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Evaluation of Nutritive, Antioxidant and Mineral Composition of Two Newly Developed Varieties of Strawberry (*Fragaria ananassa*) and their Antimicrobial Activity and Brine Shrimp Toxicity Study

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ABSTRACT

Although strawberry is produced mainly in the cold weather but recently it is a newly introduced crop in the subtropical country like Bangladesh by tissue culture technique. It is not only the excellent sources of Vitamin C, β-Carotene, dietary fiber and some other nutrients but it has also several medicinal importances for human health and nutrition as reported by researchers in different countries other than Bangladesh. The major nutrient contents as well as minerals and vitamins of two varieties, Varaity-1 (Long Conic) and Variety-2 (Globose Conic) of strawberries were analyzed. The result revealed the presence of nutrient constituent among the two varieties, Varaity-1 (Long Conic) and Variety-2 (Globose Conic) of strawberries comprising Total sugar (3.15±0.01 and 2.5±0.02%), Reducing sugar (0.0335±0.01 and 0.048±0.01%), Non-reducing sugar (3.12±0.01 and 2.4±0.01%), Starch (0.395±0.02 and 0.685±0.04%), Water soluble protein (0.785±0.01 and 0.986±0.01%), Total protein (1.2±0.02 and 1.5±0.02%), fat (0.4±0.01 and 0.6±0.02%), Vitamin C (116±0.32 and 179±0.52 mg%), Beta-carotene (3.71±0.25 and 4.15±0.30 μgm%) and minerals such as Magnesium (17.35±0.01 and 17.76±0.01 mg%), Calcium (6.40±0.20 and 11.36±0.40 mg%), Potassium (38.00±0.20 and 43.00±0.01 mg%), Copper (0.1365±0.02 and 0.0275±0.01 mg%), Iron (0.12±0.02 and 0.5415±0.01 mg%), Zinc (0.092±0.01 and 0.049±0.03 mg%), Phosphorous (0.718±0.01 and 0.627±0.02 mg%) and Sulphur (5.56±0.20 and 5.735±0.22 mg%), respectively. Results of present study suggest that Strawberry is a good source of sugar, anti-oxidants and minerals and among both the varieties, Variety-2 (Globose Conic) is rich of sugar, total protein, water soluble protein, Vitamin C, Beta-carotene, Magnesium, Calcium, Potassium, Iron and Sulphar. In this study, the aqueous extract of two varieties (Long Conic and Globose Conic) of strawberry was tested for anti-microbial activity by disc diffusion method and cytotoxicity by brine shrimp nauplii. From the results, it was found that the aqueous extract of both varieties of strawberry revealed anti-microbial activity against gram positive and gram negative bacteria. It was also found that the aqueous extract of two varieties of strawberry was not shown cytotoxic activity. Even though, the aqueous extract of two varieties of strawberry was not shown toxicity on brine shrimp nauplii but they are showing anti-bacterial activity against gram positive and

gram negative bacteria. This result clearly indicates the presence of potent bioactive principles in this aqueous extract of both varieties of strawberry which suggests a need to isolate, purify and evaluate active constituents responsible for this anti-microbial activity.

Key words: Strawberry, nutrient composition, antioxidant, minerals, antimicrobial activity, cytotoxicity

INTRODUCTION

Now a day's malnutrition is one of the common problems in the world. A large number of populations in Bangladesh have been suffering from malnutrition. There are many kinds of plants, fruits and vegetables are available in our country which is rice in various nutrients. For the ignorance and inadequate knowledge of the people, they do not know the nutritive value of most of the vegetables and fruits, as a result they can't buy right fruits for their consumption. Strawberry is one of the delicious and popular fruits in America, Europe, Japan and recently in our country, Bangladesh. The sweet fragrance, juiciness and deep red color of the fruit make the strawberries attractive. In addition to being a low caloric food, they are rich in Vitamin C, manganese, iodine, potassium, vitamin-B5, omega-3 fatty acids, dietary fiber, water, anti-oxidants and much more nutrients.

