Biochemical characterisation and evaluation of anti-cancer properties of Medicinally significant Jujube cultivars

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ABSTRACT

Jujube (*Ziziphus jujuba*), commonly known as red dates, is used in Traditional Chinese Medicine (TCM). Jujube is rich in phenolics, flavonoids, triterpenoids and have high antioxidant activity. The South Australia Jujube Growers Association (established in 2019) aimed to investigate the biochemical composition of the fruit, leaves, and seeds of five Jujube cultivars, Li 2, Chico, Shanxi Li, Sihong, and Honeyjar, to quantify the content of phenolics, flavonoids, and to assess antioxidant activity and anticancer activity. The aim of this study was to biochemically characterize the different Jujube bioresources and to determine activity against human colon cancer cells (HCT116) of crude ethanolic extracts of the leaves, fruit and seeds. Ultrasound-assisted extraction was used to extract protein and carbohydrates using water as a solvent, while extraction of phenolics, flavonoids, triterpenoids used 80% ethanol as a solvent. UPLC-MS analyses were carried out to identify bioactive compounds such as flavonoids (Rutin, Quercetin), triterpenoids (Ursolic acid) and alkaloids (Quinine), some of which were quantifiable. Antioxidant activity of these ethanolic extracts was quantified using the FRAP and cell viability was assessed in dose-response MTT assays of crude ethanolic extracts of Jujube cultivar leaves, seeds and fruit. To investigate the mineral contents (Fe, Mg, Ca, K), ICP-OES was performed by Flinders Analytical. Total dietary fibre was analysed by CSIRO (Adelaide, SA). Total protein content was highest in leaves (0.607-2.02 g/100 g DW), while total carbohydrate content (g/ 100 g DW) was highest in fruit (ranging from 33.44 (Sihong) to 58.45 (Li 2)). Of the minerals (Fe, Mg, Ca, K) investigated, Ca contents were highest in Jujube leaves (39.84 to 46.61 mg/g DW), followed by K (13.71 to 17.33 mg/g DW), Mg (3.51 to 4.13 mg/g DW), and Fe (ranged from 0.14 to 0.48 mg/g DW). Total dietary fibre was highest in seeds (56.4 to 85.5 g/ 100 g DW). Total polyphenol, total flavonoid, and total triterpenoid contents (g/ 100 g DE) were highest in leaves, 16.77 (Honeyjar) to 18.57 (Sihong), 4.28 (Honeyjar) to 5.13 (Li 2), and 190.73±3.58 (Chico) to 365.75±9.63, respectively. Antioxidant activity (mmol $FeSO_4$/ 100 g DE) of crude ethanolic extracts of leaves had 10 to 20-times higher activities (90.876±8.29 (Li 2) to 229.53±5.95 (Shanxi Li)) compared to fruit and seeds. The quantification of bioactive compounds by UPLC-MS showed that leaves are a promising source of Rutin (16.07 (Chico) to 50.98 (Shanxi Li) mg/ g DE), Quercetin (0.42 (Honeyjar) to 1.01 (Sihong) mg/ g DE), but amounts were bordering detection limit in fruit and seeds. Ursolic acid content varied with cultivar but were highest in leaves (0.24 (Honeyjar) to 3.15 (Sihong) mg/ g DE), bordering detection limits in fruit, whilst highly variable in seeds for most cultivars. LC-MS analysis confirmed the presence of quinine, but contents were below the quantification limit. 24 h dose-response tests of ethanolic crude extracts of Jujube leaves, seeds and fruit showed

that viability of HCT116 cells (human colon cancer cells) decreased at higher concentrations of leaf extracts of Li 2, Chico, Shanxi Li, while fruit and seed extracts had no effect. In conclusion, the leaves of SA Jujube cultivars are the richest source of minerals (Ca, Fe, Mg) and bio-active compounds. Leaf extracts of all Jujube cultivars may also find applications in cosmetic formulations, as they show high antioxidant activities. As crude ethanolic leaf extracts adversely affected viability of HCT116 cells, there could be potential in cancer treatments, but this requires further studies that include normal colon cells to demonstrate that such extracts do not indiscriminately reduce cell viability.

