dysenteriae*)); and 4. *In* vitro antioxidant properties *via* total antioxidant content (TAC), ferric reducing antioxidant power (FRAP), hydrogen peroxide (H_2O_2), nitric oxide (NO) and 2, 2-diphynyl-1-picrylhydrazyl (DPPH) scavenging activities.

MATERIAL AND METHODS

Chemicals and Reagents

Chemicals used, including those used for the preparation of reagents used, were of analytical grade and were procured from reputable sources.

Procurement of Johnu tisane and identification of the parent leaves used in the preparation

Jonhu tisane decoction formula was bought already prepared from the local sole producer and dispenser, Mrs Cecilia Ezemalukwuo Egbuonu (nee John Ugbo) at Ojoto, Idemili South Local Government area, Anambra State, south eastern Nigeria. The formula was inherited from the father who as a pioneer Catholic convert learnt of the diverse medicinal uses of coffee leaves-based tea from the early Irish missionary Reverend Fathers. It was accordingly prepared by heating a specified quantity of air dried leaves of coffee in a specified volume of water (w/v) to boiling and allowing it simmer before use as herbal green tea. Leaf sample in branches from the coffee plant used in the preparation was obtained from her compound for appropriate identification and deposition in the herbarium at Michael Okpara University of Agriculture Umudike as *Coffea arabica* (Voucher Number: MOH068).

Study design

The study investigated the physicochemical properties of Johnu tisane *via* the determination of moisture, ash, unsaponified matter, free fatty acid, acid value and potential of hydrogen in Johnu tisane. It investigated the antimicrobial activity of Johnu tisane against 10 (two fungal and eight bacterial) pathgogens of public health concerns. It investigated the lethality profile of Johnu tisane *via* the brime shrimp lethality or cytotoxicity test and subsequent plot of percentage mortality for the determination of linearity of and the lethal concentration for 50 % deaths, LC_{50}. The study investigated the *in vitro* antioxidant potentials of Johnu tisane *via* total antioxidant capacity (TAC), hydrogen peroxide, H_2O_2, scavenging ability, Nitric oxide scavenging activity, NOSA, ferric reducing antioxidant power (FRAP) against ascorbic acid, and 2,2-diphynyl-1-picrylhydrazyl (DPPH) scavenging activity against ascorbic acid.

Determination of physicochemical properties

Acid value, AV (mgKOH/g), Potential of hydrogen (pH) and Unsaponified matter (mg/100 g) were determined by the respective method described by Onwuka (2005). Free fatty acid (FFA) (mgKOH/g) was determined by the method of Pearson (1976). Moisture Content (%), and total ash (%) were determined by the respective method of Park (1996).

Determination of antimicrobial activity

To prepare the innocula, microorganism as used in this work were obtained from the stock culture of the microbiology laboratory of the Federal Medical Center, FMC, Umuahia, Abia State, Nigeria. Viability test for each isolate was carried out by resuscitating the organism in buffered peptone broth and thereafter sub cultured into nutrient agar medium and incubated at 37 °C for 24 hrs. The antimicrobial activity of the sample against the test organisms was determined by the cup-plate diffusion method (Ebi and Ofoefule, 1997).

Determination of *in vitro* antioxidant activities

The ferric reducing antioxidant power, FRAP (%) was determined based on the reduction of Fe^{3+} to Fe^{2+} by

antioxidant in acidic medium (Yen and Chen, 1995; Benzie and Strain, 1999). The total antioxidant capacity (TAC) of the extract was determined by the phosphomolybdate assay method (Umamaheswari and Chatterjee 2007; El-hashash et al., 2010). The scavenging activity on 2, 2-diphenyl-1-picrylhydrazyl, DPPH, free radicals was determined by photometric assay (Gyamfi et al., 1999; Mensor et al., 2001) and used ascorbic acid (vitamin C) as reference/standard (Iwalewa et al., 2008). Hydrogen peroxide, H_2O_2, scavenging ability was determined according to the method of Ruch et al. (1989) while nitric oxide radical inhibition/scavenging activity, NOSA, was estimated using the method described by Marcocci et al. (1994).

