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Phytochemical, Antioxidant and Antimicrobial Activities in the Leaf, Stem and Fruit Fractions of *Basella Alba* and *Basella Rubra*

Gabriel Olaniran Adegoke, Olaoluwa Ayodele Ojo

Department of Food Technology, University of Ibadan, Ibadan Oyo State, Nigeria

Email address:

goadegoke@yahoo.com (G. O. Adegoke), ooayodele07@yahoo.com (O. A. Ojo)

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Abstract: The overlapping nutritional and nutraceutical benefits of green leafy vegetables provides a better support for the well-being of human. This study was aimed at investigating the antioxidant, antimicrobial and phytochemical components of the methanol extracts from selected under-utilised green leafy vegetables (*Basella alba* and *Basella rubra*). The *in vitro* antioxidant activities of the extracts were measured using 1, 1-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity and ferric reducing antioxidant power, the antimicrobial activities were evaluated using the agar diffusion assay. *Basella* plant showed good fibre content up to 21.73±0.37%. The phenolics content ranged between 0.4 and 5.07mg/g GAE (Garlic Acid Equivalent) with *Basella* alba recording the highest value of 5.07mg/g GAE, while the flavonoids content of *basella* plant ranged between 4.00 and 9.87mg/gCAE (Catechin Equivalent). The radical scavenging antioxidants activities (DPPH) of *Basella* plant was more noticed in the leaves fraction with 55.32% while the Ferric ion reducing antioxidant power (FRAP) was in the range of 0.33 and 3.30mg/g and the seed fraction of *Basella alba* had the highest FRAP at 3.30mg/g. *Basella* plant extracts showed moderate antimicrobial action against *Staphylococcus aureus*, *Bacillus cereus*, *Klebsiella pneumonia*, *Aspergillus niger* and *Aspergillus flavus* at 500ppm (minimum inhibition concentration). Based on the results of the work reported herein, *Basella* plants can be said to be a good source of basic nutrients, bioactive compounds and natural antioxidants.

Keywords: *Basella alba*, *Basella rubra*, Phytochemical, Antioxidant, Antimicrobial

1. Introduction

Consumption of fruits and vegetables has attracted attention since biochemical studies have demonstrated a clear and significant positive correlation between regular intake of natural food products and reduced rates of degenerative diseases [1]. Nutraceuticals are a combination of nutritional and pharmaceutical compounds that act as medicines [2] and are becoming more widely accepted as an adjunct to conventional therapies [3]. Many of these "folk-medicines" are beneficial beyond their nutritional values because they contain a variety of plant secondary compounds such as anthocyanins, tannins, carotenoids, flavonoids, phenols and antioxidants among others [4]. Phytochemicals are also called antinutritional factors and are generally not essential for normal functioning of the body but have important therapeutic functions [5].

Antioxidants are known to interfere with the production of free radicals. Free radicals are involved in a variety of diseases and can cause damage to cellular bio-molecules like nucleic acid, proteins, lipids and carbohydrates and consequently may adversely affect immune functions [6]. Natural extract of plants may provide a new source of antimicrobial agents with potentially new mechanisms of action [7]. The use of plant extracts with known antimicrobial properties can be of great significance in therapeutic treatments and a number of studies have been conducted in different countries to establish this fact [8].

Basella (*alba* and *rubra*) known as Ceylon spinach, in south-western Nigeria it is referred to as "Amunu-tutu". It is a leafy vegetable of the family *Basellaceae* and it is a short lived perennial herb up to 4-8mm long, succulent, stem twining, slender, smooth, green (B. *alba*) or purplish (B. *rubra*) [9]. Many indigenous vegetables and fruits species are recorded to occur in the wild. In many instances, these species are able to cope with such harsh environments unfit for other crops, where they can provide sustainable productions. Some indigenous vegetables and fruits are mainly used by inhabitants for medicinal purposes [10]. The apparent lack of commercialisation and utilisation of these plants might be due to the lack of adequate medicinal information, minimal planting area and not being fully explored, but still having economic potential [11]. The objective of this study is to determine and compare the nutraceutical benefits from the leaf, stem and fruit fractions of the two species of *Basella* plants (*B. alba* and *B. rubra*) and to determine the preservative potentials of *Basella* plants as antioxidants and anti-microbial agent.

