Phytochemical Profile, Free Radical Scavenging Activity and Anticancer Potential of *Pandanus odoratissimus* Leaves Ethanol Extract

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Abstract

There is now an emerging interest in the discovery of a plant-based anticancer drug. Hence, this study determined the phytochemical profile of Pandanus odoratissimus ethanol (POE) leaves extract and investigated its preliminary anticancer capacity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (RSA) and in vitro antiproliferative activity by Saccharomyces cerevisiae yeast model. The phytochemical analysis of POE revealed the presence of flavonoids, phenols, steroids and tannins. The POE had fair amounts of total phenolic content (9.411±0.0115 mg GAE/g crude extract) and total tannin content (38.1511±1.116 mg TAE/g crude extract). The %DPPH RSA of POE significantly increased with concentration and inhibited more than 50% against DPPH. Tannic and gallic acids showed high potency in scavenging DPPH that was statistically comparable to the reference standard (ascorbic acid). In the antiproliferative assay, POE and tannic acid significantly inhibited yeast growth in a dose-dependent manner. At 1000 ppm, POE and tannic acid demonstrated the highest inhibition of viable cells at 76.04±1.08 and 86.25±0.33%, respectively. It was further revealed that the percent inhibition of cell viability and the %DPPH RSA of POE had a strong correlation (r = 0.9620). Hence, P. odoratissimus has bioactive compounds with potential chemopreventive effects.

***Keywords**:* anticancer, antiproliferative activity, DPPH free radical scavenging activity, *Pandanus odoratissimus*, phytochemical profile

1. Introduction

Cancer is the abnormality in cell division that leads to the uncontrolled proliferation and alterations, which accumulate within the normal cell into a malignant cell (Iqbal *et al*., 2017). One of the primary causes of cancer is

exposure to toxic substances in the environment resulting in genetic changes (Raheel *et al*., 2017). Reactive oxygen species (ROS) is also linked to the emergence and development of cancer (Borek, 2004). Reactive oxygen species or free radicals, such as hydroxyl radicals ($^{\bullet}OH$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), alkoxyl radical (RO) and superoxide radical ($O_2^{\bullet-}$) (Sarker and Oba, 2018a) have one or more unpaired electrons that can cause damage to the respiring cells making them highly toxic, mutagenic and carcinogenic. At higher concentrations, these reactive species can break one of the two strands in deoxyribonucleic acid (DNA) that can damage enzymes and essential proteins or cause uncontrolled lipid peroxidation and auto-oxidation reactions (Sarker and Oba, 2018b, 2020a) leading to cancer (Zahin *et al*., 2010). Also, free radicals could deactivate specific tumor-suppressing genes (Boran and Ugur, 2016); this activation will trigger uncontrolled growth and proliferation bringing about the transformation of cells to have carcinogenic properties (Rayan *et al*., 2017).

Although experts in the field made advances in the control of cancer progressions such as surgery, radiotherapy and chemotherapy, these have undesirable side effects (Shrivastava *et al*., 2016). There is now an emerging interest in creating drugs that may overcome the antagonistic impacts of some cancer medications by utilizing natural compounds, which may influence the multiple targets with decreased reactions and be effective against certain types of cancer (Padmaharish and Lakshmi, 2017). Thus, the investigation and the search for possible agents with noticeable anticancer activity and antioxidative properties are highly important nowadays.

The leaves of plants are excellent sources of natural pro-vitamin A (Sarker and Oba, 2020b), vitamin C (Sarker *et al.*, 2017a); phytochemicals like pigments such as carotenoids (Sarker *et al*., 2015a; Keles *et al*., 2016), betaxanthins, chlorophylls, betalains, betacyanins, xanthophylls (Sarker and Oba, 2021) and beta-carotene (Sarker *et al*., 2014, 2015b); phenolics specifically phenolic acids, namely different hydroxybenzoic acids and hydroxycinnamic acids; and flavonoid compounds (flavonols, flavones, flavanols, flavanones and other nonflavonoid compounds), which act as an antioxidant (Sarker *et al*., 2020a; Sarker and Oba, 2020c, 2020d). Various plant species are notable for their anticancer and antioxidative properties. New drugs derived from several plant species and their phytochemical constituents received much attention in preventing and treating cancer (Aung *et al*., 2017). Phytochemicals selectively kill cells that divide rapidly, target abnormally expressed molecular factors, eliminate oxidative stress, moderate cell growth factors, inhibit angiogenesis

in cancer tissue and induce apoptosis (Singh *et al*., 2016). Moreover, the consumption of traditional herbal plants with chemopreventive actions could be helpful to improve life expectancy strategies due to their low cost, lesser toxicity during long-term administration and availability at a large scale (Gowri and Chinnaswamy, 2011).

One plant of interest is the *Pandanus odoratissimus*, which belongs to the family *Pandanaceae* and is widely distributed in India, the Pacific Islands and the Philippines. It is a small, tortuous tree that can grow to a height of 4 m supported by aerial roots. The leaves are sword-shaped, rigid and 40-70 cm long (Adkar and Bhaskar, 2014). The Ayurveda, Unani and Siddha medical systems used the leaves of *P. odoratissimus* for treating back pain, rheumatism, wound, urinary disorder, loss of appetite, indigestion, constipation, diabetes, skin diseases and fever, to name a few. The Ayurvedic system of medicine traditionally used the sugar-based paste of *P. odoratissimus* to treat cancer (Raj *et al*., 2014a). Various phytochemical constituents present in the leaves of *P. odoratissimus* include alkaloids, tannins (Rahayu *et al*., 2013), saponins, flavonoids, steroids and phenols (Kumar *et al*., 2010).

Using two-dimensional nuclear magnetic resonance (NMR) techniques, Nonato *et al.* (1993) found out that the leaves of *Pandanus* plants contain piperidine alkaloids such as pandamarilactones, pandamine and pyrroline. Further studies suggested that these compounds could possess antioxidant, anti-inflammatory and antidiabetic activities. A study conducted by Jong and Chau (1998) isolated two phenolic compounds, namely pinoresinol and 3,4-bis-(4-hydroxy-3-methoxybenzyl) tetrahydrofuran from *P. odoratissimus* and showed strong antioxidative activities using the thiocyanate method. The study of Jong and Chau (1998) only focused on the antioxidative capacity of *P. odoratissimus* and its isolates, and they utilized a different approach in determining the antioxidative property of the plant. Londonkar and Kamble (2009) reported a high percent 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition of the leaves of *P. odoratissimus*; they made use of methanol as an extracting solvent. Some previous experimental studies on the aqueous extract of *P. odoratissimus* demonstrated its anticancer activity against cancer cell lines in vitro *and* in vivo (Raj *et al*., 2014a, 2014b). Raj *et al.* (2014a) studied the dried parts of roots and leaves of the plant extracted with methanol and water. The in vitro anticancer activity of the plant was determined through the brine shrimp lethality assay for its cytotoxicity; the onion root tip method for its antimitotic activity; and the cancer cell lines were studied using

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay. To date, no study has explicitly looked at the contribution of the tannic and gallic contents and DPPH radical scavenging activity (RSA) of *P. odoratissimus* to its overall antiproliferative capacity in the yeast cell model.

Therefore, this study determined the phytochemical profile, quantified the selected secondary metabolites and investigated the potential anticancer capacity of the ethanolic extract of the leaves of *P. odoratissimus* through its oxidative damage protecting property and antiproliferative activity in the *Saccharomyces cerevisiae* yeast cell model.

