

Comparative Analysis of Antimicrobial and Antioxidant Activities of Six Different South–East Asian Berries

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Abstract

A comparative analysis of antimicrobial and antioxidant properties of six different Southeast Asian berries (*Phyllanthus emblica*, *Averrhoa carambola*, *Musa acuminata*, *Musa balbisiana*, *Solenum torvum* and *Solenum melongena*) was performed in order to elucidate their potential health benefits. All methanolic extracts exhibited significantly higher *in vitro* antimicrobial activity when compared to the corresponding aqueous extracts. Among all the berry samples *Phyllanthus emblica* (both methanol and water extract) showed highest antimicrobial activity. The methanolic extract of *Musa balbisiana* exhibited the highest ascorbic acid content, followed by *Phyllanthus emblica*. Antioxidant and free radical scavenging properties were highest in *Phyllanthus emblica* followed by *Averrhoa carambola* and others. These berries are rich in phenolic, tannin, flavonoid, anthocyanin and mineral content. Our study revealed that *Solanum torvum* had a high Na⁺ concentration, *Phyllanthus emblica* showed elevated Ca²⁺ levels, and *Musa balbisiana* was rich in K⁺ content.

Keywords: Berry, Antioxidant activity, Antibacterial activity, Health Benefit

Introduction

Safe and nutritious food is one of the most significant factors of human health. An increasing demand of safe, nourishing and sustainable foods presents challenges for food science and industries in recent years. Diets rich in fruits and vegetables have been considered as excellent sources of antioxidants [1]. Fruits and vegetables, containing abundant dietary fiber, vitamins, and minerals, and large amounts of phytochemicals are recognized for their health benefits [2, 3]. Plants generate a high diversity of secondary metabolites with important function in the defense

against predators, microbial pathogens and abiotic stress. Oxidative stress consequences when there is an inadequate capacity of the biological system to counteract excess free radicals that have been generated [4]. The major phytochemicals like flavonoids, phenolic acids, tannins, stilbenes and lignans are mainly associated with the nutritional, antimicrobial, antioxidant as well as anti-inflammatory effect.

In botanical terminology, a berry is a simple fruit with seeds and pulp produced from the ovary of a single flower [5]. Botanical berries are not always commonly known as berries however there are several kinds of other fruits which are anonymously called berries. Most berries are delicious and provide significant health benefits because of their high levels of polyphenols, antioxidants, vitamins, minerals, fibers [6] and are consumed not only in fresh and frozen forms but also as processed and derived products, including dried and canned fruits, yogurts, beverages, sweets [7-9]. Berries such as *Phyllanthus emblica* (Amla), *Averrhoa carambola* (Star fruit), *Musa acuminata* (Small banana species), *Musa balbisiana* (banana species), *Solanum torvum* (Turkey berry), *Solanum melongena* (Musk brinjal), are of South-East Asian origin and are popularly used in the human diet either fresh or in processed forms. Moreover, there has been a growing tendency in the use of berry extracts as components in functional foods and dietary supplements, as they are rich substance of flavonoids and poly phenolic compounds [10, 11].

The rising rate of resistant microorganisms and the side effect of existing drugs lead the research on plant based safe alternatives product. Thus, the identification and development of new antimicrobial and antioxidant compounds derived from natural sources continue to be a focus of ongoing scientific investigation [12]. This study is therefore aimed at evaluating the phytochemicals composition and antioxidant and antimicrobial activities of different commonly available berries in South –East Asia.

Materials and Methods

Collection of Sample

Six different varieties of berry sample *Phyllanthus emblica*, *Averrhoa carambola*, *Musa acuminata*, *Musa babisiana*, *Solanum torvum*, *Solanum melongena* were collected from Indian Institute of Horticulture Research (IIHR) Bangalore, Karnataka (Figure 1) and were authenticated by National Ayurveda Dietetics Research Institute Bangalore, Karnataka.