2. Materials and Methods

2.1. Sample Collection

The leaves, stems and fruits of *Basella alba* and *Basella rubra* were collected from Alakia and Ajibode areas of Ibadan, Oyo state, Nigeria, respectively. The organisms used were obtained from the Department of microbiology, University of Ibadan; Gram positive Bacteria (*Staphylococcus aureus* and *Bacillus cereus*), Gram negative bacteria (*Klebsiella pneumonia*) and fungi (*Aspergillus niger* and *Aspergillus flavus*), all the chemicals used were of analytical grade.

2.2. Sample Preparation

The dried plant materials (320g) were extracted with methanol by soaking for 72 hours, followed by filtration with filter paper (whatman number 4). After extraction the solvent was evaporated to dryness using water bath at 50°C. The crude extracts (45g) were kept in refrigerator prior to further analysis. Percentage yield was determined;

$$\%\,Yield = \frac{\text{Weight of Extract}*100}{Weight\ of\ sample} \qquad (1)$$

2.3. Phytochemical Quantification of the Plants Extract

Total phenolic content was determined with spectrophotometer according to Singleton *et al.,* (1999). Total flavonoid content was measured according to colorimetric assay [13]. Tannin was quantitatively determined as reported in the manual of food quality control [14]. The method of Lee and Castle, (2001) was used for total carotenoid quantifications. Alkaloid, saponin and carotenoid content were also measured with spectrophotometer.

2.4. Antioxidant Quantification

The free radical scavenging ability of the sample against DPPH (1, 1-diphenyl-2 picrylhydrazyl) free radical was evaluated according to Ursini *et al.,* (1994), with little modification. The reducing property of the samples was determined by assessing the ability of the sample to reduce Ferric chloride (FeCl) solution as described by Pulido *et al.,* (2000).

2.5. Antimicrobial Activities of Basella Plants

Antibacterial activity and antifungal activity of the plants extracts were determined by agar diffusion method described by Hugo and Russel, (2004). *Staphylococcus aureus, Bacillus aureus*, *Klebsiella pneumonia, Aspergillus flavus* and *Aspergillus niger* were used as test organisms. The minimum inhibitory concentrations (MIC) were taken as the minimum concentration that inhibits the growth of organism.

2.6. Statistical Analysis

Results obtained were analysed with Statistical Package for Social Science (SPSS) for Windows, version 19.0, at p-values ≤0.05.

3. Result and Discussion

3.1. Percentage Yield

The results as shown in Figure 1 indicated that the leaf fraction of *Basella alba* had the highest percentage yield (24.30%), the percentage yield of *Basella rubra* fruits fraction (11.62%) was higher than *Basella alba* fruit fraction with a percentage yield of 11.13%. The variance in percentage yield of the plants across the different parts analysed could be due to variation in level of maturity, collection locations and the moisture content, but generally *Basella* plant leaves showed superior yield compared to the leaves fraction of other well-known vegetables like *Amaranthus viridis* 14.2%, *Amaranthus hybridus* 15.0%, *Lactuca Taraxicofolia* 10.7%, *Solanum aethiopicum*, 11.8% and *Telfairia occidentalis* 12.1% as described by Adetutu *et al.,* 2013.

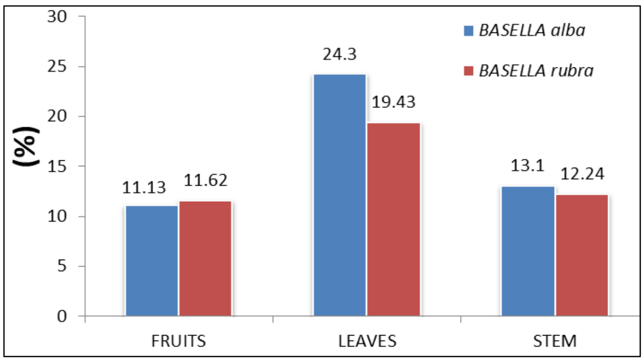

Figure 1. *Percentage yield of Basella plants.*

3.2. Phytochemical Quantification of Basella Plant

Basella alba on a whole had better phenolic content compared to *Basella rubra*. The fruit fraction *Basella alba* as shown in Figure 2, had the highest total phenolic content (5.07mg/ml) compared to other parts of *Basella alba* and *Basella rubra*. Podsędek (2007) reported that the phenolic content of *Brassica* vegetables ranged from 1.5mg/g in white cabbage to 3.37mg/g in broccoli and this shows that *Basella* plant is a good source of phenolic compounds. These findings could be due to factor(s) that influenced the phenolic contents; such as genetic variability, leaf age development and postharvest handling of the leaf samples [21].