Nature has been a good source of medicinal agents for thousands of years and an impressive number of modern drugs that have been isolated from natural sources (Pannell, 1992). Plants used for traditional medicine that contain a wide range of substances and can be used to treat chronic as well as infectious diseases. Strawberries are refreshing, fragrant, tantalizing, red beauty feast for our eyes. They also used as powerful health remedies. Strawbwrries are rich in phytonutrients, called phenols (flavonoids, proanthocyanidins, ellagitannins, gallotannins, phenolic acids) which give the fruits their bright red in color. The anthocyanins, a sub classification of phenols, have anti-inflamatory, anti-damage, anti-cancer, anti-rheumatoid and anti-osteoarthritis properties. Another phenolic compounds, ellagitannins are associated with decreased rates of cancer death and are also used as strong antibacterial agents that present in cloudberry, raspberry and strawberry (Puupponen-Pimia *et al*., 2005). It is reported that free radical theory of aging predicts that reactive oxygen species are involved in age associated functional decline. Therefore, anti-oxidants such as those present in strawberries have anti-aging effects and also reduce the occurrence of other halt conditions like muscular degeneration, high cholesterol level etc. Strawberry polyphenols have different cytotoxic effects on tumor cells (Weaver *et al*., 2009). Phenolic compounds present in strawberries selectively inhibit the growth of gastrointestine pathogens (Puupponen-Pimia *et al*., 2005).

Several research works have been done in different laboratories about the nutritional analysis and anti-microbial activity of different fruits but yet now, no research works have been done on the nutritional analysis and anti-microbial activity of strawberry fruits grown in tropical country like Bangladesh. In this study, at the first time, we report the nutrient analysis and anti-microbial activity and the cytotoxic study of the aqueous extract of two varieties (Long Conic and Globose Conic) of Strawberry that were grown and collected in Hilli region like Chittagong, Bangladesh.

MATERIALS AND METHODS

Collection of sample: The two varieties of fresh strawberries were collected from the field of Bangladesh Agricultural Research Institute (BARI), Pahartali, Chittagong, Bangladesh on February 2010 and used for the anti-microbial study and cytotoxic activity.

Procedure of nutrient analysis: The moisture and ash content of two varieties of strawberry were carried out by the standard methods of IUPAC (1979) and AOAC (1995), respectively. The other nutrient contents were determined by using the established reported methods, i.e., total sugar and starch content by the anthrone method of Jayaraman (1981), reducing sugar by dinitrosalicylic acid method of Miller (1959), non-reducing sugar by the methods of Ranganna (1979). The water soluble protein of two varieties of strawberry were determined by the method of Folin-Lowry of Lowry *et al.* (1951) and Total protein by Micro-Kjeldahl method of AOAC (1960) by using 6.25 factor to calculate protein content from nitrogen content. Vitamin C content of strawberry was determined by the standard method of AOAC (1995) while beta-carotene content was determined according to the procedure reported in the methods of vitamin assay of Jayaraman (1981). Lipid content of strawberry was determined by the method of Bligh and Dyer (1959).

Determination of mineral content: The minerals present in the two varieties of strawberry were analyzed by the procedure as described in the Analytical methods (Petersen, 2002).

Statistical analysis: The results obtained from the analysis were subjected to statistical analysis using the Statistical Package for Social Sciences (SPSS) Version 15. Means were used for the analysis of the result.

Preparation of extracts for anti-microbial and cytotoxic study: Three grams of two varieties of fresh strawberry were taken separately in a mortar and pasted well. After that 8-10 mL of sterile de-ionized distilled water was added to each sample and homogenized well. Homogenized sample was filtered by double layered muslin cloth. The mixture was then centrifuged at 2,000 rpm for 10 min at 4°C. The clear supernatant was transferred into a beaker and used for anti-microbial as well as cytotoxic study.

Biological screening

Bacteria and growth conditions: Three bacterial species, one gram positive and two Gram negative, such as *Bacillus subtilis* and *Escherichia coli* and *Salmonella typhi*, respectively were used as test organisms. Exactly 0.2 mL of overnight cultures of each organism was inoculated into 20 mL of sterile nutrient broth and inoculated for 3-5 h to standardize the culture to 10^8 cfu mL^{-1}. Each of suspension was then transferred to the plate containing nutrient agar media.