2. Methodology

2.1 Plant Material Collection and Preparation

The leaves of *P. odoratissimus* were collected within Samay, Gingoog City, Misamis Oriental, Philippines. The leaves samples were placed in a resealable plastic bag (Ziploc) and transported to the Chemistry Laboratory of the University of Science and Technology of Southern Philippines – Cagayan de Oro (USTP-CDO) Campus, Cagayan de Oro City, Philippines. The leaves collected were washed with running tap water, rinsed with distilled water, cut into small pieces and air-dried for four weeks at room temperature (30 °C). The dried leaves were homogenized using a knife mill (GM200, Retsch, Germany) and stored in plastic containers.

2.2 Plant Material Extraction

The homogenized leaves of *P. odoratissimus* (100 g) were soaked with ethanol (Analytical Grade, Scharlau, Spain) at room temperature (30 °C) for 24 h and filtered using Whatman quantitative filter paper grade 42. The extraction process was done twice. The filtrates were combined through Buchner funnel vacuum filtration (Yamato Scientific Co. Ltd., Japan). The pooled filtrate was concentrated using a rotary evaporator (Stuart RE300, Stuart, United Kingdom) at 55 °C and stored at 4 °C in a refrigerator (NR-A2007PE, National, Japan) until use.

2.3 Qualitative Phytochemical Screening

Phytochemical screening was done using the standard phytochemical methods described by Aguinaldo *et al.* (2005) with some modifications. *P. odoratissimus* ethanol (POE) extract was subjected to qualitative chemical tests to identify selected phytochemical constituents such as flavonoids using alkaline reagent test (Pandey and Tripathi, 2013); steroids through Keller-kiliani test (Aguinaldo *et al.*, 2005); phenols and tannins by ferric-chloride test (Tiwari *et al.*, 2011).

2.4 Determination of Lambda Max and Linear Dynamic Range (LDR) for Total Phenolic Content (TPC), Total Tannin Content (TTC) and DPPH RSA

The determination of lambda max (λ_{max}) and LDR was carried out using the protocol reported by Pontillas and Lapetaje (2019). Briefly, a stock solution of the test sample was made and serially diluted to achieve concentrations of the working standard solution. An aliquot of stock solution was added with reagents (see sections 2.5 and 2.6 for TPC and TTC, respectively). A working standard solution was scanned for lambda max in the specific region in the Ultraviolet-visible (UV-Vis) spectrophotometer (DR5000, Hach, United States). The absorbance of the freshly prepared working standard solutions was measured using the lambda max. A plot of absorbance versus concentration was constructed. The test sample standard solution concentration with the highest sensitivity (abs $\leq$ 1.500) was utilized in the assay. Table 1 shows the values of λ_{max} and LDR used for the assays analyzed using UV-Vis spectrophotometer.

Table 1. The values of λ_{max} and LDR used for the conduct of TPC, TTC and DPPH RSA

Assay	Test sample (concentration)	Working standard solutions (ppm)	Region scanned in UV-Vis (nm)	Lambda max (nm)	LDR (ppm)
TPC	GA (350 ppm)	1.0, 2.5, 5.0, 10, 20, 30, 35	119-900	755	1.0, 2.5, 5.0, 10
TTC	TA (225 ppm)	1.0, 5.0, 10, 15, 25, 50	200-900	740	1.0, 5.0, 10, 15
DPPH RSA	DPPH	10, 20, 30, 40, 50, 60, 70, 80, 90, 100	400-620	517	10, 20, 30, 40, 50

TPC – total phenolic content; TTC – total tannin content; DPPH RSA – 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay; GA – gallic acid; TA – tannic acid; LDR – linear dynamic range

2.5 Quantitative Determination of TPC

The quantitative analysis of the determination of total phenolic content in the ethanol extract of *P. odoratissimus* was carried out according to the Folin-Ciocalteu method (Sarker and Oba, 2020c) with modifications. Gallic acid (GA) (Analytical Grade, Scharlau, Spain) was used as the standard. POE (1.50 mL) was added with 7.5 mL of 10% (v/v) Folin-Ciocalteu reagent (Loba Chemie Pvt. Ltd., India) and 6.0 mL of 7.5% (w/v) sodium carbonate (Na_2CO_3) (Analytical Grade, RCl Labscan, Thailand). After incubating the reaction mixture at room temperature for 30 min, the absorbance was measured at 755 nm (λ_{max}) against the blank spectrophotometrically. The calibration curve of GA standard solution (1.0-10 ppm with 2.5-ppm increment) was established to determine TPC expressed as GA equivalence (GAE). The TPC was computed using Equation 1.

$$TPC = \frac{(C \cdot DF \cdot V)}{m} \quad (1)$$

where *C* is the concentration of the GA obtained from the standard calibration curve (mg/L); *DF* is the dilution factor; *V* is the volume of the extract solution (L); *m* is the weight of the dried extract (g).

2.6 Quantitative Determination of TTC

The total amount of tannins in the POE was obtained using the method of Giner-Chavez *et al.* (1997) with minor modifications. Tannic acid (TA) (Analytical Grade, Scharlau, Spain) was used as the standard. POE (3.0 mL, 4,500 ppm) was mixed with 1.50 mL of Folin's phenol reagent (Loba Chemie Pvt. Ltd., India) and 9.0 mL of 35% (w/v) Na_2CO_3 (Analytical Grade, RCl Labscan, Thailand). After the reaction mixture was incubated for 5 min at room temperature, the absorbance of the complex formed was read at 740 nm (λ_{max}) against the blank using the UV-Vis. The TTC was determined through regression analysis from the calibration curve of the TA (1.0-20 ppm with 5.0-ppm increment) expressed as TA equivalence (TAE). The TTC was computed using Equation 2.

$$TTC = \frac{(C \cdot D.F \cdot V)}{m} \quad (2)$$

where *C* is the concentration of the GA obtained from the standard calibration curve (mg/L); *DF* is the dilution factor; *V* is the volume of the extract solution (L); *m* is the weight of the dried extract (g).

2.7 Determination of the Antioxidant Activity

The antioxidant activities of POE, GA and TA were determined quantitatively using a DPPH RSA. The method was carried out using the protocol reported by Pontillas and Lapetaje (2019). A 150-ppm stock solution of DPPH (Analytical Grade, Sigma-Aldrich Chemie GmbH, Germany) in methanol (ACS, Scharlau, Spain) was prepared. A 1000 ppm stock solution of POE, GA and TA was prepared and serially diluted to 10, 50, 100, 250 and 500 ppm. DPPH solution (2.3 mL) in methanol was added to the test samples, and the final volume was made up to 7.0 mL using methanol. After the incubation for 30 min in the dark, the absorbance of the test samples and the reference standard – ascorbic acid (AA) (Analytical Grade, Scharlau, Spain) was measured at 517 nm (λ_{max}) against the blank (methanol) using the UV-Vis spectrophotometer. The percent (%) DPPH RSA for test sample concentration was calculated using Equation 3.

$$\%DPPH\ RSA = \frac{A_0 - A_1}{A_0} \times 100 \quad (3)$$

where A_0 is the absorbance of the negative control; A_1 is the absorbance in the presence of the sample.

2.8 In Vitro Antiproliferative Assay by Yeast Cell Model

The antiproliferative activities of POE and TA were determined using the yeast cell model. The assay was conducted following the methodology by Simon (2001) as reported by Raheel *et al.* (2017), Hosseinpour *et al.* (2013) and Tomar *et al.* (2018) with minor modifications.

2.8.1 Preparation of Potato Dextrose Broth (PDB)

Potato (200 g) was boiled in distilled water (1 L) for 1 h, filtered and added with 20 g glucose (Analytical Grade, Duksan Pure Chemicals, South Korea). The final volume of solution was made to 1 L using distilled water and sterilized by autoclaving (Electric Sterilizer 75X-120V, All American Electric Sterilizer, United States) at 121 °C, 15 psi for 15 min.