The flavonoid content of different parts of *Basella* species ranged between 4.00 and 9.87mg/g; the fruit fraction *Basella alba* recorded the highest flavonoid content. From the different fractions analysed, the flavonoid content of *Basella alba* was shown to be higher than of *Basella rubra* except in the stem portion.

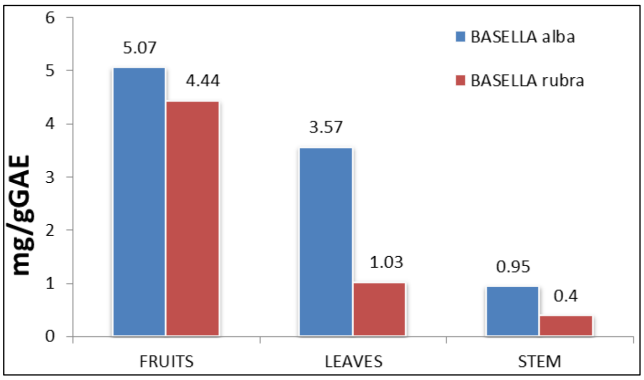

Figure 2. *Phenolic content of Basella plants.*

The results as shown in Figure 3, are in line with the total flavonoid content (TFC) of the *Telfairia occidentalis* leaf extract treated with ethanol at 7.76±0.00mg/g and *Amaranthus caudatus* leaf extract treated with ethanol at 8.40± 0.004mg/g [22].

Flavonoid intake has been associated with reduced risk from death from coronary heart disease [23]. Also, Flavonoids have long been recognized to possess anti-allergic, anti-inflammatory, antiviral, anti-proliferative, and anti-carcinogenic activities [24]; thus, *Basella* plant can be said to assist in the reduction of some ailments as indicated above.

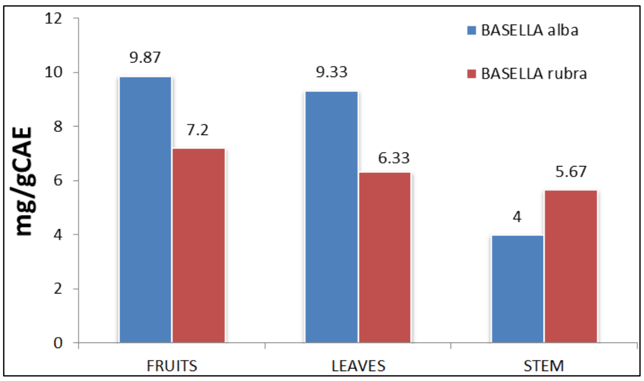

Figure 3. *Flavonoid content of Basella plants.*

Generally, the alkaloid content of *Basella* plant was low (Figure 4), the alkaloid content of *basella* plants ranged between 0.33–1.61mg/g with the leaf fraction of *Basella alba* recording the highest alkaloid content. *Basella rubra* stem had the least alkaloids content, but on a whole *Basella alba* had higher alkaloids content when compared to *basella rubra*, but the results compared with *Telfairia occidentalis* at 0.450±0.010mg/g as reported by Enujiugha et al., 2012. Alkaloids have been described as anti-nutritional factors, but at lower level (as reported herein), they have been found to show some importance as local anaesthetics and stimulants [22].

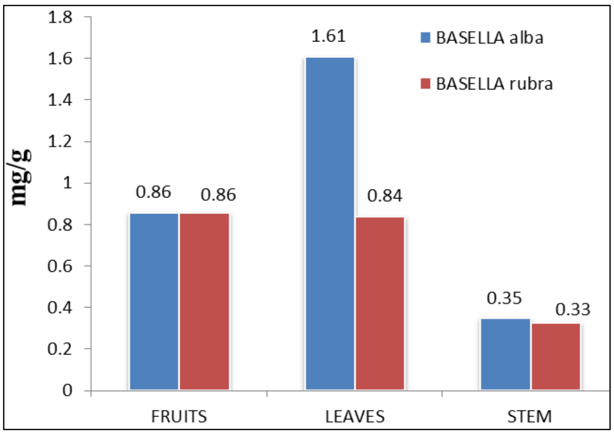

Figure 4. *Alkaloid content of Basella plants.*

Comparatively, *Basella alba* had higher percentage of saponin than *Basella rubra*. Generally, the fruits fraction recorded the highest saponin content (Figure 5), followed by the leaves fraction; the stems fraction of the plants recorded the least percentage saponins content (0.02-0.54%). At higher concentration saponin is regarded as an anti-nutritional factor, but of health benefits if present at a low percentage as reported in this study. Studies have illustrated the beneficial effects of saponins on blood cholesterol levels, cancer, bone health and stimulation of the immune system [22] and anti-inflammatory [25].