Antimicrobial assay: The disc diffusion method (Bauer *et al.*, 1966) was used to test anti-bacterial activity. Dried and sterilized filter paper discs (5 mm diameter) were soaked with known amounts of aqueous solution of two varieties of strawberry using micropipette. Discs containing the test materials were placed on Nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic disc Amoxicilin (0.05 mg $disc^{-1}$) and blank discs (soaked with distilled water) were used as a positive and negative control, respectively. These plates were then kept at low temperature in refrigerator (4°C) for 12 h to allow maximum diffusion of the compound present in strawberry samples in agar media before any growth of the organisms. The plates were then incubated at 37°C for 24 h to allow maximum growth of the microorganisms and a clear distinct zone of inhibition was visualized surround the medium. The antibacterial activity of the test samples were determined by measuring the diameter of zone of inhibition expressed in millimeter.

Cytotoxicity study: Brine shrimp lethality bioassay (Persoone, 1988) technique was applied for the determination of cytotoxic properties of aqueous extract of two varieties of strawberry.

Preparation of sea water: Thirty eight grams of NaCL was weighted, dissolved in one liter of distilled water and finally filtered through Whatman No. 1 filter paper.

Hatching of brine shrimp of eggs: Sea water was taken in a small tank and shrimp eggs were added to one side of the divided tank which was covered. The shrimps were allowed for 48 h to hatch and matured as shrimp larvae (nauplii).Then the hatched nauplii were harvested in a clean beaker.

Application of the test solution and nauplii in the vials: At room temperature 12.5, 25, 50, 100, 150 and 200 μL of the two varieties of the aqueous strawberry extracts were taken in the vials and 5 mL of the sea water was added to each vial containing 10 brine shrimp nauplii. So, the concentration of the sample in the vials were 750, 1,500, 3,000, 6,000, 9,000 and 12,000 μg mL^{-1}, respectively. These vials were used for each concentration and 5 mL sea water containing 10 nauplii were used as negative control.

Counting of the nauplii: After 24 h, the vials were observed using a magnifying glass and the number of survived nauplii in each vial were counted and noted. From this data, the percentage of mortality of the nauplii was calculated for each concentration.

RESULTS AND DISCUSSION

The amount of moisture, ash, total sugar, reducing sugar, non-reducing sugar and starch present in the two varieties of strawberry are shown in Table 1. The moisture content of two varieties, variety-1 (Long Conic) and Variety-2 (Globose Conic) of strawberry was found to be 90.96±0.04 and 90.09±0.042%, respectively. The moisture content of strawberry was reported to be 91.57% (USDA, 1999). So, our present dada is closely related to the USDA reported data. The ash content of two varieties, variety-1 (Long Conic) and Variety-2 (Globose Conic) of strawberry was found to be 0.469±0.01 and 0.539±0.02%, respectively which are little bit higher than the reported data of USDA (1999), i.e., 0.43%. The environmental as well as soil factors may be responsible for this type of difference.

Total sugar and non-reducing sugar content of two varieties, variety-1 (Long Conic) and Variety-2 (Globose Conic) of strawberry were found to be 3.15±0.01 and 2.5±0.01% and 3.12±0.02 and 2.4±0.01%, respectively. The variety-1 (Long Conic) of strawberry contained higher amount of total sugar and non-reducing sugar than Variety-2 (Globose Conic). So, variety-1 (Long Conic) strawberry is sweeter than the Variety-2 (Globose Conic) strawberry due to the presence of more non-reducing sugar.

Table 1: Moisture, ash, total sugar, reducing sugar, starch content of two varieties, variety-1 (Long conic) and Variety-2 (Globose conic) of strawberry

Varieties	Moisture (g%)	Ash (g%)	Total sugar (g%)	Reducing sugar (g%)	Non-Reducing sugar (g%)	Starch (g%)
Variety-1 of strawberry (Long conic)	90.96 ±0.04	0.469±0.01	3.15±0.01	0.0335±0.01	3.12±0.01	0.395±0.02
Variety-2 of strawberry (Globose conic)	90.09± 0.042	0.539±0.02	2.5±0.02	0.048±0.01	2.4±0.01	0.685±0.04

All values were the average of three determinations

Table 2: Water soluble protein, total protein, fat and vitamin C and Beta-carotene content of two varieties, variety-1 (Long conic) and Variety-2 (Globose conic) of strawberry

Varieties	Water soluble protein (g%)	Total protein (g%)	Fat (g%)	Vitamin C (mg%)	Beta-carotene (µgm%)
Variety -1 of strawberry (Long conic)	0.785±0.01	1.2±0.02	0.4±0.01	116±0.32	3.71±0.25
Variety-2 of strawberry (Globose conic)	0.986±0.01	1.5±0.02	0.6±0.02	179±0.52	4.15±0.30

All values were the average of three determinations

Both the two varieties of strawberry contained negligible amount of reducing sugar while variety-1 (Long Conic) contained lower amount of starch (0.395±0.02%) than Variety-2 (Globose Conic) of strawberry (0.685±0.04%) and this data is closed related to the USDA (1999) reported data (0.50%).