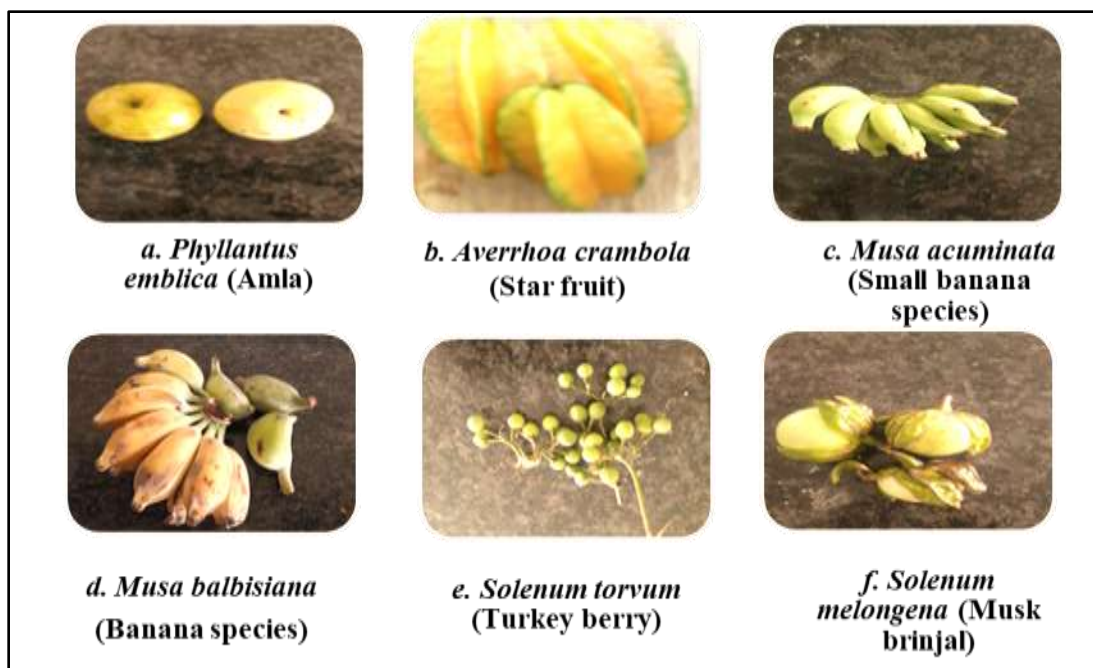


Figure 1: Six different varieties of berry samples

Extract preparation

All the samples were thoroughly cleaned, dried and powdered mechanically and sieved through No.20 mesh sieve. Samples were prepared using the solvents water and methanol. 4gm of the samples were homogenized with 20ml of the respective solvents. The crude preparations were left overnight in the shaker at room temperature and then centrifuged at 4000 rpm for 20mins. The supernatant of water extracts was then transferred in screw cap tubes for analysis. The final concentration was obtained as 200mg/ml. Methanol extracts were transferred to a pre weighed beaker and concentrated by evaporating the solvent at 60°C. The crude extracts were weighed and dissolved in a known volume of dimethyl sulfoxide (DMSO), to obtain a final concentration of 200 mg/ml.

Sources and maintenance of organisms

Gram-positive organisms (*Staphylococcus aureus*, *Bacillus subtilis*), Gram-Negative organisms (*Escherichia coli*, *Pseudomonas sp.*) were obtained and confirmed at the research laboratory of the Department of Microbiology The Oxford College of Science, Arts, Commerce and Management, Bangalore. They were maintained on Mueller-Hinton Agar medium (Oxoid, UK). Twenty-four-hour old pure cultures were prepared for use each time.

Biochemical Properties

Biochemical properties of the isolates were tested according to Bergey's Manual of Systematic Bacteriology. The following properties were determined: Catalase test, Oxidase test, Fermentation of Carbohydrates (Glucose).

Antimicrobial Assay

The effect of various berry extracts on the several bacterial strains were assayed by Agar well diffusion method and further confirmed by Disc diffusion method. Petri plates containing 20ml Muller Hinton medium were seeded with 24hr culture of bacterial strains [13, 14]. Wells were cut and 20 µl of the berry extracts (namely aqueous and methanol) were added. The plates were then incubated at 37°C for 24-48 hours. The antibacterial activity was assayed by measuring the diameter (measured in cm) of the inhibition zone formed around the well [15]. Tetracycline / Penicillin disc was used as a positive control. DMSO was used as negative control.

Estimation of Mineral Content

The aqueous extract of six different berry samples was estimated for mineral (Sodium, Potassium, and Calcium) content by Systronics ® Flame Photometer 128.

Estimation of ascorbic acid

The amount of ascorbic acid present in the different berry samples were estimated spectrophotometrically at 540 nm. [16].

Estimation of reduced glutathione

The level of reduced glutathione was estimated by the method proposed by Moron *et al.* (1979) [17]. Reduced glutathione (GSH) was measured by its reaction with DTNB (5, 5'-dithiobis-2-nitrobenzoic acid) (Ellman's reaction) to give a yellow-colored product that absorbs at 412nm.