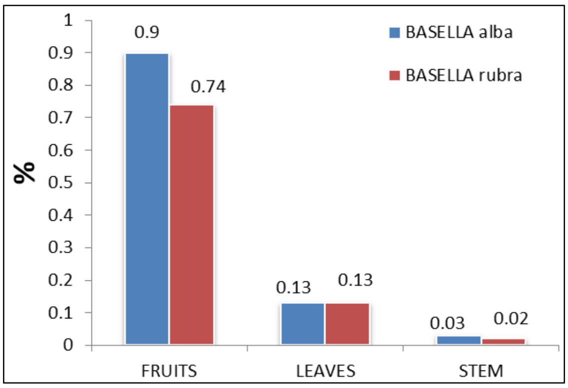

Figure 5. *Saponin content of Basella plants.*

Tannin content as indicated in Figure 6 showed that the fruits fraction of the vegetables had the highest tannin content [5.9–7.27mg/gTAE (Tannic Acid Equivalent)], closely followed by the leaf portions (1.46-3.73 mg/gTAE) and the stems fraction recorded the least tannin content. The parts that are usually consumed (stem and leaf) showed lower content of tannin which dismissed the fear of binding to major nutrients and making them unavailable (protein for instance). But tannin at lower concentration has been claimed to be of tremendous health benefits as antioxidant and anti-inflammatory [26].

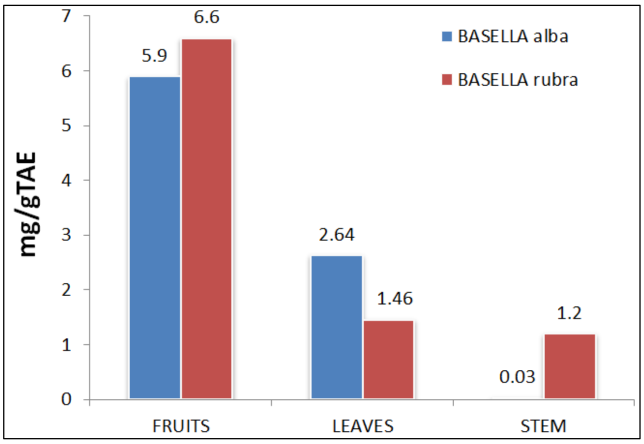

Figure 6. *Tannin content of Basella plants.*

The fruit fraction of the plants as shown in Figure 7, had the highest carotenoids content (0.28 – 0.32mg/g) followed by the leaves, but stems fraction recorded the least carotenoid content of 0.09–0.1mg/g. There was no significance difference in the carotenoids content between the fruit fraction of *Basella alba* and *Basella rubra*.

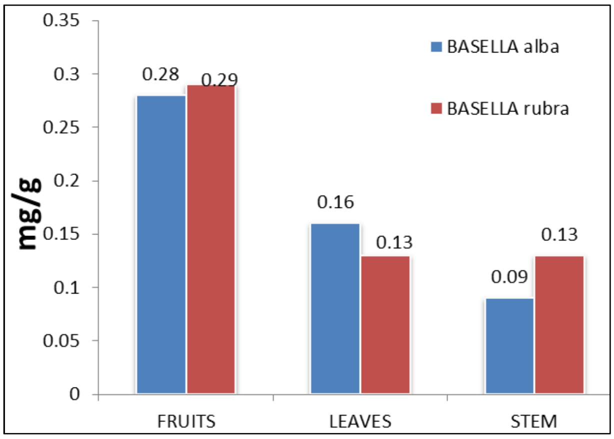

Figure 7. *Carotenoid content of Basella plants.*

From this study, the terpenoid content of *Basella* plants ranged between 0.396–0.86mg/g. *Basella alba* recorded better terpenoid content compared to *Basella rubra* (Figure 8); although the fruits fraction are less utilised but it is equally rich in terpenoid content at appreciable level, while the stem portion of *Basella rubra* had the least terpenoid content (0.396 mg/g). *Basella* plant can therefore be described as medicinal plant due to its terpenoids content; the impact of a diet rich in of fruits, vegetables and grains on reduction of cancer risk may be explained by the actions of terpenes *in vivo* [27].

