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Phytochemical analysis, antioxidant and anticancer activities of durian (*Durio zibethinus* Murr.) fruit extract

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ABSTRACT: Durian (*Durio zibethinus* Murr.) is a seasonal tropical fruit of Southeast Asia. It is a good source of carbohydrates, proteins, fibres, vitamins B and C. The fruit extract was prepared using the mixture of Ethanol and 0.2 M HCl (1:1, v/v). Phytochemical, antioxidant and anticancer properties of durian were investigated in this study. Phytochemical analysis exhibited the highest total phenolics value of 116.55±1.51 mg GAE/g extract and the highest total flavonoid value of 92.37±9.27 mg RE/g extract. In antioxidant studies, ABTS and NO assays of durian fruit extracts showed a greater value in antioxidant activity against control. Whereas, the results derived from DPPH, FRAP and other assays showed lower antioxidant potential compared to the standard. Since durian fruit possess rich phenolic content, which is the principal factor of the antioxidant activity, it shows excellent antioxidant potentials against various assay methods. MTT assay was used for the cytotoxicity of durian extract against Chang Liver cell lines. The percentage of cytotoxicity increases with the higher concentrations of durian extract. The maximum cytotoxicity (74%) of fruit extract was recorded at 100 µg/ml of the fruit extract. It shows durian fruit extract to have an excellent anti-proliferative and anticancer activities against Chang liver cell lines. Hence durian fruit can be utilized as a potential source for rich bioactive compounds with nutraceutical and pharmaceutical applications.

KEYWORDS: Durian; *Durio zibethinus*; antioxidants; anticancer; phytochemical.

1. INTRODUCTION

Fruits and vegetables are an important part of our daily diet. In recent years, several studies have reported to contain large amounts of bioactive compounds in exotic tropical fruits. They are rich in essential micro and macro nutrients and contain high levels of essential minerals and vitamins such as A, C and E. Consumption of fresh fruits is essential as they have health-promoting bioactive compounds like anthocyanins, phenolics, flavonoids and vitamins [1-2].

Durian (Scientific name: *Durio zibethinus*, family: Bombacaceae) is a seasonal tropical and climacteric fruit of Southeast Asia. As the ripe durian fruit has its unique taste and aroma, it is considered as "King of fruits". The presence of natural bioactive compounds in durian fruit plays very important role in food, pharmaceutical and cosmetic industries. Several studies have reported on various chemical constituents of this fruit, which are meant for nutritional value and its specific smell. Durian is mainly used as the source of fruit, though it is used as timber. The most common edible part of the durian is the fleshy aril that surrounds each seed. The aril is a fleshy outer part of the funicular end of the seed coat [3-7].

Both pulp (aril) and rind of durian contain a wide variety of bioactive compounds. The importance of durian fruit as a nutraceutically-valued source can be interrelated to their bioactive compounds. Fresh durian pulp is rich in Carbohydrates, proteins, dietary fibres, saturated and unsaturated fatty acids [2, 8-12]. Durian has considerably higher level of polyphenols, flavonols, flavonoids, anthocyanins and caffeic acid [13-14]. These bioactive compounds have a great potential to be used as a therapeutic agent. They can be used to treat diabetes mellitus by regulating the secretion of insulin. In addition, they can be used to treat some cardiovascular diseases by reducing serum cholesterol in blood [2, 9, 15-16]. Durian is used as an antioxidant, anti-mutagenic, anti-carcinogenic, anti-inflammatory and antimicrobial agents in various medicines owing to its phenolic compounds [17-21]. Durian fruit has conventionally been used as a remedy for infertility, but there is no substantive scientific evidence for its use. It needs to be confirmed scientifically by isolating its various chemical compounds and establishing its fertility enhancing properties [22].

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The antioxidants present in durian fruit are able to decrease the level of oxidative stress in the body. These bioactive compounds have the great potential to scavenge free radicals. Several studies have reported that, phenolic compounds are highly responsible for the antioxidant activity of durian fruit extracts. The free-radical scavenging activity of the extracts of durian was evaluated by FRAP (Ferric reducing antioxidant power assay), CUPRAC (Cupric reducing antioxidant capacity), DPPH (1, 1-Diphenyl-2-picrylhydrazyl) and ABTS (2-2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assays. In these studies, phenols, flavonoids and flavonols showed a strong antioxidant activity [2, 12, 23-24]. Different durian cultivars having same maturity and ripening stage contain different concentrations of total polyphenol content and antioxidant capacities [10].

A very few reports are available on anticancer studies of durian fruit. In MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, mature durian fruit extract of 'Mon Thong' variety had anti-proliferative activity. The extract of durian was able to reduce the growth of Calu-6 and SNU-601 cell lines by 86.8% and 88.5%, respectively [10]. Moreover, durian extracts were reported to have an inhibitory effect on nitric oxide induced cell proliferative activity in the breast cancer cell line (MCF-7) [25].

In order to elucidate the previous reports on antioxidant and anticancer properties of durian fruit extract, the present study was designed to investigate the *in vitro* antioxidant and anticancer potentials of durian fruit extract. Total phenols and total flavonoid content were also estimated by standard analytical procedures. A combination of ABTS, DPPH, FRAP, NO (Nitric oxide scavenging assay), Superoxide assay, Phosphomolybdenum assay and metal chelating assays were used to determine the antioxidant activities of a wide variety of compounds, which are present in durian fruit extract. *In vitro* cytotoxicity of durian fruit extract was studied using MTT assay on Chang liver cell lines.