DECLARATION

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Signed: M. Kishore

Date: 21/ 01/2022

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LIST OF ABBREVIATIONS

ABTS	2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)
ATCC	American Type Culture Collection
BSA	Bovine Serum Albumin
CJ	Candied Jujube
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DE	Dried Extract
DF	Dietary Fibre
DW	Dry Weight
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl Sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
FBS	Fetal Bovine Serum
FRAP	Ferric Reducing Antioxidant Power Assay
GAE	Gallic Acid Equivalents
HCT	Human Colorectal Carcinoma cell line
HPLC	High Performance Liquid Chromatography
ICP-OES	Inductive Coupled Plasma-Optical Emission Spectrometry
LDL	Low Density Lipoprotein
LC-MS	Liquid Chromatography-Mass spectrometry
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
OD	Optical Density
PBS	Phosphate Buffered Saline
QE	Quercetin Equivalents
SA	South Australia
SAJGA	South Australia Jujube Growers Association
TCM	Traditional Chinese Medicine

TDF	Total Dietary Fibre
TFC	Total Flavonoid Content
TPC	Total Phenolics Content
TTC	Total Triterpenoids Content
UAE	Ursolic Acid Equivalents
UAE	Ultrasound-Assisted Extraction
UPLC-ESI-MS	Ultra Performance Liquid Chromatography-Electrospray Ionisation-Mass Spectrometry
WA	Western Australia

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CHAPTER 1. INTRODUCTION

1.1. Introduction

Food with great health benefits can help with the prevention of diseases (Song et al. 2020). Jujube (*Ziziphus jujuba*), otherwise known as red dates or Chinese Dates, is an angiosperm that is a member of the Rhamnaceae family (Liu et al. 2020). Jujube is a subtropical and tropical plant found in arid and semi-arid regions of China, South Korea, Africa, Iran, and Europe (Abdoul-Azize 2016). China produces is the lead producer of Jujube and is also the country of origin for this fruit (Abdoul-Azize 2016), accounting for about 90% of global demand and production has risen over the last decade, due to increased demand for food and pharmaceuticals, from four to 15 million tonnes (Qiao et al. 2014), (Chen, J & Tsim 2020). The Yellow River and the region in the northwest, Shandong, Hebei, Shannxi, Shanxi, Xinjiang Uygur Autonomous Region, and Henan provinces produce almost 700 cultivars of Jujube (Liu et al. 2020). Jujube was regarded highly in ancient classical texts for medicine as one of the most nutritionally benefiting fruit. It is said that Jujube also improves sleep quality, detoxifies the body, and beautifies the skin (Lu et al. 2021). Nowadays, western society-scientists investigate Jujube as a bio-medical product, based on bioactive compound contents, whilst its medical benefits have been undisputed in Chinese Traditional Medicine (TCM), due to high nutritional value and pharmacological properties, such as anti-cancer, antioxidant, anti-inflammatory activities. The characteristics of bioactive compounds derived from plants vary according to the part of the plant and the nature of the extract used in herbal medicine. Jujube is well-known for its high polyphenol content, which has antimicrobial, antioxidant, and immunomodulatory effects (Abdoul-Azize 2016; Gao et al. 2012; Zhang, Y et al. 2021). Notably, additional biologically active compounds, including cyclopeptide alkaloids, dubbed jujubaines, dammarane saponins, and numerous flavonoids, have been extracted from this shrub, as well as unsaturated fatty acids (linoleic acid and oleic acid), while fruits contain large amounts of carbohydrate, and fibres are extracted from seed with antioxidant and antiulcerogenic properties (Ghazghazi et al. 2014). Jujubes are said to be high in amino acids, polysaccharides, polyphenols, fatty acids, triterpenic acids, nucleosides, and nucleobases (Qiao et al. 2014). Anti-inflammatory, anti-hyperglycaemic, anticancer, anti-hyperlipidemic, immunomodulatory-based activity has been identified in Jujubes (Choi et al. 2012). Alkaloids and Saponin are other biologically active compounds that are being researched as a part of the fruit, qualifying as sedatives and neuroprotective components (Wojdyło et al. 2016). The best formulation for treating insomniac patients contains Jujubes (Rajaei, A. et al. 2021b). New lines of research give further consideration of using Jujube for other medicinal benefits (Song, L et al. 2020).

1.1.1. Jujube growing conditions in South Australia

Studies relating to the characteristics of Jujube as a biological product have started since the 1950s, but the application of a detailed theoretical approach was only initiated in the 1990s. The high resilience against abiotic stress forms a major part for consideration of farming on barren soils, or in drought, saline, and alkaline environments (Liu et al. 2020). Bud and branch characteristics make the fruit unique. That respective shoot is pruned in fall, generating waste. The distinct nature of differentiating flower buds with a time of 10 days and a flowering season of nearly two months results in low fruit setting at ~1% (Liu et al. 2020). An example of a South Australian cultivar (Li 2) is shown in Figure 1.1.

Figure 1.1. Li 2 Jujube cultivar leaves, fruit and seeds, grown in the Riverland (2019-20), South Australia.

Jujube can withstand -33°C to high temperatures and can be grown in many types of soils, e.g. with high salinity or high alkalinity (Dongheng Liu, Xingqian Ye & Jiang 2016). Jujube has an outstanding tolerance to droughts. Jujube requires a small amount of winter chill to set fruit. A warm and sunny location is best suited to grow Jujube and thus SA is a suitable region for farming of Jujube. The growth conditions for SA-grown Jujube used in this study are summarised in Table 1.1.