As shown in Table 2, the water soluble and total protein content of two varieties of strawberry was found to be 0.785±0.01 and 1.2±0.02% 0.986±0.01 and 1.5±0.02%, respectively. So, among the two varieties of strawberries examined in our study, the Variety-2 (Globose Conic) of strawberry contained much higher amount of protein than the variety-1 (Long Conic) of strawberry. From this result it may suggest that both the varieties of strawberry are moderate sources of protein.

The fat content of two varieties of strawberry was found to be (0.4±0.01%) for variety-1 (Long Conic) of strawberry and (0.6±0.02%) of Variety-2 (Globose Conic) of strawberry. Among the both varieties, Variety-2 (Globose Conic) of strawberry contained higher amount of fat (0.6±0.02%) than variety-1 (Long Conic) of strawberry (0.4±0.01%). This finding indicates that strawberry is not a good source of lipid.

The Vitamin C and Beta-arotene content of two varieties, variety-1 (Long Conic) and Variety-2 (Globose Conic) of strawberry were found to be (116±0.32 mg%) and (3.71±0.25 µg%) and (179±0.52 mg%) and (4.15±0.30 µg%), respectively. Among both the varieties examined, Variety-2 (Globose Conic) of strawberry contained higher amount of Vitamin C and Beta-carotene than variety-1 (Long Conic) of strawberry. The Vitamin C and Beta-carotene content of strawberry were reported by USDA (1999) were 57 mg% and 3 µg%, respectively and our present data is much higher than the USDA reported data. This finding indicates that both varieties of strawberry are good sources of antioxidants. Beta-carotenes are precursors of Vitamin A. Animal can not synthesize it but can convert it to Vitamin A. In plants, it is very necessary for growth and development of soft tissue through its effect upon protein synthesis. Vitamin A also plays an important role in the maintenance of normal epithelial structure. The minerals content of two varieties of strawberry are presented in Table 3.

The contents of Magnesium, Calcium and Potassium of two varieties, variety-1 (Long Conic) and Variety-2 (Globose Conic) of strawberry were found to be (17.35±0.01 mg%), (6.40±0.2 mg%) and (38±0.2 mg%) and (17.76±0.01 mg%), (11.36±0.4 mg%) and (43.00±0.01 mg%), respectively.

The contents of Copper, Iron and Zinc of two varieties, variety-1 (Long Conic) and Variety-2 (Globose Conic) of strawberry were found to be (0.1365±0.02 mg%), (0.12 ±0.02 mg%) and (0.092±0.01 mg%) and (0.0275±0.01 mg%), (0.5415±0.01 mg%) and (0.049±0.03 mg%), respectively. The contents of Phosphorous and Sulphur of two varieties, variety-1 (Long Conic) and Variety-2 (Globose Conic) of strawberry were found to be (0.718±0.01 mg%) and (5.56±0.2 mg%) and (0.627±0.02 mg%) and (5.735±0.22 mg%), respectively. The present data in Table 3 clearly indicated that the Variety-2 (Globose Conic) of strawberry contained higher amount of Magnesium (17.76±0.01 mg%), Calcium (11.36±0.4 mg%) and Potassium (43.00±0.01 mg%), Iron

Table 3: Mineral content of two varieties, variety-1(Long conic) and Variety-2 (Globose conic) of Strawberry

Varieties	Magnesium (mg%)	Calcium (mg%)	Potassium (mg%)	Copper (mg%)	Iron (mg%)	Zinc (mg%)	Phosphorous (mg%)	Sulphur (mg%)
Variety -1 of strawberry (Long conic)	17.35±0.01	6.40±0.20	38.00±0.20	0.1365±0.02	0.12±0.02	0.092±0.01	0.718±0.01	5.56±0.20
Variety -2 of strawberry (Long conic)	17.76±0.01	11.36±0.40	43.00±0.01	0.0275±0.01	0.5415±0.01	0.049±0.03	0.627±0.02	5.735±0.22