2. RESULTS

2.1. Phytochemical analysis

Durian fruit extract was tested for total phenolic content and total flavonoid Content using standard analytical methods. In the test conducted on total phenolics, the highest total phenolics value was found to be 116.55±1.51 mg Gallic acid equivalent (GAE)/g extract). In the test performed on total flavonoids, the highest total Flavonoids value was 92.37±9.27 mg Rutin equivalent (RE)/g extract).

2.2. Antioxidant activities

Antioxidant activities like ABTS, DPPH, ferric reducing antioxidant power assay, nitric oxide scavenging activity, superoxide radical-scavenging activity, phosphomolybdenum assay and metal chelating activities on different concentrations of durian fruit extracts were evaluated in this study.

In ABTS radical scavenging Activity, among different concentrations (200, 400, 600, 800 and 1000 µg/ml) of durian fruit extracts tested, the highest antioxidant activity was recorded (94.18±3.38) at 1000 µg/ml of the sample concentration. The antioxidant activity of the extracts increased with higher concentrations of the extract. Durian fruit extract showed significantly higher antioxidant activity than that of standard, Ascorbic acid (Figure 1). In DPPH radical scavenging activity, the maximum radical scavenging activity was found (49.11±2.55) at 1000 µg/ml of the sample concentration. Durian fruit extract showed lower antioxidant potential than ascorbic acid (Figure 2).

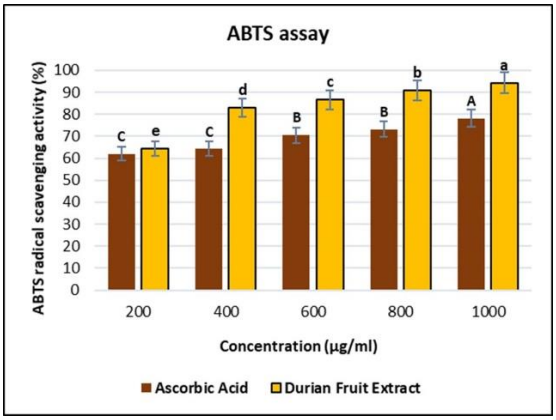

Figure 1. ABTS radical scavenging activity of different concentrations of the fruit extract of durian. Values are expressed as the mean ± standard deviation (n = 3). Samples with different letters are significantly different ($p < 0.05$).

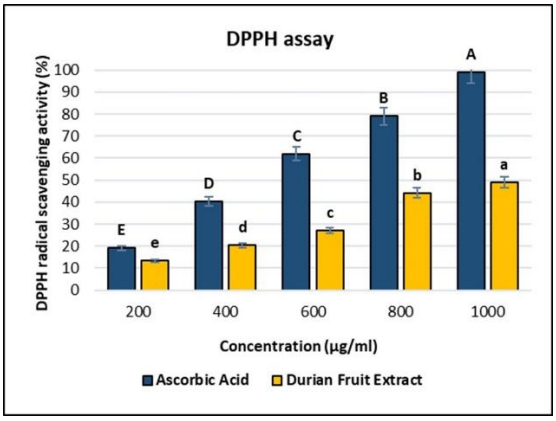

Figure 2. DPPH radical scavenging activity of different concentrations of the fruit extract of durian. Values are expressed as the mean ± standard deviation (n = 3). Samples with different letters are significantly different ($p < 0.05$).

In FRAP assay, the highest antioxidant activity was recorded (494.1 ±18.4) at 1000 µg/ml of the sample concentration. Durian fruit extract showed lower antioxidant potential than ascorbic acid (Figure 3). Nitric oxide scavenging activity exhibited the highest antioxidant value (72.02±4.21) at 100 µg/ml of the sample concentration. Durian fruit extract showed significantly higher nitric oxide scavenging activity when compared to the standard, Gallic acid. (Figure 4). In superoxide radical-scavenging activity, the maximum scavenging ability of durian fruit extract was recorded (64.03±3.37) at 250 µg/ml of the extract. In all concentrations of the sample, durian fruit extract exhibited lower scavenging activity than BHT (Figure 5).

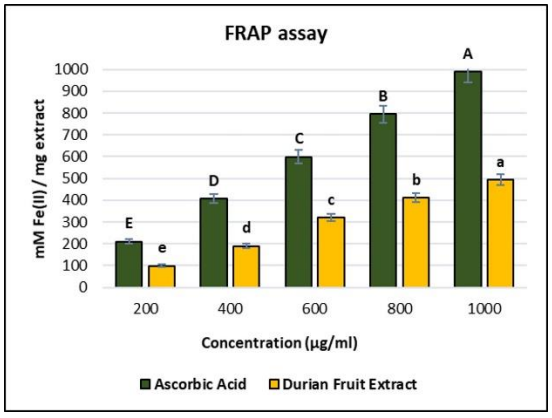

Figure 3. Ferric reducing antioxidant power assay of different concentrations of the fruit extract of durian. Values are expressed as the mean ± standard deviation (n = 3). Samples with different letters are significantly different ($p < 0.05$).

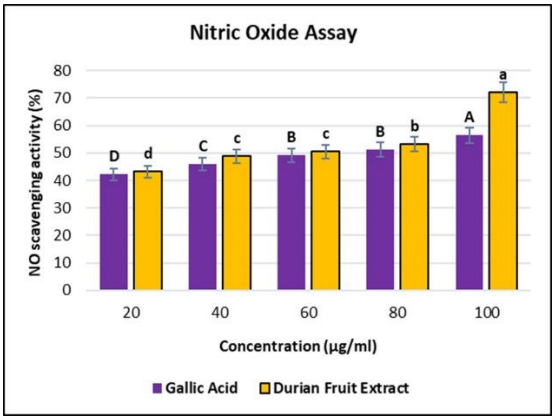

Figure 4. Nitric oxide scavenging activity of different concentrations of the fruit extract of durian. Values are expressed as the mean ± standard deviation (n = 3). Samples with different letters are significantly different ($p < 0.05$).