Determination of Brine shrimp lethality value

The brine shrimp lethality value was determined using the stock solution of actively swimming larva/nauplii obtained from freshly hatched shrimp eggs according to the method described by Sarah et al. (2017) with slight modification (sample measured in ml instead of mg).

Statistical analysis

Results were presented as mean ± standard deviation (SD). Within and between the groups, comparisons were performed by analysis of variance (ANOVA) (using SPSS 17.0 computer software package). Significant differences were compared using Duncan Multiple Range Test (DMRT), and a probability level of less than 5 % ($p < 0.05$) was considered significant.

RESULTS AND DISCUSSION

Physicochemical assessment of Johnu tisane (Table 1) uncovered the presence of moisture (92.26 %), ash (0.65 %), unsaponified matter (1.46 mg/100 g), free fatty acid (0.56 mgOH/g), acid value (1.12 mgOH/g) and potential of hydrogen (6.85). Lethality outcome of Johnu tisane using the brine shrimp lethality/cytotoxicity test (Table 2) andLC_{50} linearity plot of percentage of mortality outcome (Figure 1) uncovered a linearly-derived high lethal concentration, LC_{50} at 1000 ppm.

Table 1. Some physicochemical properties of Johnu tisane.

Physicochemical properties	Value
Free fatty acid, FFA (mgKOH/g)	0.56±0.01
Acid value, AV (mgKOH/g)	1.12±0.02
Unsaponified matter UM, (mg/100g)	1.46±0.02
Ash content, AC (%)	0.65±0.003
Potential of hydrogen, pH value	6.85±0.003
Moisture content, MC (%)	92.26±0.33

Values represent Mean ± standard deviation of triplicate determinations, n = 3

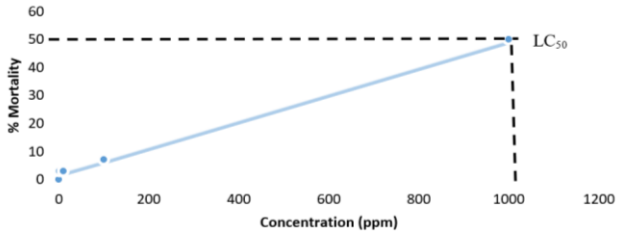

Figure 1. LC_{50} linearity plot of percentage of mortality outcome of Johnu tisane.

The antimicrobial activity test results revealed a concentration-dependent activity against the tested microbial (two fungal and eight bacterial) pathogens comparable to standards notably at the peak tested concentration. There was no measurable activity at the least tested concentration of 1/32 ml (Figure 2, Figure 3 and Table 3).

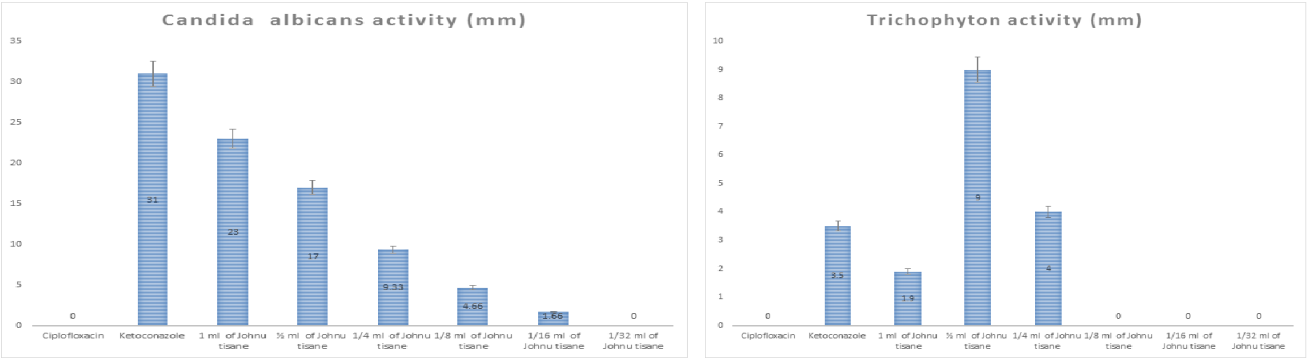

Figure 2. Activity (inhibition zone diameter, IZD, in mm) of Johnu tisane against the fungi *C. albican* and *Trichophyton* (Values represent Mean ± standard deviation of triplicate determinations, n = 3)