2.8.2 Preparation of Yeast Inoculum

Yeast was inoculated in a conical flask containing sterilized PDB (100 mL) and commercially available yeast (5 g) and incubated (Binder redLINE Incubator RI 115-U, India) at 37 °C for 24 h. Then, the seeded broth (1.0 mL) was diluted up to 10 mL with sterilized distilled water to obtain 2.54 x 10^5 cells and was diluted into 1:100 to obtain a concentration of 2.54 x 10^3 cells.

2.8.3 Cell Viability Count

The test samples (POE and TA) were prepared with 250, 500 and 1000 ppm concentrations. Test solutions with test samples, PDB (2.5 mL) and yeast inoculum (0.50 mL) were mixed in separate test tubes, and the total volume was made up to 4.0 mL using distilled water. There were three groups formed: distilled water as the negative control, POE and TA. All test samples were incubated at 37 °C for 24 h. After the incubation period, the cell suspension in each sample was added with 0.1% (w/v) methylene blue (ACS, Sisco Research Laboratories Pvt. Ltd., India) and was observed under high power (40x) microscope (Monocular T-12011C, Ken-A-Vision, United States). The number of living cells (colorless) and dead cells (appears blue) were counted for the negative control and samples treated with POE and TA. The cell viability was computed using Equations 4 and 5.

$$\% \, Cell \, viability = \frac{Total \, viable \, cells}{Total \, number \, of \, cells} \times 100 \quad (4)$$

$$\% \, Inhibition \, of \, cell \, viability = \frac{A - B}{A} \times 100 \quad (5)$$

where A is the cell viability of control; B is the cell viability of treatment.

2.9 Data Analysis

All the analyses were done in triplicates, and these values were expressed as mean and standard deviation. Statistical analysis was performed by one-way analysis of variance (ANOVA) at a 5% significance level. Tukey's honest significant difference (HSD) test was carried out when the differences by the data were significant ($p < 0.05$). MegaStat in Microsoft Excel was used for all data analyses including Pearson's correlation coefficient.

3. Results and Discussion

3.1 Phytochemical Profile of POE

A qualitative phytochemical analysis of POE extracts revealed the presence of flavonoids, phenols, steroids and tannins. The phytochemical results agreed with Kumar *et al*. (2010) and Rahayu *et al*. (2013). Ethanol was an efficient extraction solvent as all four secondary plant metabolite groups were detected. Extraction using solvents with varying polarity is one of the methods used to obtain secondary metabolites. Secondary metabolites have different functional groups classified as polar or non-polar.The recovery of polyphenols from plant tissues often involved the use of polar solvents. Ethanol has been known as a suitable solvent for extracting polyphenols and is less toxic to humans and the environment (Do *et al*., 2013). Also, it was adequate to extract sterol, flavonoid, tannins, terpenoid, saponin and alkaloids (Widyawati *et al*., 2014). Table 2 presents the results obtained from the phytochemical screening.

Table 2. Phytochemical profile of POE extract

Secondary plant metabolite	Result
Flavonoids	+
Phenols	+
Steroids	+
Tannins	+

(+) present

Flavonoids belong to a large family of polyphenol plant compounds. The different flavonoids have various biological functions, such as protection against ultraviolet radiation and phytopathogens (Ferreyra *et al*., 2012). In addition, flavonoids are well known for their health benefits in the cell signaling pathways and their antioxidant effects (Sarker and Oba, 2020b, 2021). Flavonoids could elicit beneficial effects such as antiviral, anticancer, anti-inflammatory and anti-allergic activities (Robertson, 2014). Pieces of literature have shown that the leaves of different vegetable amaranth species, such as *Amaranthus spinosus* and *A. viridis* (Sarker and Oba, 2019a), *A. blitum* (Sarker and Oba, 2020e), red morph amaranth (Sarker and Oba, 2019b), stem amaranth (Sarker *et al.*, 2020a), salt-tolerant vegetable amaranth (Sarker *et al*., 2020b) and green morph amaranth (Sarker *et al*., 2020c) had high total flavonoid content with a potent antioxidant activity which conform with the present findings. Phytosterols, commonly called plant steroids, possess antioxidant activities due to forming an allylic free radical and transforming

to other stable free radicals (Yoshida and Niki, 2003). Sultan (2015) reported that phytosterols have cholesterol-lowering properties and help reduce cholesterol levels in humans and prevent cancer. The results, as mentioned earlier, showed that the leaves of *P. odoratissimus* were rich in potential secondary metabolites that could exhibit beneficial health effects.

3.2 TPC and TTC

Table 3 presents the results from the determination of TPC and TTC found in POE extract. The data in Table 3 indicated that the TPC of POE was lower than the TTC. The lower values of TPC could be due to the presence of the unquantifiable other chemical constituents. Some researchers reported that the Folin reagent could oxidize other substances resulting in the poor specificity of the assay (Baang *et al*., 2015). The data also showed that TPC and TTC increased as the concentration of POE increased. POE contained a higher amount of TTC.

Table 3. TPC and TTC of POE extract

POE concentration (ppm)	TPC (mg GAE/g crude extract)	TTC (mg TAE/g crude extract)
500	5.248±0.284	22.063±0.480
1000	9.411±0.0115	38.151±1.116
Published value	3.5 to 10% (w/w)[a]	N/A[b]

Results are the mean of triplicate determinations ±SD; TPC – total phenolic content; TTC – total tannin content; POE – *P. odoratissimus* ethanol extract; [a]published value of TPC from Londonkar and Kamble (2009); [b]no available published value of the TTC in the leaves of *P. odoratissimus*.

Giner-Chavez *et al*. (1997) reported a similar methodology on the Folin-Ciocalteu method; the notable difference was the volume of reagents added to the test solution. Also, the lambda max used to measure the absorbance of the test solution was different in both assays. Phenolic compounds have distinct responses in Folin-Ciocalteu, and the molecular antioxidant response of phenolic compounds varied considerably to their chemical structure (Kahkonen *et al*., 1999). Londonkar and Kamble (2009) found that the TPC of *P. odoratissimus* leaves ranged from 3.5 to 10% (w/w). A direct comparison between the published and the experimental values could not be made due to the differences in the unit and the nature of analysis; however, the present findings agree that the *P. odoratissimus* leaves had a

significant amount of total phenols. On the one hand, no published data was found on the total tannins in the *P. odoratissimus* leaves.

A lot of researchers have become more interested in phenolic compounds due to their potent antioxidant properties (Sarker *et al*., 2018a, 2018b; Sarker and Oba, 2020b). It was revealed from the literature that the leaves of different vegetable amaranth such as *A. hypochondriacus* (Sarker and Oba, 2020f), drought-tolerant vegetable amaranth (Sarker and Oba, 2020d), *A. gangeticus* (Sarker and Oba, 2020g), *Amaranthus* leafy vegetable (Sarker and Oba, 2018c) and *A. tricolor* (Sarker and Oba, 2018d) had high TPC with strong antioxidant activity that was in agreement with the present findings. Tannin may inhibit lipid peroxidation in cellular oxidizing states and quench critical free radicals (Wisman, 2008). Previous studies showed that *Psychotria carthagenensis* had a high level of condensed tannins (Formagio *et al*., 2014) and ethanol extracts of *Cytinus* species (*Cytinus hypocistis* and *C. ruber*) contained significant quantities of gallotannins (Maisetta *et al*., 2019). Both studies yielded an important insight that the plant species tested exhibited a radical quenching ability due to tannins. The TPC and TTC detected in the POE may aid the claim that the plant possessed antioxidant and therapeutic properties.