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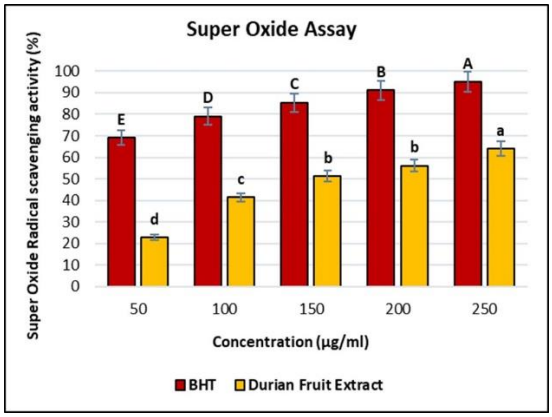

Figure 5. Super oxide radical scavenging activity of different concentrations of the fruit extracts of durian. Values are expressed as the mean ± standard deviation (n = 3). Samples with different letters are significantly different ($p< 0.05$).

The Phosphomolybdenum assay was used to determine the ability of durian fruit extract to reduce Mo(VI) to Mo(V) and subsequent formation of green phosphate/Mo(V) complex at an acid pH. The results were expressed as mg of ascorbic acid equivalents/g. The fruit extract of durian showed Phosphomolybdenum activity of 166.66±5.35 mg AAE/g, which was lower than that of the standard BHT (337.93±4.19 mg AAE/g) (Figure 6). In metal chelating activity, the decolorization of red colour of the reaction mixture depends on the reduction of ferrous ions by fruit extract. The results were expressed as mg EDTA equivalents/g. The fruit extract of durian showed the metal chelating activity value of 72.22±3.67 mg EDTA/g extract. Metal chelating activity of durian fruit extract was lower than that of BHT (262.88±3.09 mg EDTA/g extract (Figure 7).

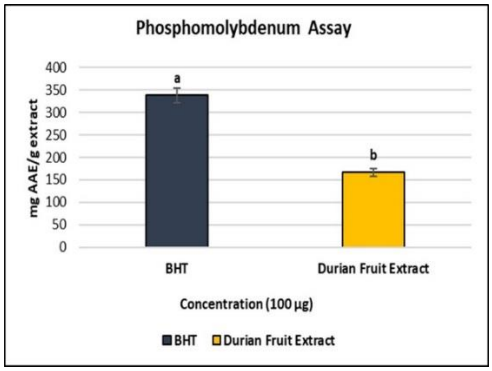

Figure 6. Phosphomolybdenum assay of the fruit extract of durian. Values are expressed as the mean ± standard deviation (n = 3). Samples with different letters are significantly different ($p< 0.05$).

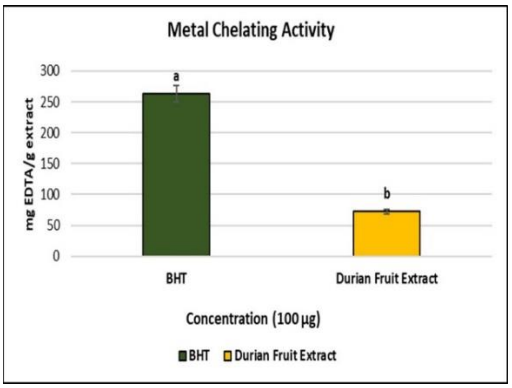

Figure 7. Metal chelating activity of the fruit extract of durian. Values are expressed as the mean ± standard deviation (n = 3). Samples with different letters are significantly different ($p< 0.05$).

2.3. Anticancer activity (*In Vitro* Cytotoxicity - MTT Assay)

In vitro cytotoxicity of durian fruit extract was evaluated at different concentrations (0, 25, 50, 75 and 100 µg/ml) against Chang liver cells. The cytotoxicity of Durian fruit extract was dose dependent. The maximum cytotoxicity (74%) was found at 100 µg/ml and the moderate cytotoxicity (66%, 58 %) at 75 µg/ml and 50 µg/ml respectively. The minimum cytotoxicity (56 %) was recorded at 25 µg/ml of the durian fruit extract. Control did not exhibit cytotoxic activity as expected. The fruit extract of durian showed Moderate to severe cytotoxic reactivity to Chang liver cell lines after 24hr treatment (Figure 8).

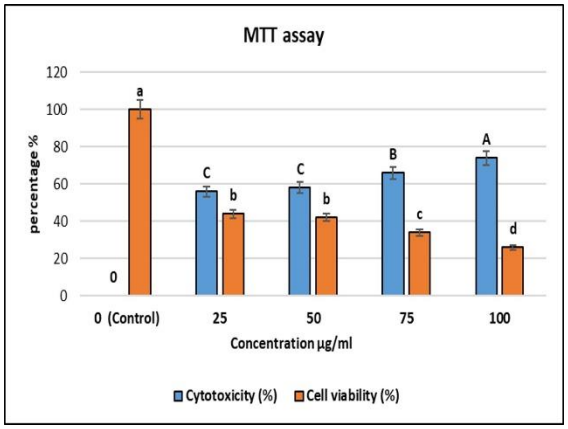

Figure 8. MTT assay for anticancer activity of different concentrations of the fruit extract of durian against Chang liver cell line. Values are expressed as the mean ± standard deviation (n = 3). Samples with different letters are significantly different ($p < 0.05$).