Table 2. Activity of Johnu tisane against the bacteria *E. coli*, *N. gonorrhoeae*, *S. typhi* and *P. Mirabilis.*

	Escherichia coli	*Neisseria gonorrhoeae*	*Salmonella typhi*	*Proteus Mirabili*
Ciplofloxacin	43.67±1.53	42.00±1.00	47.00±0.58	34.00±1.00
Ketoconazole	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1 ml of Johnu tisane	29.67±0.58	36.00±1.00	20.00±0.58	17.67± 0.58
½ ml of Johnu tisane	19.00±1.00	14.00±1.00	9.00±0.58	8.00±1.00
1/4 ml of Johnu tisane	10.33±0.58	7.66±0.57	3.67±0.33	2.67±.58
1/8 ml of Johnu tisane	4.00±1.00	3.00±1.00	0.00±0.00	0.00±0.00
1/16 ml of Johnu tisane	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1/32 ml of Johnu tisane	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Values represent Mean ± standard deviation of triplicate determinations, n = 3

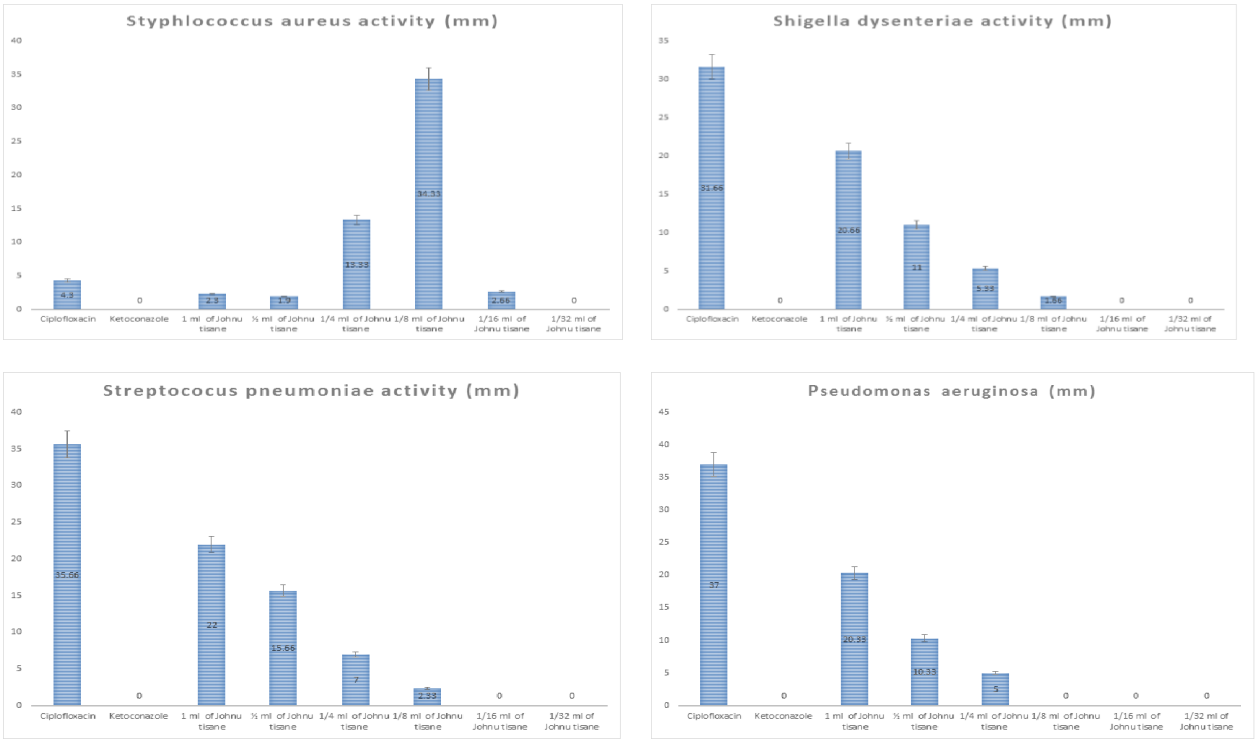

Figure 3. Activity of Johnu tisane against the bacteria *S. aureus, S. dysenteriae*, *S. pneumoniae* and *P. aerouginosa* (Values represent Mean ± standard deviation of triplicate determinations, n = 3)