All values were the average of three determinations

(0.5415±0.01 mg%) and Sulphar (5.735±0.22 mg%) other than variety-1 (Long Conic) of strawberry while the Copper (0.1365±0.02 mg%), Zinc (0.092±0.01 mg%) and Phosphorus (0.718±0.01 mg%) contained of variety-1 (Long Conic) of strawberry is higher than the Variety-2 (Globose Conic) of strawberry. The minerals contained in the two varieties of strawberry may play important role in human nutrition.

Magnesium, calcium and potassium in the human required for building red blood cell and for body mechanism (FAO/WHO/IAEA, 1996). Calcium plays a major role in CNS function and it is essential for nerve impulse conduction and activates some enzymes which generate neurotransmitters (Watts, 1997) and also as we know it is one of the most important elements in the diet because it is a structural component of bones, teeth and soft tissues and is essential in many of body's metabolic processes.

Phosphorous is also a constitute of bones and teeth and acts together with calcium for making structure of bone and plays a significant role in CNS function. It is also constituent of phosphoproteins. Phospholipids are also involved in nerve conduction. Phosphate is the primary iron in extra and intracellular fluid. It helps absorption of dietary constituents which help to maintain the blood at a slightly alkaline level regulates enzyme activity and is involved in the transmission of nerve impulses (Karade *et al*., 2004). Potassium has many functions for protein synthesis, activation of many enzymes, stimulation of the movement of the intestinal tracts etc., excess of potassium can produce neurological disturbances such as numbness of hand and feet (Watts, 1997). Iron plays a significant role in oxygen transport in the body and it required for the synthesis of DNA. It is also essential for the activation of enzymes involved in brain neurotransmitters (Watts, 1997). Zinc is extremely important for numerous body functions. Zinc deficiencies are associated with mental impairments. Zinc deficiency may be associated with mental lethargy, emotional disorder and irritability (Watts, 1997).

Sulphur is an integral part of many proteins and enzymes that maintain good health. In human, sulphar is an essential component of proteins involved in oxygen transport (Dallman, 1986). The present data clearly indicate that strawberry contain good amount of antioxidants, various nutrients and minerals and these components help to fulfill the deficiencies of the requirement of our body and also help our people to prevent malnutrition, night blindness, anemia etc.

The results of antibacterial study of aqueous extract of two varieties of strawberry are presented in Table 4 for variety-1 (Long Conic) and for Variety-2 (Globose Conic). In this study, one gram positive, *Bacillus subtilis* and two gram negative bacteria, *E. coli* and *Salmonella typhi* were used. The antibacterial study of aqueous extract of two varieties of strawberry was compared to standard drug Amoxicilin.

Table 4: Antibacterial activity of aqueous extract of two varieties, a) Variety-1 (Long Conic) and b) Variety-2 (Globose Conic) of Strawberry by disc-diffusion method

	Diameter of zone of inhibition (mm)			
	Variety-1 (Long conic)		Negative control	Positive control amoxicillin
Bacteria	5 mg disc^{-1}	10 mg disc^{-1}	10 µL of sterilized water	0.05 mg disc^{-1}
Gram positive				
Bacillus subtilis	6	14	0	45
Gram negative				
E. coli	6	8	0	40
Salmonila typhi	6	12	0	45
	Diameter of zone of inhibition (mm)			
	Variety-2 (Globose conic)		Negative control)	Positive control amoxicillin
Bacteria	5 mg disc^{-1}	10 mg disc^{-1}	10 µL of sterilized water	0.05 mg disc^{-1}
Gram positive				
Bacillus subtilis	6	12	0	45
Gram negative				
E. coli	6	10	0	45
Salmonila typhi	6	14	0	45