3.3 Antioxidant Activity

The results obtained from the DPPH RSA were summarized in Table 4 and illustrated in Figure 1. As shown in Table 4, the scavenging effect of GA, TA, POE extract and reference standard (AA) on the DPPH radical at 50-500 ppm decreased in order: AA > TA > GA > POE. The one-way ANOVA results showed no significant difference ($p > 0.05$) between the concentration and the %DPPH RSA in GA and TA. The results implied that the %DPPH RSAs of GA and TA were independent of the concentration. On the other hand, the concentration and %DPPH RSA of POE were statistically different ($p < 0.05$); this suggests that the radical scavenging capacity of POE depends on the concentration. The %DPPH RSA of POE also increased with increasing concentration. The DPPH RSA of the AA) reached a saturation point at 250 ppm. After it peaked at this point, the DPPH radical scavenging ability of AA began to decrease when concentration doubled (500 ppm). A possible reason for this anomaly could be due to some interfering substances (Huang *et al*., 2004).

Table 4. %DPPH RSA of GA, TA and POE extract against %DPPH RSA of reference standard (AA)

Concentration (ppm)	%DPPH RSA			
	GA	TA	POE	AA
10	94.50^{c}±0.31	94.91^{c}±0.17	5.85^{a}±0.21	85.93^{b}±0.40
50	94.53^{b}±0.43	94.94^{b}±0.43	3.93^{a}±0.08	96.07^{c}±0.05
100	94.64^{a}±0.09	95.07^{b}±0.05	5.91^{c}±0.24	96.08^{d}±0.05
250	94.75^{b}±0.05	95.10^{b}±0.37	24.30^{a}±0.60	96.17^{c}±0.02
500	94.83^{b}±0.21	95.32^{b}±0.19	52.99^{a}±1.63	95.94^{b}±0.16

Results are the mean of triplicate determinations ±SD; GA – gallic acid; TA – tannic acid; POE – *P. odoratissimus* ethanol extract; AA – ascorbic acid; for each row, means followed by common letters are not significantly different. One-way ANOVA determined significant differences ($p < 0.05$) followed by the Tukey's HSD test.

At 50, 100 and 250 ppm, statistical results revealed that the %DPPH RSA of GA and TA significantly differed ($p < 0.05$) from the reference standard (AA). The radical scavenging capacities of GA and TA at 10 ppm were statistically higher ($p < 0.05$) than that of the AA. Thus, GA and TA had high potency in scavenging DPPH radicals because both samples exhibited a good antioxidant action at low concentrations. At 500 ppm, there was no significant difference ($p > 0.05$) in the percentage of radical scavenging activities of GA, TA and the reference standard. The DPPH inhibition may be considered the same in GA, TA and the reference standard in this concentration. Tukey's HDS test showed that the %DPPH RSA of the POE significantly differed ($p < 0.05$) from the reference standard. The scavenging capacity of POE was statistically lower compared to GA and TA. This outcome may happen apparently as GA and TA were pure (analytical grade) and known to elicit a high antioxidant activity. The POE may contain some interference from other chemical components present in the plant, thereby affecting the results.

As depicted in Figure 1, among the test samples, TA exhibited the highest capacity in scavenging DPPH radical followed by GA. Polyphenols act as antioxidants preventing damage from free radicals and blocking the stage of cancer initiation. Polyphenolic compounds also prevent DNA damage caused by free radicals or cancer-causing agents through various mechanisms including direct radical scavenging (Ramos, 2008).

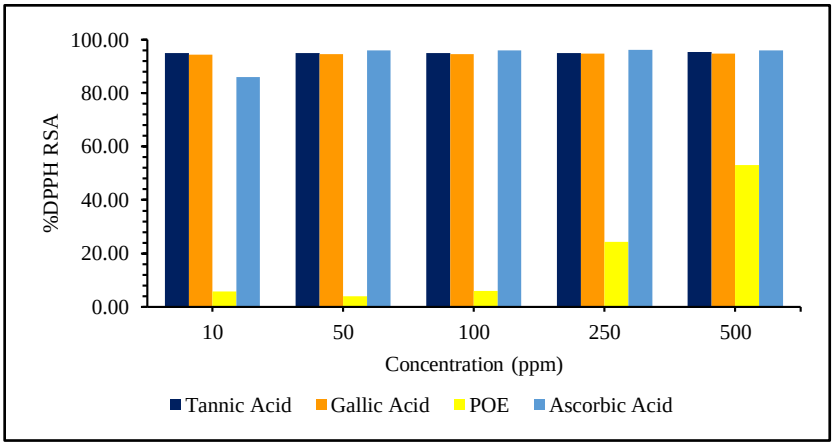


Figure 1. Comparison of the %DPPH RSA of GA, TA and POE extract against the reference standard (AA) at different concentrations

Since GA and TA are phenolic compounds, the result of this study agrees to the standing notion that phenolic compounds have high potency in scavenging free radicals. The highest radical scavenging activity of TA documented in the present investigation concurred with Gulcin *et al*. (2010). TA was investigated for its DPPH inhibition capacity and showed 95.1% RSA at 45 µg/mL (~45 ppm). Rafiee *et al*. (2018) reported that the free radical-scavenging capacity of most phenolic compounds largely depends on their structure and degree of polymerization. The multiple phenol groups surrounding the structure of TA allow them to stabilize its oxidized (radical) forms by electronic delocalization in the π-conjugated system. One of the most important mechanisms of action of phenolic antioxidants is to transform free radicals into inactive products. For these reasons, TA and its derivate are efficient free radical scavengers. Accordingly, some factors are responsible for the antioxidant activity of phenolics including the number and position of the hydroxyl group, the presence of other functional groups and their location in hydroxyl groups. GA has three hydroxyl groups bonded to the aromatic ring in an ortho position; this arrangement is considered the primary basis of the radical scavenging efficiency of GA (Badhani *et al*., 2015).

In this study, the highest percent radical scavenging of POE was 52.99±1.63% at 500 ppm. The value seemed low compared with that in the literature reported by Londonkar and Kamble (2009) that the methanolic leaf extracts of *P. odoratissimus* had the highest percent inhibition on DPPH (87.52%). The difference in the results may be due to ethanol as an extracting solvent in the present study. The study by Londonkar and Kamble (2009) used methanol as

a solvent, and methanol is known to possess higher polarity than ethanol. There could be a possibility that more phenolic compounds were extracted in methanol since phenolic compounds are much polar. The amount of extracted polar compounds such as phenolic constituents could affect the RSA because polar compounds are known to exhibit antioxidative actions (Walag and Del Rosario, 2020).

Furthermore, the low level of free radical scavenging activity of POE extract could be due to the drying method employed in the study; some antioxidants may be exposed to further oxidation. Nevertheless, the POE had the potential capacity to scavenge free radicals because it inhibited more than 50% against DPPH as presented in Table 4. The present findings were corroborative to the findings of different vegetable amaranth species such as *A. tricolor* (Sarker and Oba, 2018e), salt-tolerant *A. tricolor* (Sarker *et al*., 2018c), salt-tolerant *Amaranthus* leafy vegetables (Sarker and Oba, 2018f) and vegetable amaranth (Sarker *et al*., 2017b) exhibiting strong antioxidant activity. The POE contained sufficient amounts of total tannin and total phenolics. The standards (GA and TA), employed to quantify the phenols and tannins in POE, were subjected to DPPH radical scavenging assay to confirm whether TTC and TPC of the POE could contribute to the antioxidant potency. The GA and TA exhibited likely results in the inhibition of DPPH radicals because their %DPPH RSAs were statistically comparable to the reference standard. The general findings emerged from the analysis that the phenols and tannins in POE might be significant contributors to the free scavenging activity in this study. The findings of the present investigation concurred with the results published by Londokar and Kamble (2009) and Jong and Chau (1998) that *P. odoratissimus* could inhibit free radicals, which could assert the therapeutic properties of the plant.