3. DISCUSSION

Previous investigations reported that, the bioactive compounds like polyphenols, flavonoids, flavonols, tannins, anthocyanins, caffeic acid, vanillic acid, quercetin, hesperidin, p-coumaric acid, myricitin, apigenin and campherol were present in many varieties of durian fruit. Whereas the tannin content was very low in durian [23]. Phenolic acids (caffeic acid, p-coumaric acid, sinnamic acid, vanillic acids) and flavonoids (quercetin, morin, myricitin, campherol, apigenin) containing higher antioxidant activity were found in durian [26]. Durian fruit extract was found to possess a total phenolic content of 79.15 mg GAE/100 g, in which myricitin, campherol, and cinnamic acid were the major compounds [27]. The total polyphenols of durian rind was reported to have 33.77 mg GAE/g [28]. While, in the seed extracts of durian, total polyphenols was 3.67 mg GAE/g [29]. In this present study, phytochemical analysis on durian fruit extract showed the highest total phenolics value of 116.55±1.51 mg GAE/g extract and the highest total flavonoid value of 92.37±9.27 mg RE/g extract.

Durian is used as antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory and antimicrobial agents in various medicines owing to its phenolic compounds. Phenolic compounds are highly responsible for the antioxidant activity in exotic fruits [17]. The free-radical scavenging activity of phenolic extracts of durian showed a strong antioxidant activity for FRAP, CUPRAC and ABTS [14]. Polyphenols have a strong endothelium-dependent vasodilator activity and inhibits nitric oxide degeneration. Therefore it is used as antioxidants. In exotic fruits like durian, flavonoids and flavonols are responsible for their higher antioxidant activity [17, 30]. Durian fruit extracts exhibited the potential to scavenge the ABTS+ radical cation and the free radical DPPH [28].

Polyphenol content in the ripe durian fruit was much higher than that of unripe and overripe fruit [4,23]. Durian fruit at different stages of ripening was investigated and found that, a fruit diet is an additive to common foods for the prevention of cardiovascular and other diseases [31]. Ripe durian extracts showed higher antioxidant Capacity in FRAP and CUPRAC assays, when compared to overripe durian fruit extracts [32]. A wide variety of durian cultivars having same maturity and ripening stage were reported to have different concentrations of total polyphenol content and antioxidant capacities [14]. Furthermore, some compounds such as quercetin, caffeic acid and apigenin were detected only on ripening or over-ripened stages, while some of them (hesperidin, p-coumaric acid, and quercetin) were not found at the same stage [24]. Caffeic

acid and quercetin are the principal anti-oxidants present in durian fruit [33]. Quercetin shows a vast range of pharmacological properties. It is the richest and most active flavonoid in human diet [4].

As stated earlier, durian possess rich phenolic content in their fruits, which is the principal factor of the antioxidant activity, they exhibit excellent antioxidant activities against various assay methods. In this study, ABTS and NO assays of the fruit extracts of durian showed significantly higher value in antioxidant activity against control. Whereas, in the results derived from DPPH, FRAP, Super oxide assay, phosphomolebdnum assay and metal chelating assay, the antioxidant potential was lower when compared to the standard. The crude extracts are made of a mixture of several scavenging compounds which could act in a synergetic manner to enhance the antioxidant activity. Moreover, the antioxidant activity depends on the availability and the ability of these extracts to give hydrogen or electron atom [34, 35].

In vitro investigations reported on durian fruit extract to have active anti-proliferative and anticancer potentials against cancer lines. In MTT assay, mature durian fruit extract of 'Mon Thong' variety had anti-proliferative activity against Calu-6 and SNU-601 cell lines by 86.8% and 88.5%, respectively [10]. Moreover, durian fruit extracts was found to have inhibitory effect on nitric oxide induced cell proliferative activity in the breast cancer cell line (MCF-7) [25]. Mature durian sample exhibited the highest antiproliferative activity. This is due to the presence of high antioxidant activity as well as the amount of flavonoids and other bioactive compounds. The results on cell proliferation can be explained as a complex effect of flavonoids, flavonols and ascorbic acid in mature durian [36].

In our study, the percentage of cytotoxicity increased with the higher concentrations of durian extract against Chang liver cell line. It shows durian fruit extract to have an excellent anti-proliferative and anticancer activities against Chang liver cell line. Since we have used one cancer cell line for cytotoxicity, it is recommended that further studies are required to confirm cytotoxicity of durian fruit extract using more cell lines. Durian can be utilized as a potential source of high value bioactive compounds with nutraceutical and functional food additive applications.

4. CONCLUSION

Durian fruit has rich nutraceutical values. Since many bioactive compounds are present in durian, the edible parts of durian fruit (pulp) and its residues (rind and seed) are very much useful for food, pharmaceutical and cosmetic applications. Durian fruit is rich in dietary fibres, carbohydrates, vitamins, amino acids and minerals. So it can be used as a food additive in our daily food consumption. Durian fruit possess rich phenolic content, which is the principal factor of the antioxidant activity. İt exhibited excellent antioxidant potentials against various assay methods and anticancer activities against chang liver cell lines. The presence of antioxidant and anticancer potentials of durian can effectively be utilized to replace synthetic chemicals, which are the components of drugs to treat many diseases. Regular intake of durian fruit helps easy digestion and prevents cardiovascular diseases, blood pressure, insomnia, anemia, sexual dysfunction, cancer and ınfertility diseases. It is also used as an antiaging factor. Hence durian fruit can be utilized as a potential source of bioactive compounds with nutraceutical and pharmaceutical applications.