Estimation of total phenolic by Folin-Ciocalteu assay

The concentration of total phenolic in the methanol extracts of different berries were determined by the Folin-Ciocalteu assay as per the method of Harbertson and Spayd 2006 [18]. In this study Gallic acid was considered as experimental standard.

Determination of Tannin

The tannin concentration from different berry samples were determined by Folin-Ciocalteu method [19].

Determination of total flavonoid content

Total flavonoids content (TFC) of different berry extracts were determined by following Kim K.S et al, (2003) method [20]. The TFC was expressed in mg quercetin equivalents for 100g of fresh material (FW). Concentration values of extracts were obtained from Quercetin standard curve. TFC was calculated by using the following formula

$$\text{TFC} = (R \times DF \times V \times 100) / W$$

Determination of anthocyanin content

Total anthocyanins were determined according to the pH differential spectroscopic method by Cheng and Breen (1991). The results were calculated similarly to Giusti and Wrolstad (2001) [21].

$$\text{Asp} = (\text{A}_{510} - \text{A}_{700})_{\text{pH } 1.0} - (\text{A}_{510} - \text{A}_{700})_{\text{pH } 4.5}$$

The content of total anthocyanins (TA) was calculated as follows:

$$TA = (Asp \times M \times DF \times 1000) / (\lambda \times \varepsilon \times m)$$

DPPH scavenging effect

The ability of the plant berry extracts to scavenge the stable free radical DPPH was assayed by the method of Mensor *et al.* (2001) [22]. The radical scavenging activity was calculated as follows:

$$\text{Scavenging activity (\%)} = [100 - \{A518 (\text{sample}) - A518 (\text{blank})\} / A518 (\text{blank})] \times 100$$

Statistical analysis

Statistical analysis of data was conducted using one-way Analysis of Variance (ANOVA) using SPSS 17.0 software. Values in the tables / figures indicate the mean values $\pm$ SD based on independent three determinations ($n = 5$). Least Significant Difference (LSD) test was used to assess the differences between control and different treatments; $p < 0.05$ was considered statistically significant.

Result and discussion

Antimicrobial assay

The antimicrobial activity of six different berry extracts (aqueous and ethanol) was determined against two gram-positive and gram-negative bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas sp.* respectively). The result of antimicrobial assay was shown in Table 1. Aqueous extract showed the least antibacterial activity compared to alcohol extracts. The methanolic extract of *Phyllanthus emblica* exhibited the highest zone of inhibition against *Staphylococcus aureus* and *Pseudomonas sp.* compared with the other test organisms. However, methanol extract of *Solenum melongena* exhibited highest zone of inhibition against *E.coli*.

Table 1: Antimicrobial Assay of the Methanol Extract of Six Berry Samples.

Diameter of zone of inhibition (cm)				
<i>Ethanol Extract Concentration in 50mg/ Disc</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Pseudomonas sp.</i>
<i>Phyllanthus emblica</i>	2.30 $\pm$ 0.1 ^a	1.15 $\pm$ 0.2 ^a	1.20 $\pm$ 0.2 ^b	2.00 $\pm$ 0.1 ^b
<i>Averrhoa carambola</i>	1.15 $\pm$ 0.2 ^a	0.90 $\pm$ 0.4 ^c	1.10 $\pm$ 0.3 ^a	1.05 $\pm$ 0.3 ^c
<i>Musa balbisiana</i>	1.00 $\pm$ 0.1 ^a	0.80 $\pm$ 0.1 ^a	0.90 $\pm$ 0.1 ^b	0.90 $\pm$ 0.2 ^c
<i>Musa acuminata</i>	0.95 $\pm$ 0.3 ^a	0.80 $\pm$ 0.3 ^a	1.05 $\pm$ 0.3 ^a	1.00 $\pm$ 0.1 ^b
<i>Solenum torvum</i>	0.95 $\pm$ 0.2 ^a	1.00 $\pm$ 0.2 ^b	1.20 $\pm$ 0.3 ^c	0.95 $\pm$ 0.2 ^a
<i>Solenum melongena</i>	0.90 $\pm$ 0.3 ^a	1.00 $\pm$ 0.2 ^a	1.80 $\pm$ 0.1 ^b	0.95 $\pm$ 0.2 ^a
+ ve Control	+	+	+	+
- ve Control	-	-	-	-

Values are means $\pm$ S.E.M ($n=5$), ND= not Detected, - = no inhibition, $p < 0.05$

a, b, c are the 1st, 2nd and 3rd highest value obtained by that particular concentration on that particular organism.