3.4 Antiproliferative Activity by S. cerevisiae Yeast Cell Model

The results of the percent cell viability of yeast proliferation of POE and TA at different concentrations are shown in Table 5. A graphical representation of percent cell viability against concentration is depicted in Figure 2. Based on the results, the POE and TA were good inhibitors of yeast cell growth, and the percent cell viability observed was dose-dependent with increasing concentration.

Table 5. Antiproliferative activity of POE and TA at different concentrations

Concentration (ppm)	Percent (%) cell viability		Percent (%) inhibition of cell viability	
	POE	TA	POE	TA
250	88.12^{b}±0.56	67.54^{c}±2.57	4.65±0.60	26.92±2.78
500	46.45^{d}±1.41	35.35^{e}±1.73	49.74±1.52	61.75±1.88
1000	22.14^{a}±1.00	12.71^{b}±0.30	76.04±1.08	86.25±0.33

Results are the mean of triplicate determinations ± SD; negative control (distilled water) percent cell viability = 92.42±0.68%; POE – *P. odoratissimus* ethanol extract; TA – tannic acid; for each row, means followed by common letters are not significantly different by the Tukey's HSD test ($p < 0.05$).

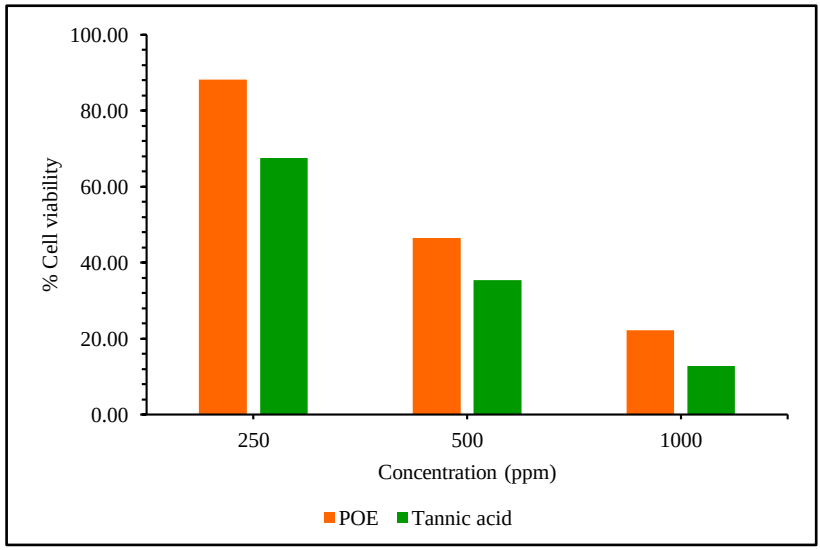
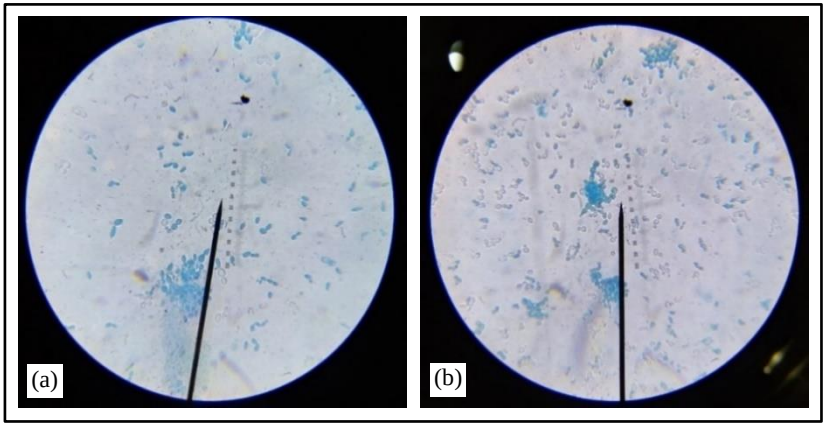


Figure 2. Comparison of the percent cell viability of yeast treated POE extract and tannic acid at different concentrations

The statistical results revealed that POE and TA at 250-1000 ppm significantly reduced ($p < 0.05$) the percent cell viability of yeast compared to the negative control. At 1000 ppm, POE and TA elicited the highest antiproliferative effects on the yeast cells. The yeast inhibition of POE significantly differed from TA at different concentrations. The results implied that the POE and TA possessed antiproliferative nature. Photomicrographs of yeast cells treated with POE and TA are shown in Figure 3.

TA and POE may inhibit DNA replication, possibly due to the topoisomerase II, a key enzyme in DNA replication (Chandrappa *et al.*, 2013). Topoisomerases are enzymes associated with DNA that break and then close

either one (Topo I) or two (Topo II) DNA strands during replication or transcription allowing these strands to pass to each other. Several compounds are known as "Topo II poisons" interfere with these processes by inhibiting the relegation of double-stranded DNA breaks and improving the formation of cleaved enzymatic complexes. Some polyphenols, including tannins, are Topo II poisons (Ferguson, 2001). Numerous anticancer compounds show inhibition of topoisomerase, and this property could, therefore, be used to detect and discover anticancer compounds (de Mejia *et al*., 2005).



Colorless and light blue yeast cells were viable and dead, respectively.

Figure 3. Photomicrographs of yeast cells treated with POE (a) and TA (b) at 1000 ppm

Considering the fair amounts of total phenolic and total tannins detected in POE, their presence could likely be attributed to the antiproliferative effect. Yáñez *et al*. (2004) found a correlation between the state of structural oxidation and the position, number and nature of the substituents of the polyphenols and their antiproliferative effects. GA, one of the compounds tested in the study of Yáñez *et al*. (2004), prevented cellular growth due to its hydroxylated aromatic structure. Furthermore, polyphenols can alter cell cycle-specific proteins that may impact the growth and proliferation of cancer cells. Cell cycle control points such as G1/S and G2/M are also significant targets for polyphenols (Araújo *et al*., 2011).

The present findings of the study agreed with Raj *et al*. (2014b) that *P. odoratissimus* could inhibit cell proliferation. The previous study showed that *P. odoratissimus* could significantly reduce calu-6-cells from proliferation without affecting the normal lung fibroblast cells and peripheral blood mononuclear cells. Similar effects on antiproliferative activity of POE in this

study were also documented using *Ficus benghalensis* (Raheel *et al*., 2017), *Matricaria chamomilla* (Hosseinpour *et al*., 2013), *Revia hypocrateriformis* (Saboo *et al*., 2012) and *Vitis vinifera* (Periyanayagam *et al*., 2013). The previous studies demonstrated that the tested plant species elicited potent antiproliferative activity against yeast cells in a dose-dependent manner. Also, the plant species were phytochemically rich in flavonoids, phenols, polyphenolic and triterpenoid constituents. Periyanayagam *et al*. (2013) pointed out that the antiproliferative activity of *V. vinifera* was due to its phenolic compounds and antioxidant capacity. This result is consistent with the present findings. A strong correlation (r = 0.9620) between percent inhibition of cell viability and percent DPPH radical scavenging activity (%DPPH RSA) of POE was established using Pearson-product moment correlation as presented in Table 6.