Similarly, *in vitro* antioxidant results revealed concentration-dependent total antioxidant capacity, TAC, nitric oxide radical scavenging activity, NOSA and hydrogen peroxide scavenging activity (Figure 4).

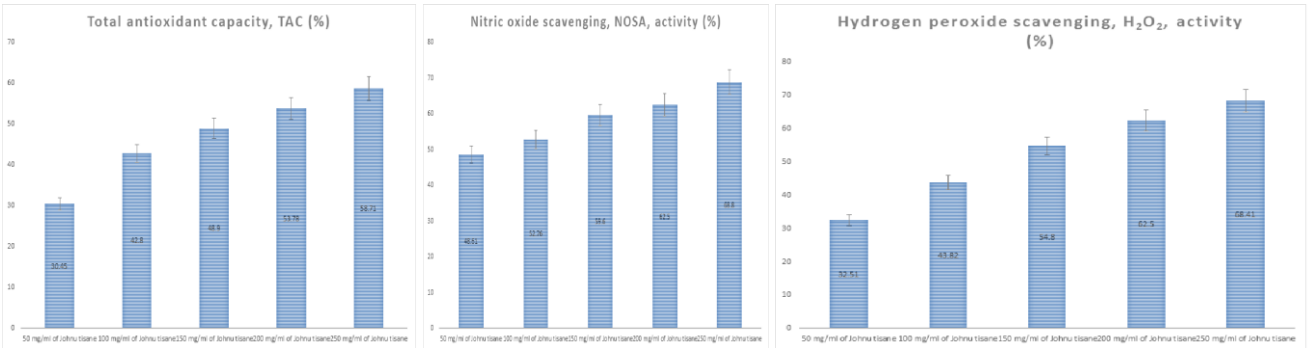

Figure 4. Total antioxidant capacity, TAC, Nitric oxide scavenging activity, NOSA and hydrogen peroxide scavenging activity of Johnu tisane (Values represent Mean ± standard deviation of triplicate determinations, n = 3)

In a similar pattern, 2-diphynyl-1-picrylhydrazyl scavenging (DPPH) activity and ferric reducing antioxidant power (FRAP) increased concentration-dependently in comparison with the standard but a measurable FRAP at the least concentration (25 µg/ml) unlike the reference antioxidant (Figure 5).

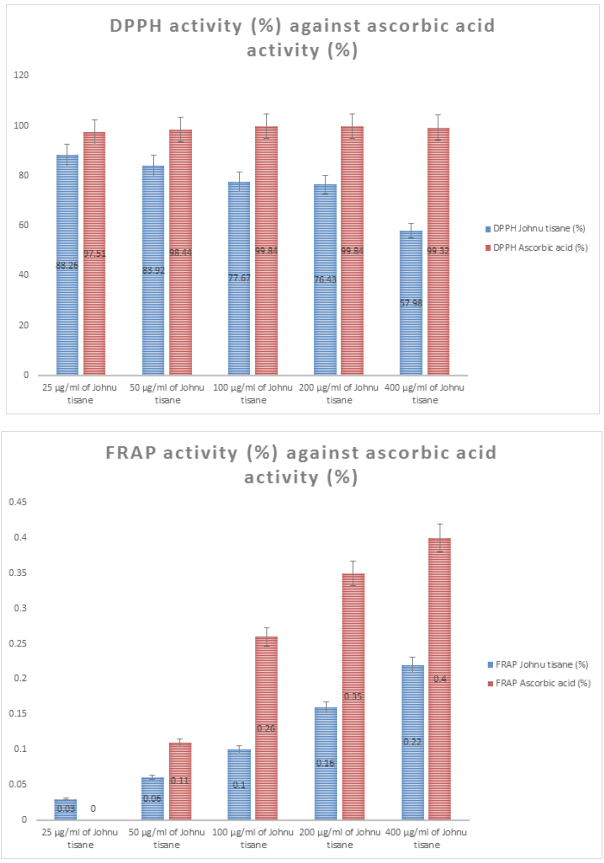

Figure 5. DPPH and FRAP activity (%)of Johnu tisane against reference/standard antioxidant, ascorbic acid activity.