Estimation of Ascorbic acid

Among all berry samples investigated, *Musa balbisiana* (methanol extract) showed highest 22425.32 ± 3556.36 $\mu\text{g/g}$ value of Ascorbic acid concentration followed by *Phyllanthus emblica* (Figure 2). Our finding suggested that *Musa balbisiana* could be a potential rich source of vitamin C which need further investigation. Ssekiwoko et al (2014) did reveal similar result [23] and suggested high ascorbic acid content in *Musa balbisiana*, which is as per our line of observation. A considerable level of ascorbic acid was detected in *Phyllanthus emblica*, which is consistent with findings reported by other studies [24].

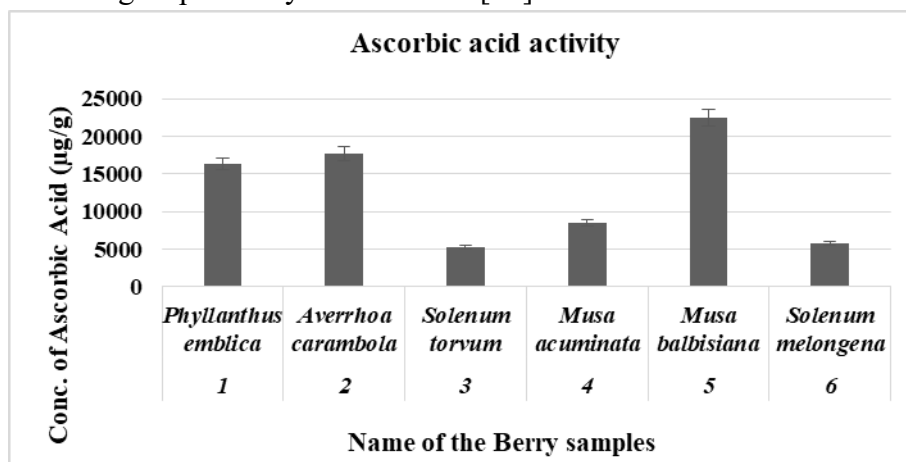


Figure 2: Estimation of Ascorbic acid concentration in six berry samples

Estimation of Glutathione (GSH)

Induction of GSH level is an important protective mechanism to minimize oxidative damage in plants. The reduced glutathione activity of *Phyllanthus emblica* extract showed the highest value (3520 ± 40.1 $\mu\text{g/g}$) followed by *Solenium torvum* (Figure 3). The protective effects of *Phyllanthus emblica* bioactive compounds against oxidative damage had been demonstrated in HepG2 cells by Shivananjappa and Joshi [25]. Rani P et al. (2004) [26] reported that grapes contain the highest levels of reduced glutathione. Consistent with these reports, the present study revealed a high concentration of GSH in *Phyllanthus emblica*, *Solanum torvum*, and *Solanum macrocarpon*.