5. MATERIALS AND METHODS

5.1. Materials

All the chemicals and reagents used in this study were of analytical grade. The fresh fruits of durian (*Durio zibethinus* Murr.) were collected in the month of June from the State Horticulture farm, Burliar, which is located near Mettupalayam in Coimbatore district, Tamilnadu, India. The fruit was identified and authenticated (Voucher specimen No. CBE/GAC/021/16) by the kind assistance of botanists from the PG and Research Department of Botany, Government Arts College, Coimbatore, Tamilnadu.

5.2. Preparation of durian fruit extract

The Durian fruits were made free from mud and other impurities and dried in shade. The edible parts (aril) were weighed (750 gm) and chopped into small pieces without using steel knives. The chopped pulp was dried in shade and was then powdered. The samples were ground in order to pass through a 0.5-mm sieve and stored at -20^0 C for further analysis. Powdered durian samples (1 g) were placed in a small vial and 15 ml of a binary mixture composed of Ethanol and 0.2 M HCl (1:1, v/v) was added. The samples were kept in Super sonication bath for 40 min at 40^0 C. The extracts were separated from the solid matter by filtration, condensed to 5 ml, and analyzed for the contents of bioactive compounds.

5.3. Phytochemical analysis

5.3.1. Total phenolic content

The total phenolic content of the extract of durian pulp was determined as per the method described by Makkar (2003) [37]. An aliquot of 100 µl (1 mg/1 ml) of the extract was taken in a test tube and made up to a volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%; w/v) were added sequentially in each tube. Immediately after vortexing the reaction mixture, the tubes were placed in the dark for 40 min. The absorbance was recorded at 725 nm against the reagent blank. The amount of total phenolics was calculated as pyrogallol equivalents from the calibration curve.

5.3.2. Total flavonoid content

The total flavonoid content of durian pulp extract was determined by using the method described previously by Zhishen *et al* (1999) [38]. An aliquot of 100 µl (1 mg/1 ml) extract was taken in a test tube and made up to a volume of 1 ml with distilled water and followed by 150 µl of 5 % $NaNO_2$ solution. After 5 min, 150 µl of 10 % $AlCl_3$ solution was added and allowed to stand for 6 min. Then 2 ml of 4% NaOH solution was added to the mixture. Immediately distilled water was added to make the final volume of 5 ml and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm against water blank. Rutin was used as a standard compound for the quantification of total flavonoid. All the values were expressed as gram of rutin equivalent per 100 g of extract.

5.4. Antioxidant activities

5.4.1. ABTS radical scavenging activity

ABTS assay was performed according to the method of Re *et al* (1999) [39]. ABTS stock solution was prepared by mixing 7mM ABTS stock solution with 2.45 mM potassium persulfate (1/1, v/v) and allowing the mixture to stand in the dark at room temperature for 12–16 h until the reaction was complete and the absorbance was stable. The ABTS solution was diluted with ethanol (about 1:89 v/v) to an absorbance of 0.700 ± 0.05 at 734nm. The photometric assay was conducted by adding 0.9ml of ABTS solution to different concentrations (200, 400, 600, 800 and 1000 µg/ml) of durian pulp extract and incubated for 15 min, measurement was taken immediately at 734 nm. The antioxidant activity of the tested samples were calculated by determining the decrease in absorbance at different concentrations by using the following equation:

$$\text{ABTS Radical Scavenging Activity (\%)} = ((Ac-At)/Ac) \times 100 \quad \text{[Eq. 1]}$$

Where, At and Ac are the respective absorbance of tested samples and ABTS was expressed as µmol.

5.4.2. DPPH radical scavenging activity

The antioxidant activity of durian pulp extract was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH, according to the method of Alessandra Braca *et al* (2001)[40]. Durian pulp extracts were taken in various concentrations (200, 400, 600, 800 and 1000 µg/ml) and the volume was adjusted to 100 µl with methanol. About 3 ml of a 0.1 mM methanolic solution of DPPH was added to the aliquots of samples and mixed well. Negative control was prepared by adding 100 µl of methanol in 3 mL of 0.1 mM methanolic solution of DPPH. Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration. The tubes were allowed to stand in dark for 30 minutes at room temperature. The absorbance of the sample was measured at 517 nm against the blank. The radical scavenging activity was calculated as follows:

$$\text{DPPH radical scavenging activity (\%)} = (\text{Control OD - Sample OD/ Control OD}) \times 100 \quad \text{[Eq. 2]}$$

5.4.3. FRAP (Ferric reducing antioxidant power) assay

FRAP assay was performed by the method prescribed by Benzie and Strain (1996) [41]. FRAP assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of 2,4,6-tri (2-pyridyl)- s-triazine (TPTZ), forming an intense blue Fe^{2+} -TPTZ complex with an absorption maximum at 593 nm. Different

concentrations of the durian pulp extract (200, 400, 600, 800 and 1000 µg/ml) and standard were added to 2.7 ml of FRAP reagent (10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10.0 mM TPTZ solution and 1 part of 20.0 mM $FeCl_3.6H_2O$ solution) and the reaction mixture was incubated at 37°C for 30 min and the increase in absorbance at 593 nm is measured. The antioxidant capacity based on the ability to reduce ferric ions of sample was calculated from the linear calibration curve and expressed as mmol.