3.3. Ferric Reducing Antioxidant Power (FRAP) of Basella Plants

Table 1 showed the antioxidant activities of the vegetables based on the ferric ion reducing abilities, with the result ranging between 0.33–3.30mg/g. The fruit fraction of *Basella alba* (BAF) showed the highest ferric reducing ability and *Basella rubra* stem (BRS) had the least reducing power.

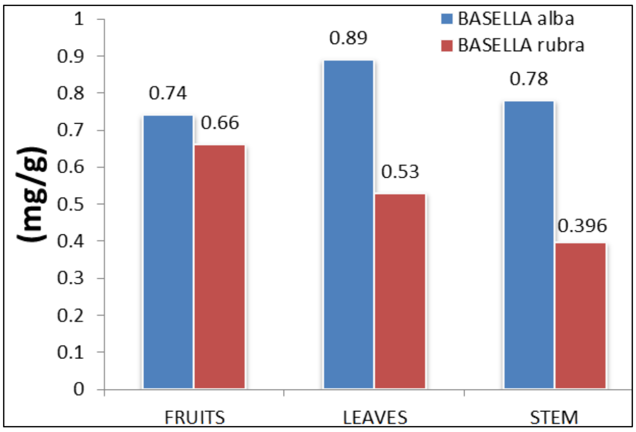

Figure 8. *Terpenoid content of Basella plants.*

As shown in Table 1, *Basella alba* generally had better ferric reducing ability to *Basella rubra* comparing the different parts analysed, but when compared with butylated Hydroxyl Toluene (BHT)(used as standard), the synthetic antioxidant showed better ability to reduce ferric ion (F^{3+} to F^{2+}). Reports has shown that butylated Hydroxyl Anisole (BHA) has carcinogenic effects in non-rodents (pigs, monkeys) and causes lesion formation in the rat stomach whereas BHT has carcinogenic effects in the liver of rats and mice[28]; necessitating the use of natural plant extracts as antioxidant in food mix, although more studies is needed in the area of products compatibility.

3.4. Radical Scavenging Antioxidant Activity

Table 1 showed the antioxidant activity of the selected vegetable samples determined using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay. DPPH generates free radicals; the assay measures the ability of various solvent extracts to scavenge these free radicals.

The lower the concentration of DPPH radicals in solution the higher is the ability of the extract to scavenge free radicals. *Basella alba* leaf (BAL) fraction showed the highest scavenging ability against DPPH (stable radical) with 55.07%, closely followed by *Basella rubra* leaves (BRL); with the fruit fraction recording the least radical scavenging activities (Table1). The scavenging activities of *Basella* species against DPPH ranged between 55.07% and 15.40%. The total antioxidant activities of *Basella* plant obtained in this study were in agreement with what was reported by Olajire and Azeez, (2011) with little variation; and this could be due to methods used for the analysis and the medium of extraction as pointed out by Li *et al.,* (2008). The results were in agreement with previous reports that *brassica* group vegetables are effective antioxidants); and *brassica* crops are among those vegetables that have the highest antioxidant activity [39].

Table 1. *Antioxidant Activities of Basella plants and Amaranthus caudatus.*

Sample	FRAP (mg/g)	DPPH (%)
BAF	3.30±0.30[a]	15.40±0.07[e]
BAL	2.46±0.20[b]	55.07±0.49[a]
BAS	1.37±0.03[c]	27.50±0.53[d]
BRF	2.99±0.10[a]	16.58±0.54[e]
BRL	0.89±0.5[d]	51.27±0.61[b]
BRS	0.33±0.06[e]	35.58±0.02[c]
BHT	34.47	82.86

*Mean values with different letters are significantly different (p< 0.05) along the column

Significant correlation was obtained between antioxidant activity and phytochemical content, indicating that bioactive compounds contributed significantly to antioxidant activity of the investigated vegetables. As shown in Table 2, there was a good linear correlation between the total phenolic content and the ferric reducing antioxidant power (FRAP) of the extracts (r^2=0.862). These results indicated that the ferric reducing antioxidant power of each extract might be mostly related to the phenolic hydroxyl group.