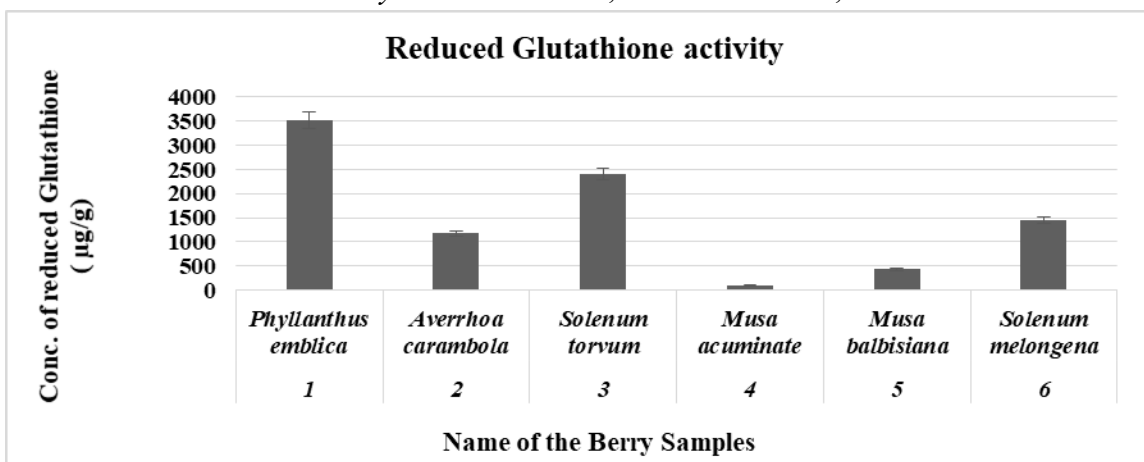


Figure 3: Estimation of reduced glutathione in six berry samples

Estimation of total Phenolic, Flavonoids, Tannin and Anthocyanins

The methanol extract of *Phyllanthus emblica* showed highest concentration of phenolic and flavonoids content (0.09 ± 0.0001 GAE/g, 0.062 ± 0.001 quercetin/g dry weight respectively) among all the six berry extracts (Table 2) which was supported by previous observation by Himani Badoni and others (2016) [27]. Among all the berry samples investigated, methanol extract of *Phyllanthus emblica* and *Averrhoa carambola* showed highest concentration of tannin (0.09 ± 0.0005 GAE/g) followed by *Musa acuminata*. Gina Borges and co-workers (2010) [28] reported a similar pattern of observations. Anthocyanin content was observed highest in *Averrhoa carambola*.

Table 2: Total Phenolic, Tannin, Flavonoid & Anthocyanin content in Six Berry Samples

Parameters	Unit	<i>Phyllanthus emblica</i>	<i>Averrhoa carambola</i>	<i>Musa balbisiana</i>	<i>Musa acuminata</i>	<i>Solenum torvum</i>	<i>Solenum melongena</i>
Total Phenolic Content	mg of GAE/ g. of Extract.	0.09 ± 0.0001	0.08 ± 0.0001	0.07 ± 0.0002	0.07 ± 0.0003	0.075 ± 0.0002	0.08 ± 0.00035
Tannin Content	mg of GAE/ g. of Extract	0.09 ± 0.0005	0.09 ± 0.0005	0.031 ± 0.0002	0.08 ± 0.0003	0.027 ± 0.0006	0.06 ± 0.0004
Total Flavonoid Content	μg of Quercetin / g. of Extract.	0.062 ± 0.001	0.052 ± 0.002	0.007 ± 0.0003	0.005 ± 0.0005	0.005 ± 0.0005	0.006 ± 0.0002
Anthocyanin content	mg /g. of Extract.	1.53 ± 0.859	19.04 ± 5.507	4.71 ± 1.917	3.13 ± 0.957	5.47 ± 1.548	5.94 ± 2.997

Estimation of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging effect

DPPH, a free radical was used for assessing antioxidant activity and the reduction of this radical by an antioxidant results in a loss of absorption at 517 nm [29]. The radical scavenging activities of the samples were expressed in terms of % inhibition of DPPH. Correlation between free radical scavenging activity and the phenolic content have also been reported for various fruits and vegetables [30]. Highest free radical scavenging activity was observed (Figure 4) in the *Phyllanthus emblica* ($190.4 \pm 0.35\%$ per g) followed by *Averrhoa carambola*. A moderate free radical scavenging activity was observed among banana species, however, *Solenum torvum*, showed very low value. Himani and others (2016) [27] measured the free radical scavenging activity of *emblica* species by measuring the scavenging of DPPH radicals and each value were expressed as mean $\pm$ standard error. The data obtained by them supported our investigation.