Table 6. Correlation between the %DPPH RSA and percent inhibition of cell viability in POE

	%DPPH RSA	%ICV
%DPPH RSA	1.00	
%ICV	0.962*	1.00

%DPPH RSA – percent DPPH radical scavenging activity; %ICV – percent inhibition of cell viability; *strong correlation

The result posited that the inhibitory effect on DPPH radicals strengthens the antiproliferative potential of *P. odoratissimus.* Oxidative cellular damage events are often associated with oxidative stress. The correlation of the antioxidant and the antiproliferative activities of POE could be beneficial for preventive or therapeutic purposes.

4. Conclusion and Recommendation

Significant outcomes of the present investigation revealed that POE extract elicited anticancer potential in terms of its phytochemical profile, DPPH scavenging activity and yeast growth inhibition capacity. The findings of the study supported the idea that the secondary plant metabolites (flavonoids, phenols, steroids and tannins) present in the POE might have a synergistic effect that influenced the free radical scavenging activity and the antiproliferative effect of the plant extract. In addition, the results

demonstrated that *P. odoratissimus* could be a potential source of antioxidants and antiproliferative action that could be useful for treatments of diseases and could be a candidate for plant-based drug development. Also, it could be a possible subject for further confirmatory testing. Further research and chemical analysis on the pure compounds and their impact on the DPPH inhibition and cell proliferation are needed to determine their mechanism of action.

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6. References

Adkar, P.P., & Bhaskar, V.H. (2014). *Pandanus odoratissimus* (Kewda): A review on ethnopharmacology, phytochemistry, and nutritional aspects. Advances in Pharmacological, 2014, 120895. https://doi.org/10.1155/2014/120895

Aguinaldo, A.M., Espeso, E.I., Guevarra, B.Q., & Nonato, M.G. (2005). Phytochemistry. In B.Q. Guevarra (Ed.), A guidebook to plant screening: Phytochemical and biological (pp. 23-60). Philippines: University of Santo Tomas.

Araújo, J.R., Gonçalves, P., & Martel, F. (2011). Chemopreventive effect of dietary polyphenols in colorectal cancer cell lines. Nutrition Research, 31, 77-87. https://doi.org/10.1016/j.nutres.2011.01.006

Aung, T.N., Qu, Z., Kortschak, R.D., & Adelson, D.L. (2017). Understanding the effectiveness of natural compound mixtures in cancer through their molecular mode of action. International Journal of Molecular Sciences, 18(656), 1-20. https://doi.org/10.3390/ijms18030656

Baang, R.P., Del Rosario, R.M., & Palmes, N. D. (2015). Phytochemical profiles and antioxidant activity of selected indigenous vegetables in Northern Mindanao, Philippines. International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering, 9(8), 769-774.

Badhani, B., Sharma, N., & Kakkar, R. (2015). Gallic acid: A versatile antioxidant with promising therapeutic and industrial applications. RSC Advances, 1(1), 1-54. https://doi.org/10.1039/C5RA01911G

Boran, R., & Ugur, A. (2016). The mutagenic, antimutagenic, and antioxidant properties of *Hypericum lydium*. Pharmaceutical Biology, 55(1), 402-405. https://doi.org/10.1080/13880209.2016.12422146

Borek, C. (2004). Dietary antioxidants and human cancer. Integrative Cancer Therapies, 3(4), 333-341. https://doi.org/10.1177/1534735404270578

Chandrappa, C., Govindappa, M., & Anil Kumar, N. (2013). In vitro antimitotic, antiproliferative, and DNA fragmentation assay of ethanol extract of *Carmona retusa* (Vahl.) Masam. Applied Cell Biology, 2(2), 52-57.

de Mejia, E.G., Song, Y., Ramirez-Mares, M., & Kobayashi, H. (2005). Effect of yerba mate (*Ilex paraguariensis*) tea on topoisomerase inhibition and oral carcinoma cell proliferation. Journal of Agricultural and Food Chemistry, 53(6), 1966-1973. https://doi.org/10.1021/jf048158g

Do, Q., Angkawijaya, A., Tran-Nguyen, P., Huynh, L., Soetaredjo, F., Ismadji, S., & Ju, Y.-H. (2013). Effect of extraction solvent on total phenol content, total flavonoids content, and antioxidant activity of *Limnophila aromatica*. Journal of Food and Drug Analysis. https://doi.org/10.1016/j.jfda.2013.11.001

Ferguson, L.R. (2001). Role of plant polyphenols in genomic stability. Mutation Research, 475(1-2), 89-111. https://doi.org/10.1016/s0027-5107(01)00073-2

Ferreyra, M.F., Rius, S.P., & Casati, P. (2012). Flavonoids: Biosynthesis, biological functions, and biotechnological applications. Frontiers in Plant Science, 3, 1-15. https://doi.org/10.3389/fpls.00222

Formagio, A.N., Volobuff, C.F., Santiago, M., Cardoso, C.L., Vieira, M.D., & Pereira, Z.V. (2014). Evaluation of antioxidant activity, total flavonoids, tannins and phenolic compounds in *Psychotria* leaf extracts. Antioxidants, 3(4), 745757. https://doi.org/10.3390/antiox3040745

Giner-Chavez, B.I., Van Soest, P.J., Robertson, J.B., Lascano, C., Reed, J.D., & Pell, A.N. (1997). A method for isolating condensed tannins from crude plant extracts with trivalent ytterbium. Journal of the Science of Food and Agriculture, 74, 359-368. https://doi.org/10.1002/(SICI)1097-0010(199707)74:3<359

Gowri, S., & Chinnaswamy, P. (2011). Evaluation of in vitro antimutagenic activity of *Caralluma adscendens* Roxb. in bacterial reverse mutation assay. Journal of Natural Product and Plant Resource, 1(4), 27-34.

Gulcin, I., Huyut, Z., Elmastas, M., & Aboul-Enein, H.Y. (2010). Radical scavenging and antioxidant activity of tannic acid. Arabian Journal of Chemistry, 3, 43-53. https://doi.org/10.1016/j.arabjc.2009.12.008

Hosseinpour, M., Mobini-Dehkordi, M., Saffar, B., & Teimori, H. (2013). Antiproliferative effects of *Matricaria chamomilla* on *Saccharomyces cerevisiae*. Journal of HerbMed Pharmacology, 2(2), 49-51.

Huang, D.-J., Lin, C.-D., Chen, H.-J., & Lin, Y.-H. (2004). Antioxidant and antiproliferative activities of sweet potato (*Ipomoea batatas* [L.] Lam 'Tainong 57') constituents. Botanical Bulletin-Academia Sinica, 45(3), 179-186.

Iqbal, J., Abbasi, B.A., Mahmood, T., & Kanwal, S. (2017). Plant-derived anticancer drugs: A green anticancer approach. Asia Pacific Journal of Tropical Biomedicine, 1-23. https://doi.org/10.1016/j.apjtb.2017.10.016

Jong, T.-T., & Chau, S.-W. (1998). Antioxidative activities of constituents isolated from *Pandanus odoratissimus*. Phytochemistry, 49(7), 2145-2148.

Kahkonen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.-P., Pihlaja, K., Kujala, T.S., & Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agricultural and Food Chemistry, 47(10), 3954-3962. https://doi.org/10.1021/jf990146

Keles, D., Ozgen, S., Saracoglu, O., Ata, A., & Ozgen, M. (2016). Antioxidant potential of Turkish pepper (*Capsicum annuum* L.) genotypes at two different maturity stages. Turkish Journal of Agriculture and Forestry, 40, 542-551. https://doi.org/10.3906/tar-1601-24

Kumar, D., Kumar, S., Kumar, S., Singh, J., Sharma, C., & Aneja, K.R. (2010). Antimicrobial and preliminary phytochemical screening of crude leaf extract of *Pandanus odoratissimus* L. Pharmacology Online, 2, 600-610.