5.4.4. Nitric oxide scavenging activity

The nitric oxide scavenging activity of durian pulp extract was evaluated according to the method of Sreejayan and Rao (1997) [42]. 3 ml of 10 mM Sodium nitroprusside in 0.2 M phosphate buffered saline (pH 7.4) was mixed with different concentrations (20, 40, 60, 80 and 100 µg/ml) of durian pulp extract and incubated at room temperature for 150 min. After incubation time, 0.5 ml of Griess reagent (1% sulfanilamide, 0.1% naphthyl ethylene diamine dihydrochloride in 2% H_3PO_4) was added. The absorbance of the chromophore formed was read at 546nm. Standard and the same mixture of the reaction without sample extracts were employed as positive and negative control. Percentage radical scavenging activity of the sample was calculated as follows:

NO scavenging activity (%) = (Control OD - Sample OD/ Control OD) x 100 [Eq. 3]

5.4.5. Superoxide radical scavenging activity

Superoxide radical activity was estimated by the method of Beauchamp and Fridovich (1971) [43]. Different concentrations (50, 100, 150, 200 and 250 µg/ml) of durian pulp extract and standards (BHT) were taken in triplicates. 3 ml of reaction mixture containing 50 mM sodium phosphate buffer (pH 7.6), 20 µg of riboflavin, 12 Mm of EDTA and 0.1 mg of NBT were added to the extracts in test tubes. The reaction was initiated by illuminating the reaction mixture with samples for 90 seconds. The illuminated reaction mixture without sample was used as the negative control. The unilluminated reaction mixture without plant sample was used as the blank. Immediately after illumination, the absorbance was measured at 590 nm against the blank. The scavenging activity (%) of superoxide anion generation was calculated using the following equation:

Superoxide radical scavenging activity (%) = ((Control OD-Sample OD)/ Control OD) X 100 [Eq. 4]

5.4.6. Phosphomolybdenum assay

Phosphomolybdenum assay was performed according to the method described by Prieto *et al* (1999) [44]. In this assay, Mo (IV) is reduced to Mo (V) and forms a green Phosphomolybdenum complex. The reagent was prepared by adding 0.6 M sulphuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate with sodium phosphate and ammonium molybdate in the desired volume of 0.6 M H_2SO_4. A standardized concentration of 100 µl (1 mg/1 ml) of durian pulp extract was prepared in triplicate in test tubes. 1 ml of the reagent solution was added. A reaction mixture without sample or standard was used as blank. The reaction mixture was Incubated in a water bath at 95° C for 90 minutes and then cooled at room temperature. The absorbance of the mixture was measured at 765 nm against a blank. The results were expressed in ascorbic acid equivalent antioxidant capacity (AEAC) per gram extract.

5.4.7. Metal chelating activity

The metal chelating activities of durian pulp extract was estimated using the method of Dinis *et al* (1994) [45]. An aliquot of 0.1 ml of the extract, 0.6 ml distilled water and 0.1 ml ferrous chloride (2 mmol/l) was well mixed and incubated for 30s. Then, 0.2 ml ferrozine (5 mmol/l) was added to the mixture and incubated for 10 min at room temperature. The absorbance was measured at 562 nm with UV–Vis spectrophotometer. 0–2µg of EDTA was used as a standard for the preparation of calibration curve. The metal chelating ability of antioxidant was expressed as mg EDTA/g.

5.5. Anticancer activity

5.5.1. In vitro cytotoxicity - MTT assay

Chang Liver is an established and well-characterized cell line that has demonstrated reproducible results. Hence, this cell line was selected for MTT assay. The cell line was maintained in Minimum essential medium supplemented with foetal bovine serum. The culture medium of the Chang liver monolayer was replaced with fresh medium. Test samples in Triplicates were added in the cells. After incubation at 37±1°C for 18 hrs, MTT was added in all the wells and incubated for 4 hrs. After incubation, DMSO was added in the wells and read at 570 nm using photometer. Cytotoxicity and cell viability were calculated as follows:

$$\text{Cytotoxicity} = [(\text{Control} - \text{Treated})/\ \text{Control}] \times 100 \quad \text{[Eq. 5]}$$

$$\text{Cell viability} = (\text{Treated}\ /\ \text{Control}) \times 100 \quad \text{[Eq. 6]}$$

5.6. Statistical analysis

Statistical analysis were conducted by using SPSS Statistics 26 software (IBM Corporation, Armonk, NY) and one-way analysis of variance (one-way ANOVA, Tukey's test). A statistical significance of $p < 0.05$ was considered to be significant.

Author contributions: Concept – V.S., R.D.; Design – V.S., R.D.; Supervision – R.D.; Resources – V.S., R.D.; Materials – V.S., R.D.; Data Collection and/or Processing – V.S.; Analysis and/or Interpretation – V.S., R.D.; Literature Search – V.S.; Writing – V.S.; Critical Reviews – V.S., R.D.

Conflict of interest statement: The authors declared no conflict of interest in the manuscript.