Discussion

Tea prepared from coffee leaves has relevance in ethno-medications due to its pharmacologic activities resulting from its rich phytoconstituents. Jonhu tisane is a typical *Coffea arabica* leaf decoction formula used in ethno-medication against rheumatoid arthritis, obesity, inflammation of the lower limbs during pregnancy and fetal overweight. It, however, has no supporting scientific basis for these activities amidst possible variations in coffee leaves based tea due to processing methods, species and location reported recently (Ding et al., 2022). This study investigated physicochemical, antimicrobial, lethality and *in vitro* antioxidant profiles of Johnu tisane by acceptable methods. Results uncovered the presence of high moisture (92.26 %), but low ash (0.65 %), unsaponified matter (1.46 mg/100 g), free fatty acid (0.56 mgKOH/g), acid value (1.12 mgKOH/g), and potential hydrogen (6.85) in Johnu tisane. These indicated that Johnu tisane is relatively low in impurities, minerals and keeping quality with likelihood of rancidity and requisite physicochemical mix for acceptable taste, consumption and bioactivity. The ash content of Jonhu tisane (0.65 %) indicates a negligible presence of inorganic minerals and other noncombustible components hence negligible impurities and inadequate mineral content compared to 3.52 % in unprocessed *Vigna aconitifolia* (moth bean) seeds (Opara et al., 2017). The ash content reported herein is in line with the proposal of Ifemeje et al. (2020) that ash content should not exceed 5.54 % in order to maintain purity and quality of tea during storage. Moisture content (%) correlates with increased microbial activity leading to depreciation in quality hence is an essential determinant and indicator of keeping quality and safety (Arsenoaia et al., 2023; Aruna, 2023). The moisture content of the sample (Johnu tisane) recorded herein was quite above the proposed acceptable value of 6.5 % (Ifemeje et al., 2020); 12.87 % reported for unprocessed *Vigna aconitifolia* (moth bean) seeds (Opara et al., 2017) and 3.42, 3.78 % respectively in seed and peel of Carica papaya (Egbuonu et al., 2016) which will have a negative implication on its stability, shelf life or keeping quality and safety as water promotes microbial growth and can lead to spoilage. This is an intriguing but interesting finding in relation to the actual long keeping quality of Johnu tisane. The formula when prepared and preserved in a corked plastic container with no formal preservative stayed for a very long time, and used on dilution with hot water. It is therefore probable that Johnu tisane has inherent antimicrobial potential that could sufficiently inhibit microbial growth and activity expected to result from its high moisture content noted herein. Alternatively according to Ifemeje et al. (2020), the markedly low ash content of Johnu tisane may impact against the expected high moisture-related reduction in its keeping quality. Ash content is usually an indicator of the cleanliness and purity of a sample, so low ash content as observed herein suggests that the Johnu tisane is relatively free from impurities that could have promoted the buildup of microbes and microbial activities.

Acid value is a relative measure of rancidity due to free fatty acids formed during the decomposition of glycerides. Hence, acid value is often used as a general indicator of the condition and edibility and the capacity of a sample to neutralize organic acids to yield free fatty acids following hydrolysis of glycerides *via* either lipase enzyme catalysis or in the presence of air and possibly bacteria (Aruna S.O., 2023). High acid value indicates lesser quality probably due to a variety of agents, including presence of moisture. In this study, the acid value of Johnu tisane (1.12±0.02 mgKOH/g) is comparatively low for rancidity to occur hence will be edible (Nwachoko et al., 2023)). Similarly, high free fatty acids content indicates capacity to undergo hydrolysis of triacylglycerol and release free fatty acids, inadequate processing and storage conditions or presence of contaminants notably microorganisms which serve as