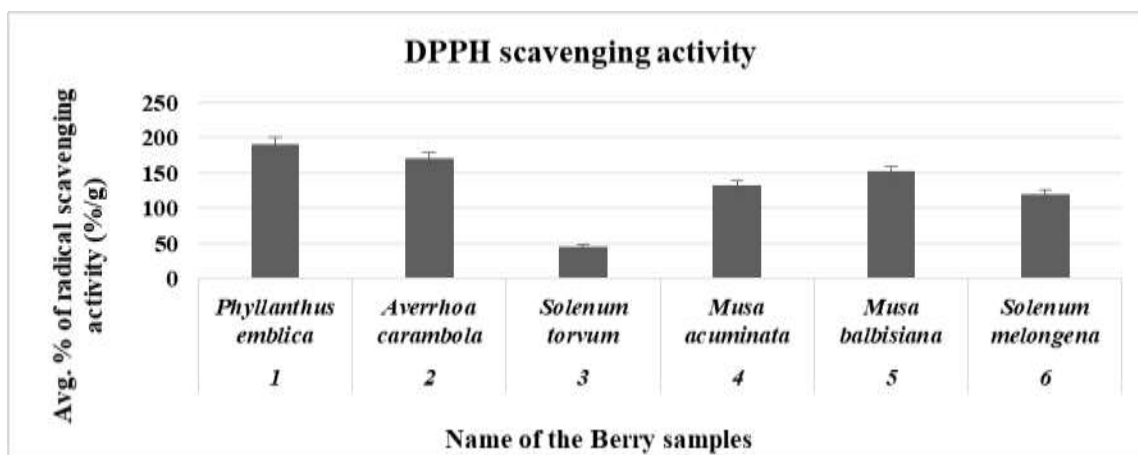


Figure 4: Estimation of DPPH Scavenging Activity in six berry samples

Estimation of Mineral Content

As per our investigation, a high concentration of Calcium is present in *Phyllanthus emblica* aqueous extract. Potassium and Sodium ions are present in high concentration in *Solenium torvum* (Figure 5). Venkatesh K Mutalik and others [31, 32] did mineral content estimation particularly potassium level, in *Phyllanthus emblica* aqueous extract by flame photometer with similar observation.

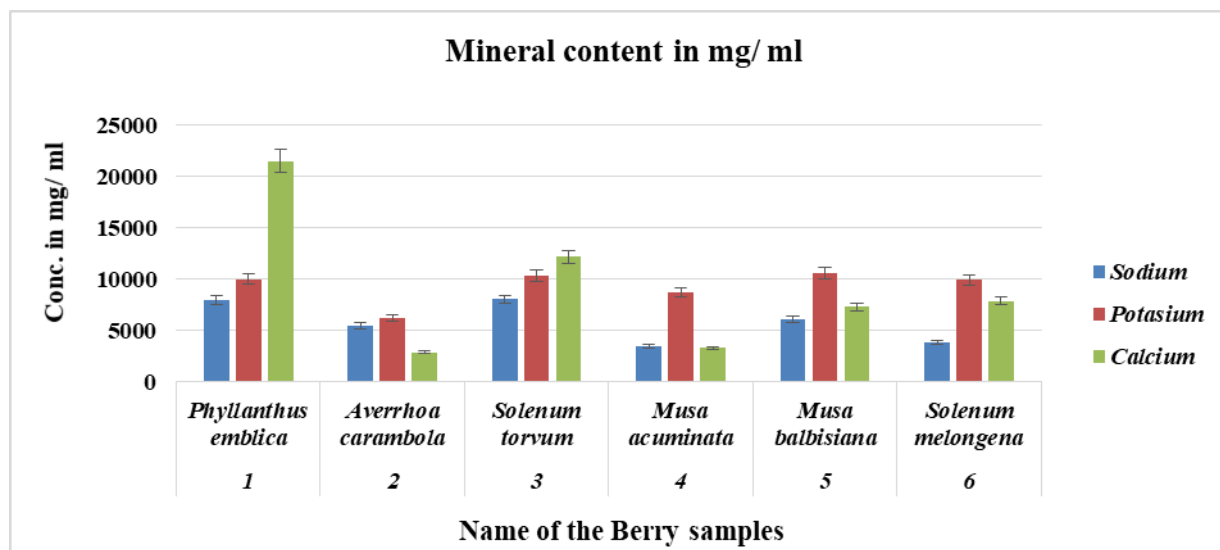


Figure 5: Estimation of Mineral Content in the aqueous extract of six berry samples

Conclusion:

Berries are a good source of natural antioxidants. They offer a vast range of nutrients that help to maintain optimal health and enhance immunity. They are rich in ascorbic acid, reduced glutathione, phenolic and flavonoid content. They are also rich in vitamins and minerals. Since they are rich in antioxidants, they also have a free radical scavenging activity and play many important roles in the body.

There have been many studies going on different properties of these berries but there are only a few studies available on the comparative aspect on antimicrobial and antioxidant properties of these different types of berries. We have chosen South East Asian berries mainly which are native of India because they have high nutritive food value and are easily available. They are also cost effective and can be considered in daily food habit. These berries show high antimicrobial property so it can be used as a replacement of chemical preservatives.

In today's lifestyle, people prefer fast food over balanced diet which ultimately leads to different disorders. Since berries are cost effective and rich with many essential substances and minerals therefore production and popularization of berry-based food product would be our future perspective.

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Ethical Issues

Not applicable.

Conflict Of Interest

The authors declare no conflict of interest.

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