It has been found that both the aqueous extract of strawberry showed antibacterial activity against all the three bacteria, i.e., *Bacillus subtilis, E. coli* and *Salmonella typhi* (Table 4). However, variety-1 and variety-2 showed strong anti-bacterial activity against *Bacillus subtilis* (Zone of inhibition, 6 and 6 mm for 5 mg disc^{-1} and zone of inhibition, 14 and 12 mm for 10 mg disc^{-1}, respectively) and *Salmonella* typhi (Zone of inhibition, 6 and 6 mm for 5 mg disc^{-1} and zone of inhibition, 12 and 14 mm for 10 mg disc^{-1}, respectively). It is remarkable that in case of both the varieties for *Bacillus subtilis and Salmonella typhi*, the zone of inhibition was increased correspondingly that is more than double with increasing concentration. While in case of variety-2 for *E. coli*, the zone of inhibition was found to be increased about 66% but for variety-1 that was found to be about 33%. Since *Bacillus subtilis* and *Salmolella typhi* are the frequent candidates causing food born illnesses and also *Salmonella typhi* is a causative agent for typhoid and paratyphoid infection, so, the experimental strawberry varieties might be useful as a supplement of medicine to prevent food borne illnesses as well as typhoid and paratyphoid infections.

Table 5 shows the results of the brine shrimp lethality bioassay after 24 h of exposure to the aqueous extract of two varieties of strawberry and the negative control. The LC_{50} value (Concentration at which 50% mortality of the brine shrimp nauplli occur) was determined from the graph by extrapolation and the LC_{50} values of the two varieties of strawberry, Variety-1 (Long Conic) and Variety-2 (Globose Conic) were found to be about 5 and 6 mg mL^{-1}, respectively. From the results of brine shrimp lethality bioassay, it is concluded that the aqueous extract of both varieties of strawberry are non toxic or very less toxic on the brine shrimp as they gave LC_{50} values much greater than 100 µg mL^{-1} as it was also established that root extracts of *Terminalia brownie* is considered to be non-toxic since it showed LC_{50} values greater than 100 mg mL^{-1} on the brine shrimps (Mbwambo *et al*., 2007). Even though, the aqueous extract of both varieties of strawberry exhibited very low toxicity on Brine shrimp but they are showing strong antibacterial activity

Table 5: Brine shrimp lethality bioassay of the aqueous extract of two varieties of strawberry

Test sample	Volume of sample	Concentration of sample (μg mL^{-1})	Log concentration (Log C)	No. of total shrimp (each vial)	No. of survival Shrimp (average)	(%) of Mortality	LC_{50} (mg mL^{-1})
Control	0	0	0	10	10	0	
Variety -1 of	12.5	750	2.87	10	10	10	5.0
strawberry	25	1500	3.17	10	9	20	
(Long conic)	50	3000	3.47	10	8	30	
	100	6000	3.77	10	7	60	
	150	9000	3.95	10	4	80	
	200	12000	4.07	10	2	100	
Variety-2 of	12.5	750	2.87	10	9	10	6.0
strawberry	25	1500	3.17	10	8	20	
(Globose conic)	50	3000	3.47	10	7	30	
	100	6000	3.77	10	5	50	
	150	9000	3.95	10	2	80	
	200	12000	4.07	10	0	100	

against *Bacillus substilis* and *Salmonella typhi as* (Puupponen-Pimia *et al*., 2005) also reported that berries especially cranberry, cloudberry, raspberry, strawberry and bilberry possess clear anti-microbial activity against salmonella and staphylococcus. In conclusion, the present result is very much encouraging in relation to antimicrobial activities and indicating the presence of potent bioactive compounds in strawberry and might be useful as bioactive agent against various diseases. Further detailed studies are needed to isolate and purify bioactive compounds in the aqueous extract as well as different solvent extract of two varieties of strawberry to evaluate their precise roles.

CONCLUSION

In conclusion, the nutritional study suggests that strawberry contains a good amount of nutritional components. We know Vitamin C and Vitamin A deficiency are the big problem in the developing country, like Bangladesh. So, intake of adequate amount of Vitamin C and Beta-carotene rich strawberries developed in the Hilly region like Chittagong, Bangladesh may eliminate both the Vitamin C and Vitamin A deficiency diseases and may help to prevent cancer since both vitamins have anti-oxidant properties. From this study, it is also concluded that intake of both varieties of strawberries may eliminate Calcium, Potassium, Magnesium, Sulphur and Iron deficient symptoms and diseases.

It is also concluded that the aqueous extract of both the varieties of strawberry can be a good source of anti-microbial agents with potential applications in food as well as pharmaceutical industries to control food born illnesses caused by *Bacillus subtilis* and *Salmonella typhi*.

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