REFERENCES

[1] Contreras - Calderón J, Calderón - Jaimes L, Guerra - Hernández E, García - Villanova B. Antioxidant capacity, phenolic content and vitamin C in pulp, peel and seed from 24 exotic fruits from Colombia. Food Res Int. 2011; 44: 2047–2053. [CrossRef]

[2] Gorinstein S, Poovarodom S, Leontowicz H, Leontowicz M, Namiesnik J, Vearasilp S, Haruenkit R, Ruamsuke P, Katrich E, Tashma Z. Antioxidant properties and bioactive constituents of some rare exotic Thai fruits and comparison with conventional fruits *in vitro* and *in vivo* studies. Food Res Int. 2011; 44: 2222–2232. [CrossRef]

[3] Berry SK. Cyclopropene fatty acids in some Malaysian edible seeds and nuts: 1. Durian (*Durio zibethinus* Murr.). Lipids. 1979; 15: 452–455. [CrossRef]

[4] Leontowicz H, Leontowicz M, Jesion I, Bielecki W, Poovarodom S, Vearasilp S, Aguilar G, Sánchez M, Trakhtenberg S, Gorinstein S. Positive effects of durian fruit at different stages of ripening on the hearts and livers of rats fed diets high in cholesterol. Eur J Integr Med. 2011; 3: 169–181. [CrossRef]

[5] Srianta I, Hendrawan B, Kusumawati N, Blanc P J. Study on durian seed as a new substrate for angkak production. Int Food Res J. 2012; 19: 941–945.

[6] Subhadrabandhu S, Ketsa S. Durian-king of tropical fruit. CABI Publishing, New Zealand, 2001.

[7] Voon YY, Hamid NSA, Rusul G, Osman A, Quek SY. Characterization of Malaysian durian (*Durio zibethinus* Murr.) cultivars: Relationship of physicochemical and flavour properties with sensory properties. Food Chem. 2007; 103: 1217–1227. [CrossRef]

[8] Ho LH, Bhat R. Exploring the potential nutraceutical values of durian (*Durio zibethinus* L.) – An exotic tropical fruit. Food Chem. 2015; 168: 80–89. [CrossRef]

[9] Devalaraja S, Jain S, Yadav H. Exotic fruits as therapeutic complements for diabetes, obesity and metabolic syndrome. Food Res Int. 2011; 44: 1856–1865. [CrossRef]

[10] Haruenkit R, Poovarodom S, Vearasilp S, Namiesnik J, Sliwka-Kaszynska M, Park YS, Heo BG, Cho JY, Jang HG, Gorinstein S. Comparison of bioactive compounds, antioxidant and anti-proliferative activities of Mon Thong durian during ripening. Food Chem. 2010; 118: 540–547. [CrossRef]

[11] Ali MM, Hashim N, Aziz SA, Lasekan O. Exploring the chemical composition, emerging applications, potential uses, and health benefits of durian: A review. Food Control. 2020; 113(107189): 1-11. [CrossRef]

[12] Chingsuwanrote P, Muangnoi C, Parengam K, Tuntipopipat S. Antioxidant and anti-inflammatory activities of durian and rambutan pulp extract. Int Food Res J. 2016; 23(3): 939-947.

[13] Bate-Smith EC. Plant phenolics in foods. In the Pharmacology of Plant Phenolics. Proceedings of a symposium held at Oxford, April 1958. J.W. Fairbairn (Ed.). Academic Press, New York, London, 1959, pp.133-147.

[14] Toledo F, Arancibia-Avila P, Park YS, Jung ST, Kang SG, Heo BG, Drzewiecki J, Zachwieja Z, Zagrodzki P, Pasko P, Gorinstein S. Screening of the antioxidant and nutritional properties, phenolic contents and proteins of five durian cultivars. Int J Food Sci Nutr. 2008; 59: 415–427. [CrossRef]

[15] Leontowicz M, Leontowicz H, Jastrzebski Z, Jesion I, Haruenkit R, Poovarodom S, Katrich E, Tashma Z, Drzewiecki J, Trakhtenberg S, Gorinstein S. The nutritional and metabolic indices in rats fed cholesterol-containing diets supplemented with durian at different stages of ripening. Biofactors. 2007; 29: 123–136. [CrossRef]

[16] Aziz ANA, Jalil MAM. Bioactive Compounds, Nutritional Value and Potential Health Benefits of Indigenous Durian (*Durio Zibethinus* Murr.): A Review. Foods. 2019; 8(96): 1-18. [CrossRef]

[17] Ashraf MA, Maah MJ, Yusoff I, Mahmood K, Wajid A. Study of antioxidant potential of tropical fruit. Int J Biosci Biochem Bioinforma. 2011; 1(1): 53–57. [CrossRef]

[18] Aruan DGR, Barus T, Haro G, Simanjuntak P. Toxicity and antioxidant activities of extract of n-hexane, H_2O, and ethyl acetate from the leaves of durian, *Durio zibethinus* L. Rasayan J Chem. 2019; 12(2): 947-950. [CrossRef]

[19] Sumitha S, Vasanthi S, Shalini S , Chinni SV, Gopinath SC, Anbu P, Bahari MB, Harish R, Kathiresan S, Ravichandran V. Phyto-Mediated Photo Catalysed Green Synthesis of Silver Nanoparticles Using *Durio Zibethinus* Seed Extract: Antimicrobial and Cytotoxic Activity and Photocatalytic Applications. Molecules. 2018; 23: 3311. [CrossRef]

[20] Sah BP, Pathak T, Sankar DS, Suresh DB. Phytochemical Investigations on the Fruits of *Durio zibenthinus* Linn. For Antimicrobial Activity. Int J Pharm Sci Res. 2014; 5(12): 878-891.