The same trend was noticed for all the phytochemicals studied, and it showed that as the phytochemicals content increases, the FRAP antioxidant power of these vegetables also increases. Significant correlation was also noticed among the phytochemical contents of the plant (*Basella* plants and *Amaranthus caudatus*). As shown in Table 2, there was a positive correlation between the phenolic and flavonoid at r^2=0.645 (0.01 levels), also the correlation between alkaloid content to phenolic content and total flavonoid was also significant at r^2=0.419 and r^2=0.801 respectively.

Table 2. *Correlation between the Phytochemicals and the Antioxidant Activities of Basella plants.*

	Phenolics	Flavonoids	Alkaloids	Saponins	Tannins	Carotenoids	Terpenoids	FRAP	DPPH
Phenolics	1.00								
Flavonoids	0.819**	1.00							
Alkaloids	0.579*	0.764**	1.00						
Saponins	0.864**	0.616**	0.187*	1.00					
Tannins	0.902**	0.683*	0.352*	0.942**	1.00				
Carotenoids	0.887**	0.667**	0.293**	0.940**	0.988**	1.00			
Terpenoids	0.537**	0.397*	0.595**	0.184*	0.174*	0.154*	1.00		
FRAP	0.968**	0.728**	0.541**	0.841**	0.852**	0.830**	0.637**	1.00	
DPPH	-0.451*	0.008*	0.452*	-0.718**	-0.588**	-0.631**	0.000	-0.485*	1.00

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

3.5. Antimicrobial Activities of Basella Plants Extract

Basella plant extracts showed antimicrobial activity against tested organism, although the degree of inhibition varied with tested organism and extract concentration. *Staphylococcus aureus* was inhibited at a concentration of 500ppm and above, whereas the extracts showed considerable inhibition against the fungi especially *Aspergillus niger* at lower concentration. *Basella alba* stem extract inhibited *Klebsiella pneumonia* better than other extracts studied. As shown in Table 3, the results of different concentrations of *basella* extracts were weak compared with the drugs used as standard (Ampicilin and Flucanazole); and antimicrobial activity was found to be dose dependent against the tested organism. The antimicrobial effect of methanol extracts against these organisms may be due to the ability of the methanol to extract some of the active properties of these plants like phenolic compounds, saponin and other secondary metabolites which were reported to be antimicrobial agent [32]. From the Table 3, the antimicrobial activity of the extracts showed better inhibition against gram-negative bacteria compared to gram-positive bacteria; with the fungi been the most susceptible. The reason for higher sensitivity of the gram-negative bacteria than gram-positive bacteria could be ascribed to the difference in cell wall constituents and their arrangement [33].

Table 3. *Antimicrobial activities of Basella alba and Basella rubra extract.*

	Inhibition zone (mm)				
Sample/ Concentration	Bacillus cereus	Staphylococcus aureus	Klebsiella pneumonia	Aspergillus flavus	Aspergillus niger
BAF 1000ppm	9	3	8	3.5	4.0
500ppm	4	2	5	1.0	1.0
250ppm	2	NI	4	NI	NI
BAL 1000ppm	7.0	6	8.5	9.5	10.0
500ppm	6.0	2	7.5	5.0	8.5
250ppm	2.0	NI	3.0	3.5	6.00
BAS 1000ppm	12.0	2.5	15.5	9.0	7.5
500ppm	8.0	NI	6.0	5.5	5.0
250ppm	6.0	NI	5.0	2.0	3.5
BRF 1000ppm	8.0	2.5	5.5	4.5	4.0
500ppm	5.0	1.0	2.0	2.0	1.5
250ppm	NI	NI	NI	NI	NI
BRL 1000ppm	6	5.5	7.5	9.0	10.5
500ppm	4.5	2	4.0	7.5	8.0
250ppm	1.5	NI	1.0	5.0	5.5

	Inhibition zone (mm)				
Sample/ Concentration	**Bacillus cereus**	**Staphylococcus aureus**	**Klebsiella pneumonia**	**Aspergillus flavus**	**Aspergillus niger**
BRS 1000ppm	4	3.0	3.5	7.0	7.5
500ppm	3	1.0	NI	3.0	4.0
250ppm	NI	NI	NI	NI	1.0
STD 1000ppm	28	NG	23.5	27	29
500ppm	25	NG	23.0	25	28
250ppm	21	NG	22.5	22	24
CONTROL	19.0	22.5	42.5	34.5	36.0

BAF= *Basella alba* fruit, BAL= *Basella alba* leave, BAS= *Basella alba* stem, BRF= *Basella rubra* fruit, BRL= *Basella rubra* leaf, BRS= *Basella rubra* stem, Std- Ampicillin and Flucanazole, Control- Methanol (Pure extract), NI- No inhibition.