Londonkar, R., & Kamble, A. (2009). Evaluation of free radical scavenging activity of *Pandanus odoratissimus*. International Journal of Pharmacology, 5(6), 377-380. https://doi.org/10.3923/ijp.2009.377.380

Maisetta, G., Batoni, G., Caboni, P., Esin, S., Rinaldi, A.C., & Zucca, P. (2019). Tannin profile, antioxidant properties, and antimicrobial activity of extracts from two Mediterranean species of parasitic plant *Cytinus*. BMC Complementary and Alternative Medicine, 19, 82. https://doi.org/10.1186/s12906-019-2487-7

Nonato, M.C., Garson, M.J., Truscott, R.W., & Carver, J.A. (1993). Structural characterization of piperidine alkaloids from *Pandanus amaryllifolius* by inverse-detected 2D NMR techniques. Phytochemistry, 34(4), 1159-1163.

Padmaharish, V., & Lakshmi, T. (2017). Anticancer activities of medicinal plants – An update. Journal of Pharmaceutical Sciences and Research, 9(4), 432-440.

Pandey, A., & Tripathi, S. (2013). Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. Journal of Pharmacognosy and Phytochemistry, 2(5), 115-119.

Periyanayagam, K., Kasirajan, B., Karthikeyan, V., Indumathi, R., & Kumuda, T. (2013). *Vitis vinifera* L (Vitaceae) leaves towards antimitotic and antiproliferative activity in anticancer drug discovery. Innovare Journal of Health Science, 1(3), 32-35.

Pontillas, P., & Lapetaje, J.E. (2019). Antioxidant capacity, antibacterial activity, and toxicity of the methanol and hexane leave extracts of Damong Bulang (*Dactyloctenium aegyptium*), Dalupang (*Urena lobata*), and Mayana (*Plectranthus scutellarioides*) (Thesis). University of Science and Technology of Southern Philippines, Cagayan de Oro City, Misamis Oriental, Philippines.

Rafiee, S., Farhoosh, R., & Sharif, A. (2018). Antioxidant Activity of gallic acid as affected by an extra carboxyl group than Pyrogallol in various oxidative environments. European Journal of Lipid Science and Technology, 120(11), 1800319. https://doi.org/10.1002/ejlt.201800319

Rahayu, S.E., Handayan, S., Jalip, I.S., & Noverita. (2013). Antifungal and preliminary phytochemical screening of leaf extracts of *Pandanus odoratissimus* L. Proceedings of the 4th International Conference on International Green Technology, Malang, Indonesia, 101-105.

Raheel, S., Saddique, Z., Iram, M., & Afzal, S. (2017). In vitro antimitotic, antiproliferative and antioxidant activity of stem bark extracts of *Ficus beghalensis* L. South African Journal of Botany, 111, 248-257. https://doi.org/10.1016/j.sajb.2017.03.037

Raj, G.G., Varghese, H., Kotagiri, S., Swamy, B.V., Swamy, A., & Pathan, R.K. (2014a). Anticancer studies of aqueous extract of roots and leaves of *Pandanus odoratissimus* f. ferreus (Y. Kimura) Hatus: An in vitro approach. Journal of Traditional and Complementary Medicine, 4(4), 279-284. https://doi.org/10.4103/2225-4110.129199

Raj, G.G., Varghese, H., Kotagiri, S., & Swamy, V. (2014b). Evaluation of the anticancer potential of aqueous extract of *Pandanus odoratissimus* (Y. Kimura) Hatus. forma ferreus, by in vivo ascitic tumor model in Swiss albino mice. Pharmacology Journal, 6(1), 57-62. https://doi.org/10.5530/pj.2014.1.9

Ramos, S. (2008). Cancer chemoprevention and chemotherapy: Dietary polyphenols and signaling pathways. Molecular Nutrition & Food Research, 52, 507-526. https://doi.org/10.1002/mnfr.200700326

Rayan, A., Raiyn, J., & Falah, M. (2017). Nature is the best source of anticancer drugs: Indexing natural products for their anticancer bioactivity. PLoS One, 12(11), 1-12. https://doi.org/10.1371/journal.pone.0187925

Robertson, S. (2014). What are flavonoids? Retrieved from http://www.news-medical.net/health/What-are-Flavonoids.aspx

Saboo, S., Khadabadi, S., & Tapadiya, G.G. (2012). In vitro evaluation of antimitotic, antiproliferative, dna fragmentation and anticancer activity of chloroform and ethanol extracts of *Revia hypocrateriformis*. Asian Pacific Journal of Tropical Disease, 2, S503-S508. https://doi.org/10.1016/S2222-1808(12)60211-4

Sarker, U., Islam, M., Rabbani, M., & Oba, S. (2014). Genotypic variability for nutrient, antioxidant, yield, and yield contributing traits in vegetable amaranth. Journal of Food, Agriculture and Environment, 12(3&4), 168-174. https://doi.org/10.1234/4.2014.5378

Sarker, U., Islam, M., Rabbani, M., & Oba, S. (2015a). Variability, heritability, and genetic association in vegetable amaranth (*Amaranthus tricolor* L.). Spanish Journal of Agricultural Research, 13(2). https://doi.org/10.5424/sjar/2015132-6843

Sarker, U., Islam, T., Rabbani, G., & Oba, S. (2015b). Genotype variability in composition of antioxidant vitamins and minerals in vegetable amaranth. Genetika, 47(1), 85-96. https://doi.org/10.2298/GENSR1501085S

Sarker, U., Islam, M., Rabbani, M., & Oba, S. (2017a). Genotypic diversity in vegetable amaranth for antioxidant, nutrient and agronomic traits. Indian Journal of Genetics and Plant Breeding, 77(1), 173-176. https://doi.org/10.5958/0975-6906.2017.00025.6

Sarker, U., Islam, M., Rabbani, M., & Oba, S. (2017b). Phenotypic divergence in vegetable amaranth for total antioxidant capacity, antioxidant profile, dietary fiber, nutritional and agronomic traits. Acta Agriculturae Scandinavica Section B-Soil & Plant Science, 68(1), 67-76. https://doi.org/10.1080/09064710.2017.1367029

Sarker, U., Islam, M., Rabbani, M., & Oba, S. (2018b). Variability in total antioxidant capacity, antioxidant leaf pigments and foliage yield of vegetable amaranth. Journal of Integrative Agriculture, 17(5), 1145-1153. https://doi.org/10.1016/S2095-3119(17)61778-7

Sarker, U., Islam, M., & Oba, S. (2018c). Salinity stress accelerates nutrients, dietary fiber, minerals, phytochemicals, and antioxidant activity in *Amaranthus tricolor* leaves. PLoS ONE, 13(11), e0206388. https://doi.org/10.1371/journal.pone.0206388

Sarker, U., Islam, M., Rabbani, M., & Oba, S. (2018c). Antioxidant leaf pigments and variability in vegetable amaranth. Genetika, 50(1), 209-220. https://doi.org/10.2298/GENSR1801209S

Sarker, U., & Oba, S. (2018a). Catalase, superoxide dismutase and ascorbate-glutathione cycle enzymes confer drought tolerance of *Amaranthus tricolor*. Scientific Reports, 8, 16496. https://doi.org/10.1038/s41598-018-34944-0

Sarker, U., & Oba, S. (2018b). Drought stress effects on growth, ROS markers, compatible solutes, phenolics, flavonoids, and antioxidant activity in *Amaranthus tricolor*. Applied Biochemistry and Biotechnology, 186, 999-1016. https://doi.org/10.1007/s12010-018-2784-5