The Jujube cultivars grown in the Riverland, Adelaide plains, Barossa and Southeast parts of South Australia (Table 1.1). A wide range of 40-45 cultivars are grown across the South Australia in these parts.

Table 1.1. Growing conditions of Jujube in South Australia

Climatic condition	Soil condition
Annual average temperature (22°C)	The Riverland - calcareous sand topsoil up to 1.5 m deep limestone marl Adelaide plains and Barossa - clay/loam soils of good depth for horticulture Southeast - sand of varying pH (between 5.5 and 8.5) over clay or rock
Average rainfall	Average rainfall varies from 259-550 mm, but irrigation would be provided in commercial plantings
Average temperature during flowering season	26 °C
Minimum-maximum temperature in SA	Temperature ranges from -4 C to 48 °C

1.2. Problem statement and significance of this research

The major challenges faced by SA agriculture are changes in climatic conditions such as increasing temperature, decreased rainfall resulting in shortages of water availability, unstable weather patterns, unseasonal floods and drought conditions (Department of Environment 2021). One of the most problematic issues for agriculture with climate change are rogue weather events, flooding, drought, large temperature fluctuations within a season etc., as this affects the biology of the crops. Consequently, the productivity of traditional crops such as peaches, apples, cherries, and citrous has been reduced, which has led the SA and WA agriculture industries to put into jeopardy (Bureau of Agricultural Economics 1982). The increasing average temperature rate in South Australia is rising faster than the global land area coverage. The average temperature in SA state was between 2008-2018 was recorded to be 0.95 °C. It is noted that, average temperature has increased than the average temperature noted in 1980 (Fig. 1.2). Since 2005, SA state has seen 9/10 hottest years (Department for Environment and water 2021). Climate variation projections depicts the increment in occurrence of storms, heatwaves, higher rainfall intensities, prolonged droughts and further acceleration in sea level rise over next 10 years, stated by Bureau of Meteorology, and CSIRO. This trend could impact the agriculture sector in the state. (Department for Environment and water 2021).

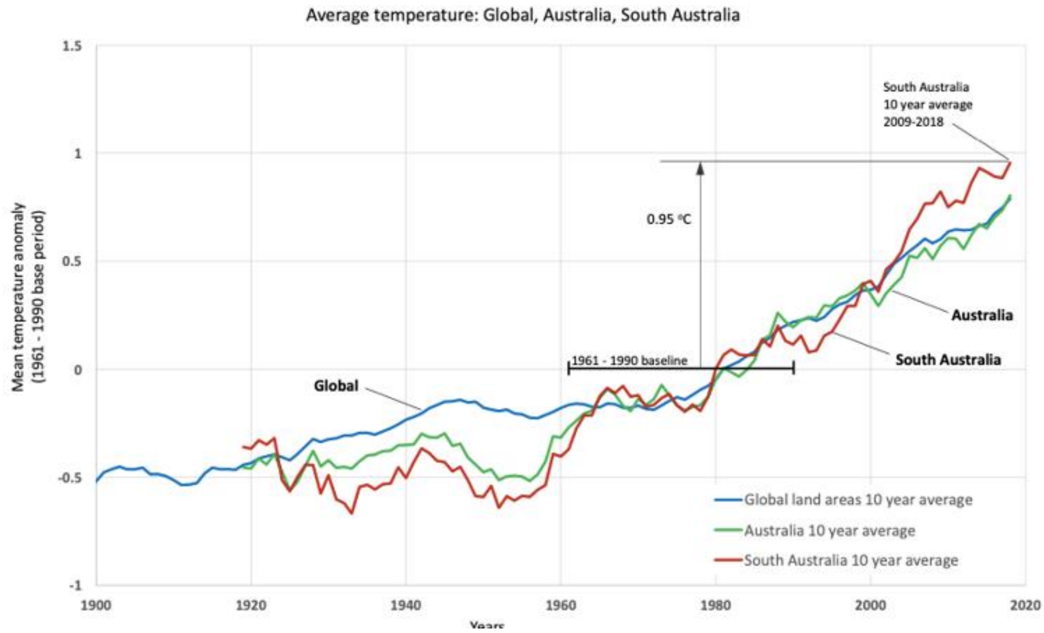

Figure 1.2. Changes in average temperature (Global, Australia and South Australia) from 1910 to 2018 (Department for Environment and water 2021).

To tackle the challenges of climate change, different crop varieties and cropping practices are being developed in South Australia. Accordingly, the South Australian agriculture sector should coordinate with government, and find alternative crops to grow in the state. Jujube is one of the alternative crops to grow in SA and WA.