the source of the enzyme lipase that catalyzes the hydrolysis reaction. This study recorded a low free fatty acid concentration of 0.56±0.01 mgKOH/g and supported the apparent purity, edibility and stability of Johnu tisane. Potential hydrogen (pH) measures the hydrogen ion activity as it serves as a significant parameter in acid-base neutralization and an indicator of fluid drinking quality and safety (Vijayakumar et al., 2019). Generally, coffee is expected to have a pH value between 5 and 6. As would be expected, a potential hydrogen (pH) value of 6.85 in Johnu tisane was recorded in this study as against the value of 4.90 reported for *Citrullus lanatus* seed oil (Egbuonu et al., 2015). This suggests that Johnu tisane coffee leaf decoction is a slightly acidic solution. This further confirms its relatively low acid value, edibility and low toxicity. In support, results also showed linearly-derived high lethal concentration, LC_{50} of 1000 ppm, which indicates its unbroken high safety margin or low toxicity when consumed by animal, hence edible for animal consumption. Time dependent variation of combined processing methods could affect some physicochemical properties of plant food sources (Egbuonu et al., 2014), hence studies aimed at optimizing the mix of these parameters in Johnu tisane are warranted and recommended.

Acid value correlated negatively, while the composition of unsaponified matter correlated positively, with antioxidant activity (Malecka, 2002). Thus, the mix of unsaponified matter (mg/100 g) (1.46) and acid value (mgOH/g) (1.12) compositions of Johnu tisane reported herein suggested its high antioxidation capacity since acid value and in particular the composition of unsaponified matter correlated negatively and positively, respectively with antioxidant activity according to Malecka (2002). Results of the investigation of the *in vitro* anti-oxidation profile of Johnu tisane revealed concentration-dependent increase in total antioxidant capacity, TAC, nitric oxide scavenging activity, NOSA and hydrogen peroxide scavenging activity. Similarly, 2, 2-diphynyl-1-picrylhydrazyl scavenging (DPPH) activity and ferric reducing antioxidant power (FRAP) increased concentration-dependently in comparison to standard but recorded a measurable FRAP at the least concentration (25 µg/ml) unlike the reference antioxidant. These indicated high *in vitro* antioxidation potential of Johnu tisane (coffee leaves decoction) irrespective of concentration and a basis for possible *in vivo* antioxidant roles. A strong antioxidant activity was reported earlier, but for samples of coffee beans (Tsedale et al., 2020). The implication is that Johnu tisane has significant hydrogen donating property and could donate hydrogen to pair up with lone pair of the free radicals to scavenge or break the free radical chains (Mbinda and Musangi 2019). In support, a dose dependent increase in FRAP observed herein which indicated antioxidant activity accompanied by reducing power is a strong pointer that Johnu tisane has a proton-donating property and could exert a strong free radical inhibiting or scavenging activity and probable *in vivo* antioxidant role (Uroko et al., 2022). Also, its concentration dependent increase in TAC indicates the cumulative antioxidant capacity of an antioxidant to scavenge free radicals attributable to high phenolic content (Frempong et al., 2021). The phenolic content of Johnu tisane was not determined in this study which is a notable limitation underscoring the need to elucidate the phenolic content and other pharmacologically active compounds in Johnu tisane. Coffea arabica leaves have abundant antioxidant chemicals and resultant teas demonstrated antioxidant activities *in vitro* and *in vivo* (Monteiro et al., 2020). The present outcomes and suppositions thereto strongly support the ethno-medicinal uses and actions of Johnu tisane. Potent antioxidants, including ascorbic acid commonly used in DPPH radical scavenging assay as a standard/reference compound for measuring *in vitro* antioxidant activity, could scavenge the oxidant or free radicals-prone DPPH due to their hydrogen or electron donating ability (Adesegun et al., 2017). Oxidative stress due to excess oxidants and free radicals is fundamental to pathogenic states and antioxidants through antioxidant defense mechanisms that mop up excess free radicals to prevent oxidative stress *in vivo* significantly modulated pathogenic states in animals (Ponnampalam et al., 2022). Thus, the possible pharmacologic activities of Johnu tisane may be *via* antioxidant metabolic pathways warranting further, particularly, mechanistic studies to elucidate its metabolic roles and routes in experimentally challenged animal diseased models.