Sarker, U., & Oba, S. (2018c). Drought stress enhances nutritional and bioactive compounds, phenolic acids and antioxidant capacity of *Amaranthus* leafy vegetable. BMC Plant Biology, 18, 258. https://doi.org/10.1186/s12870-018-1484-1

Sarker, U., & Oba, S. (2018d). Response of nutrients, minerals, antioxidant leaf pigments, vitamins, polyphenol, flavonoid and antioxidant activity in selected vegetable amaranth under four soil water content. Food Chemistry, 252, 72-83. https://doi.org/10.1016/j.foodchem.2018.01.097

Sarker, U., & Oba, S. (2018e). Augmentation of leaf color parameters, pigments, vitamins, phenolic acids, flavonoids and antioxidant activity in selected *Amaranthus*

tricolor under salinity stress. Scientific Reports, 8, 12345. https://doi.org/10.1038/s41598-018-30897-6

Sarker, U., & Oba, S. (2018f). Salinity stress enhances color parameters, bioactive leaf pigments, vitamins, polyphenols, flavonoids and antioxidant activity in selected *Amaranthus* leafy vegetables. Journal of the Science of Food and Agriculture, 99, 2275-2284. https://doi.org/10.1002/jsfa.9423

Sarker, U., & Oba, S. (2019a). Nutraceuticals, antioxidant pigments, and phytochemicals in the leaves of *Amaranthus spinosus* and *Amaranthus viridis* weedy species. Scientific Reports, 9, 20413. https://doi.org/10.1038/s41598-019-50977-5

Sarker, U., & Oba, S. (2019b). Protein, dietary fiber, minerals, antioxidant pigments and phytochemicals, and antioxidant activity in selected red morph *Amaranthus* leafy vegetable. PLoS ONE, 14(12), e0222517. https://doi.org/10.1371/journal.pone.0222517

Sarker, U., Oba, S., & Daram, M. (2020a). Nutrients, minerals, antioxidant pigments and phytochemicals, and antioxidant capacity of the leaves of stem amaranth. Scientific Reports, 10, 3892. https://doi.org/10.1038/s41598-020-60252-7

Sarker, U., Hossain, M., Iqbal, M., & Oba, S. (2020b). Bioactive components and radical scavenging activity in selected advance lines of salt-tolerant vegetable amaranth. Frontiers in Nutrition, 7, 587257. https://doi.org/10.3389/fnut.2020.587257

Sarker, U., Hossain, M., & Oba, S. (2020c). Nutritional and antioxidant components and antioxidant capacity in green morph *Amaranthus* leafy vegetable. Scientific Reports, 10, 1336. https://doi.org/10.1038/s41598-020-57687-3

Sarker, U., & Oba, S. (2020a). The response of salinity stress-induced *A. tricolor* to growth, anatomy, physiology, non-enzymatic and enzymatic antioxidants. Frontiers in Plant Science, 11, 1354. https://doi.org/10.3389/fpls.2020.559876

Sarker, U., & Oba, S. (2020b). Leaf pigmentation, its profiles and radical scavenging activity in selected *Amaranthus tricolor* leafy vegetables. Scientific Reports, 10, 18617. https://doi.org/10.1038/s41598-020-66376-0

Sarker, U., & Oba, S. (2020c). Nutraceuticals, phytochemicals, and radical quenching ability of selected drought-tolerant advance lines of vegetable amaranth. BMC Plant Biology, 20, 564. https://doi.org/10.1186/s12870-020-02780-y

Sarker, U., & Oba, S. (2020d). Phenolic profiles and antioxidant activities in selected drought-tolerant leafy vegetable amaranth. Scientific Reports, 10, 18287. https://doi.org/10.1038/s41598-020-71727-y

Sarker, U., & Oba, S. (2020e). Nutrients, minerals, pigments, phytochemicals, and radical scavenging activity in *Amaranthus blitum* leafy vegetables. Scientific Reports, 10, 3868. https://doi.org/10.1038/s41598-020-59848-w

Sarker, U., & Oba, S. (2020f). Nutritional and bioactive constituents and scavenging capacity of radicals in *Amaranthus hypochondriacus*. Scientific Reports, 10, 19962. https://doi.org/10.1038/s41598-020-71714-3

Sarker, U., & Oba, S. (2020g). Polyphenol and flavonoid profiles and radical scavenging activity in leafy vegetable *Amaranthus gangeticus*. BMC Plant Biology, 20, 499. https://doi.org/10.1186/s12870-020-02700-0

Sarker, U., & Oba, S. (2021). Color attributes, betacyanin, and carotenoid profiles, bioactive components, and radical quenching capacity in selected *Amaranthus gangeticus* leafy vegetables. Scientific Reports, 11, 11559. https://doi.org/10.1038/s41598-021-91157-8

Shrivastava, V., Sijoria, R., Dey, V.N., Pandey, N.K., Jadhav, A., & Wanjari, M. (2016). Antimitotic and antiproliferative activity of stem bark of *Oroxylum indicum*. Journal of Basic and Applied Research International, 13(3), 147-154.

Simon, J.A. (2001). Yeast as a model system for anticancer drug discovery. Emerging Therapeutic Targets, 5(2), 177-195.

Singh, S., Sharma, B., Kanwar, S.S., & Kumar, A. (2016). Lead phytochemicals for anticancer drug development. Frontiers in Plant Science, 7, 1667. https://doi.org/10.3389/fpls.2016.01667

Sultan, A. (2015). Steroids: A diverse class of secondary metabolites. Medicinal Chemistry, 5(7), 310-317. https://doi.org/10.4172/2161-0444.1000279

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and extraction: A review. Internationale Pharmaceutica Sciencia, 1(1), 98-105.

Tomar, P., Dey, Y.N., Sharma, D., Wanjari, M.M., Gaidhani, S., & Jadhav, A. (2018). Cytotoxic and antiproliferative activity of *Kanchanar guggulu*, an ayurvedic formulation. Journal of Integrative Medicine, 16(6), 411-417. https://doi.org/10.1016/j.joim.2018.10.001

Walag, A.P., & Del Rosario, R.M. (2020). Total flavonoids content, total phenolics content, and antioxidant activities of *Acanthaster planci* and *Linckia laevigata* collected from Carmen, Agusan del Norte, Philippines. Malaysian Journal of Biochemistry & Molecular Biology, 23, 77-85.

Widyawati, P., Budianta, T.W., Kusuma, F.A., & Wijaya, E. (2014). Difference of solvent polarity to phytochemical content and antioxidant activity of *Pluchea indicia* less leaves extracts. International Journal of Pharmacognosy and Phytochemical Research, 6(4), 850-855.

Wisman, K.N. (2008). Cytotoxicity and anti-inflammatory activity of polyphenolics in natural soils (Thesis). Miami University, Oxford, Ohio, United States.

Yáñez, J., Vicente, V., Alcaraz, M., Castillo, J., Benavente-García, O., Canteras, M., & Teruel, J.L. (2004). Cytotoxicity and antiproliferative activities of several phenolic compounds against three melanocytes cell lines: Relationship between structure and activity. Nutrition and Cancer, 49(2), 191-199. https://doi.org/10.1207/s15327914nc4902_11

Yoshida, Y., & Niki, E. (2003). Antioxidant effects of phytosterol and its components. Journal of Nutritional Science and Vitaminology, 49(4), 277-280. https://doi.org/10.3177/jnsv.49.277

Zahin, M., Aqil, F., & Ahmad, I. (2010). Broad spectrum antimutagenic activity of antioxidant active fraction of *Punica granatum* L. peel extracts. Mutation Research/ Genetic Toxicology and Environmental Mutagenesis, 703, 99-107. https://doi.org/10.1016/j.mrgentox.2010.08.001