3.6. Minimum Inhibition Concentration (MIC)

Extract of leaf fraction of the plants showed antimicrobial activity against the tested organisms at 250ppm, except *Staphylococcus aureus* that was inhibited at a higher concentration of 500ppm. The minimum inhibition concentration of *Basella alba* leaves extract against the tested organism showed that *Aspergillus niger* was inhibited at 100ppm, the same trend was noticed for the extract of stem fraction *Basella alba*, while the minimum inhibition concentration of the fruit extract of *Basella rubra* against the tested organism was at 500ppm, which was considerably weak compared to the other extracts. *Basella rubra* leaves extracts showed inhibition at a minimum concentration of 100ppm against the fungi, while the minimum inhibition concentration of the stem extract of *Basella rubra* against *Aspergillus niger* was at 250ppm, 500ppm against *Aspergillus flavus*, *Bacillus cereus*, *Staphylococcus aureus* and 1000ppm against *klebsiella pneumonia* (Table 4).

It was noticed that an increase in the concentration of the extract increased the antimicrobial activity as shown (Table 4) by the diameter of inhibition zone. The fact that organisms may need higher concentrations of extracts to inhibit or kill them may be due to their cell wall components. Antimicrobial agents with a low activity against an organism have a high Minimum Inhibition Concentration (MIC) while a highly active antimicrobial agent gives a low MIC [34].

Table 4. *Minimum Inhibition Concentration of Basella alba and Basella rubra extract.*

	Minimum Inhibition Concentration (ppm)				
Sample	**Bacillus cereus**	**Staphylococcus aureus**	**Klebsiella neumonia**	**Aspergillus flavus**	**Aspergillus niger**
BAF	250	500	100	500	500
BAL	250	500	250	250	100
BAS	250	1000	100	250	100
BRF	500	500	500	500	500
BRL	250	500	250	100	100
BRS	500	500	1000	500	250
STD	100	100	100	100	100

BAF= *Basella alba* fruit, BAL= *Basella alba* leaf, BAS= *Basella alba* stem, BRF= *Basella rubra* fruit, BRL= *Basella rubra* leaf, BRS= *Basella rubra* stem, ACF= *Amaranthus caudatus* fruit, ACL= *Amaranthus caudatus* Leaf, ACS= *Amaranthus caudatus* stems, STD= Standard

4. Conclusion

Based on the results of the work reported herein, lesser known vegetables can be said to be potential sources of bioactive compounds, natural antioxidant and antimicrobial agents. From the study conducted on the fruit, leaf and stem fractions of *Basella* plants, the extracts contains active compounds; flavonoid, saponin, alkaloid, tannin, terpenoid, carotenoid and phenol. In view of this, *basella* plant can be used as folk medicine to prevent or delay the onset of some diseases. The health implication of synthetic antioxidants led to the search of natural sources of antioxidants; and from the study conducted on *Basella* plant, it has been found that the extracts the plant can be a good replacement for synthetic antioxidants, especially the leaf fraction. The seed fraction of *basella* plant has also been shown to be a good source of oil and may be developed for oil production.

This study has shown *Basella* plants to be good source of several phytonutrients, it is therefore recommended that *Basella* plant be added to meals containing vegetables as it will not only provide basic nutrients but also health promoting compounds; and also add variety to diets. Consumption of indigenous plants should be encouraged as this will prevent excessive importation of foreign goods and prevent so many diseases that have been attributed with imported foods, and it will also encourage active participation in farming.

The results obtained might then be considered sufficient for further studies on the isolation and identification of the active components; and to evaluate the possible synergism among extract components for improved antioxidant and antimicrobial activities. Also there is need for compatibility study with different food composition to ensure the effectiveness of these extracts as potential preservative agents.

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