The antimicrobial activity of Johnu tisane was explored to assess the probability that the decoction has inherent antimicrobial potential that could sufficiently inhibit microbial growth and activity expected to result from its high moisture content noted in this study. The antimicrobial activity results showed concentration-dependent activity against the tested microbes which were comparable to standards notably at the peak. These revealed the inhibition of wide range of microbial pathogens and an indication of broad spectrum activities of Johnu tisane against varied pathogenic microbes. Thus, the concentration-dependent antimicrobial activity of Johnu tisane observed herein aligns with the mooted inherent antimicrobial potential which could inhibit microbial activities that could have reduced its keeping quality when preserved. It also supports the diverse medicinal uses of Johnu tisane since microbial pathogens underlie the pathogenesis of many diseases of public health concerns (GBD 2019 Antimicrobial Resistance Collaborators, 2022). *Pseudomonas aeruginosa* resists many antibiotics while *Staphyloccus Aureus, E. coli* and *K. pneumoniae* causes various diseases, including diarrhea, urinary tract infections and pneumonia among others (Akinpelu et al., 2015). *Trichophytons* are infectious dermatophytes that cause tinea capitis and

ringworm among (especially African) children (Akinpelu et al., 2015). The susceptibilities of these pathogens to Johnu tisane corroborate its local uses against varied diseases and infections that may be caused by microbial pathogens and thus demonstrated its significant therapeutic potential and possibility to serve as a good source of antimicrobial compound that can be employed in pharmaceutical industries for the formulation of potent antimicrobial drugs. Johnu tisane inhibited these pathogens even at lower, albeit crude, concentration and thus could be used in preventing opportunistic infections caused by these pathogens. These support the inferred possibility in this study of inherent antimicrobials in Johnu tisane inhibiting the growth and activity of opportunistic microbes that could have impaired its keeping quality due to its high moisture content reported herein. The study outcomes provoke and warrant time-dependent studies on Johnu tisane. These also provoke an investigation on the general impact of variation of ash to moisture mix on potential hydrogen, overall microbial and antimicrobial activities and the attendant shelf life or keeping quality, bioactivity and anti-oxidative roles to provide a novel deep insight on food packaging and preservation in relation to *in-vivo* anti-oxidative outcomes in healthy and unhealthy animal models. This is imperative because, as recorded herein, a strong antioxidant activity that correlated positively with the antibacterial activity was reported for samples of coffee beans (Tsedale et al., 2020) and concomitant elicitation of antioxidant and antimicrobial activities could offer synergistic preventive *cum* therapeutic benefits against animal diseases.

CONCLUSIONS

Thus, Johnu tisane demonstrated low minerals and keeping quality; requisite physicochemical mix for consumption and bioactivity; high safety margin; antimicrobial potency; and requisite anti-oxidation capacity for possible *in vivo* antioxidant role. These provided scientific support for its current ethno-medicinal uses; underscored the need to elucidate its pharmacologically active compounds; mechanistic roles in animal models; and the impact of ash to moisture mix variation on potential hydrogen, microbial and antimicrobial activities in relation to shelf life, bioactivity and possible *in vivo* anti-oxidative roles. These would provide a novel deep insight on packaging and preservation in relation to preserving diet/drug quality, safety, bioactivity and *in vivo* anti-oxidative outcomes.

***Authors' Contributions*:** Egbuonu A.C.C., Alaebo P.O. and Onuoha U.N. designed and supervised the study. Njoku C.J. and Obike C.A. assembled the draft manuscript. Nlemadim S.O., Chukwu B.I., Iwejuo S.M., Amaechi O.G., Obiefuna V.I., Onuoha B.N., Okoli D.N., Nwokeoma P.C. and Eze C carried out the laboratory work under supervision. All authors read and approved the final version of the manuscript.

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