[21] Amanah A, Pratamawati TM, Taslimah M, Primanagara R. Potential polysaccharide gel from *Durio zibethinus* var.*Raja galuh* rind extract towards *Klebsiella pneumoniae* bacteria. J Phys. Conf Ser. 2019; 1146 (012029): 1-7. [CrossRef]

[22] Ansari RM. Potential use of durian fruit (*Durio zibenthinus* Linn) as an adjunct to treat infertility in polycystic ovarian syndrome. J Integr Med. 2016; 14(1): 22–28. [CrossRef]

[23] Arancibia-Avila P, Toledo F, Park YS, Jung SJ, Kang SK, Heo B K, Lee SH, Sajewicz M, Kowalska T, Gorinstein S. Antioxidant properties of durian fruit as influenced by ripening. LWT-Food Sci Technol. 2008; 41: 2118–2125. [CrossRef]

[24] Poovarodom S, Haruenkit R, Vearasilp S, Namiesnik J, Cvikrová M, Martincová O, Ezra A, Suhaj M, Ruamsuke P, Gorinstein S. Comparative characterisation of durian, mango and avocado. Int J Food Sci Tech. 2010; 45: 921–929. [CrossRef]

[25] Jayakumar R, Kanthimathi MS. Inhibitory effects of fruit extracts on nitric oxide-induced proliferation in MCF-7 cells. Food Chem. 2011; 126: 956–960. [CrossRef]

[26] Haruenkit R, Poovarodom S, Leontowicz H, Leontowicz M, Sajewicz M, Kowalska T, Delgado-Licon E, Rocha-Guzmán NE, Gallegos-Infante JA, Trakhtenberg S, Gorinstein S. Comparative study of health properties and nutritional value of durian, mangosteen, and snake fruit: experiments i*n vitro* and *in vivo*. J Agric Food Chem. 2007; 55(14): 5842–5849. [CrossRef]

[27] Fu L, Xu BT, Xu XR, Gan RY, Zhang Y, Xia EQ, Li HB. Antioxidant capacities and total phenolic contents of 62 fruits. Food Chem. 2011; 129: 345–350. [CrossRef]

[28] Wang L, Li X. Antioxidant activity of durian (*Durio zibethinus* Murr.) shell in vitro. Asian j pharm biol res. 2011; 1(4): 542–551.

[29] Deng GF, Shen C, Xu XR, Kuang RD, Guo YJ, Zeng LS, Gao LL, Lin X, Xie JF, Xia EQ, Li S, Wu S, Chen F, Ling WH, Li HB. Potential of fruit wastes as natural resources of bioactive compounds. Int J Mol Sci. 2012; 13: 8308–8323. [CrossRef]

[30] Abete I, Goyenechea E, Zulet MA, Martínez JA. Obesity and metabolic syndrome: potential benefit from specific nutritional components. Nutr Metab Cardiovasc Dis. 2011; 21(2): B1-B15. [CrossRef]

[31] Miller KL, Liebowitz RS, Newby LK. Complementary and alternative medicine in cardiovascular disease: a review of biologically based approaches. Am Heart J. 2004; 147: 401-411. [CrossRef]

[32] Charoensiri R, Kongkachuichai R, Suknicom S, Sungpuag P. Beta-carotene, lycopene and alpha-tocopherol contents of selected Thai fruits. Food Chem. 2009; 113: 202–207. [CrossRef]

[33] Dembitsky VM, Poovarodom S, Leontowicz H, Leontowicz M, Vearasilp S, Trakhtenberg S, Gorinstein S. The multiple nutrition properties of some exotic fruits: Biological activity and active metabolites. Food Res Int. 2011; 44 (7): 1671– 1701. [CrossRef]

[34] Djacbou DS, Anatole PC, Cabral BP, Veronique PB. Comparison of *in vitro* antioxidant properties of extracts from three plants used for medical purpose in Cameroon: *Acalypha racemosa, Garcinia lucida* and *Hymenocardia lyra*. Asian Pac J Trop Biomed. 2014; 4: S625–S632. [CrossRef]

[35] Devi KP, Suganthy N, Kesika P, Pandian SK. Bioprotective properties of seaweeds: *in vitro* evaluation of antioxidant activity and antimicrobial activity against food borne bacteria in relation to polyphenolic content. BMC Complement Altern Med. 2008; 8: 38. [CrossRef]

[36] Campbell JK, King JL, Harmston M, Lila M A, Erdman JW Jr. Synergistic effects of flavonoids on cell proliferation in Hepa-1c1c7 and LNCaP cancer cell lines. J Food Sci. 2006; 71: S358–S363. [CrossRef]

[37] Makkar, Harinder PS. Quantification of tannins in tree and shrub foliage: A laboratory manual, Kluwer academic publishers, Dondrecht, The Netherlands, 2003, pp.49-51.

[38] Zhishen J, Mengecheng T, Jianming W. The determination of flavonoid contents on mulberry and their scavenging effects on superoxide radical. Food Chem. 1999; 64: 555–559. [CrossRef]

[39] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999; 26: 1231–1237. [CrossRef]

[40] Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I. Antioxidant principles from *Bauhinia terapotensis*. J Natl Prod. 2001; 64: 892-5. [CrossRef]

[41] Benzie, Strain J. The ferric reducing ability of plasma (FRAP) as a measure of ''antioxidant power'': the FRAP assay. Anal biochem. 1996; 239: 70–76. [CrossRef]

[42] Sreejayan N, Rao MNA. Nitric oxide scavenging by cucuminoids. J Pharm Pharmacol. 1997; 49:105-107. [CrossRef]

[43] Beauchamp C, Fridovich I. Superoxide dismutase: Improved assay and an assay applicable to polyacrylamide gels. Anal Biochem. 1971; 44(1): 276–287. [CrossRef]

[44] Prieto P, Pineda M, Anguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of Vitamin E. Anal Biochem. 1999; 269: 337-341. [CrossRef]

[45] Dinis TCP, Madeira VMC, Almeida LM. Action of phenolic derivatives (acetaminophen salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys. 1994; 315: 161–169. [CrossRef]