In general, for western societies, however, the fruit of Jujubes are unattractive in taste and texture, in addition to having a fairly low-income potential. Therefore, growers are looking to upgrade products, producing beers, vinegar, cakes etc. from the fruit (South Australia Jujube Growers Association 2019). While this increases the income potential, it does not exploit the potential bioactive market opportunity. In order to capitalise on that, growers must know the biochemical composition of and contents thereof in the fruit and must validate bioactivity.

The second problem affecting growers is the annual generation of pruning waste and leaf litter, the latter due to the deciduous nature of the shrubs. The resulting waste may also provide an opportunity for additional product development, utilising this underutilized resource for bioactive compound development, if the biochemical profile identifies any opportunities.

Therefore, the principal aim of this research was to conduct the biochemical profiling on fruit, leaves and seeds of five common SA-grown Jujube cultivars, to lay the foundation for future studies looking at product development and required validation of bioactivities, ideally from purified compounds.

1.3. Colorectal cancer (bowel cancer) in Australia

Colorectal cancer or bowel cancer develops in the inner lining of the bowel, usually preceded by the growth of polyps turning into cancer if not detected. Colorectal cancer (CRC) is the 3rd most common cancer in Australia. In 2019, CRC was the 2nd most common cause of cancer deaths in Australia and estimated CRC cases were 15,540 in 2021. Due to the claimed anticancer properties of Chinese Jujube, this study investigated if crude ethanolic extracts of Jujube leaves, seeds and fruit showed any growth inhibition on a colorectal cancer cell line (HCT116). A CRC cell line was chosen over other potential cancer cell line candidates derived from other organs, because in traditional Chinese medicine Jujube fruit are dried and administered orally unextracted. Hence, bioactive contents would transit the gut, where effects would be expected to take place, if indeed Jujube fruit have anti-cancer properties.

1.4. Research gaps

At present, the Jujube industry in Australia is growing due to significant changes in climatic conditions, adversely affecting traditional agricultural crops. It is well known that biochemical profiles of plants change in response to soils, fertilisation, climate, season, and other environmental conditions. The fundamental research on the biochemical composition of Jujube grown in South Australia has not been investigated to date. Such baseline data are however needed to quantify the impacts of environmental and climate conditions on the biochemical profile of Jujube cultivars is still unknown. Therefore, the basic chemical profile of South Australian-grown Jujube fruit was investigated. As Jujube are deciduous trees, annual leave waste may have the potential to add value to fruit production, if the biochemical profile shows significant amounts of bioactives. The biochemical profile of Jujube leaves, and bioactive compound contents has been poorly studied. Therefore, this study included estabilishing the biochemical profile of leaves and seeds as well to determine the potential for value-add co-product development.

1.5. Aims and objectives

- To characterize the biochemical composition of SA Jujube (*Ziziphus jujuba Mill*.) cultivars and quantify bioactive compounds
- To screen bioactive extracts for their reported anti-cancer effectiveness

Objectives

- To establish nutritional profile of Jujube

- To establish suitable green extraction techniques for quantification of alkaloids, triterpenoids, flavonoids, the polysaccharides, proteins and fibre contents of different Jujube cultivars
- To use MTT assay on the Colon cancer cells (HCT-116), to test for cell death induction of extracted compounds

CHAPTER 2. REVIEW OF LITERATURE

2.1. Jujube industry in the World and in Australia

Chinese Jujube is one of the most valuable fruit crops in China, being grown in nearly every area. and. South Korea has an industrial production facility of approximately 5,000 ha and an estimated production capacity of 20,000 tonnes annually, but this is insufficient to meet domestic and global demand. Over 45 other countries, including Australia, have successfully imported Chinese Jujube for cultivation, though on a small scale, demonstrating the Chinese Jujube's adaptability (Liu et al. 2020). CSIRO introduced the Jujube germplasm to Australia in 1993 (Dongheng Liu, Xingqian Ye & Jiang 2016).

Its cultivation and use dates back to the Neolithic era, about 7,000 years ago. It has expanded across China, covering a total of 2 million ha and producing over 8 million tonnes annually (Dongheng Liu, Xingqian Ye & Jiang 2016). Xinjiang, Shandong, Hebei, Shaanxi, Shanxi, and Henan account for more than 90% of Jujube output (Dongheng Liu, Xingqian Ye & Jiang 2016). At present, it is among the most widely grown fruit varieties, the most produced dried fruit, and the primary source of income for China's 20 million farmers (Qiao et al. 2014). Jujube cultivation has expanded to at least 48 countries, since it was imported into neighbouring nations such as Japan and Korea 2,000 years ago. Commercial Jujube production has grown at various levels in China, Italy, Iran, South Korea, the United States, Israel, and Australia, among other countries (Zhang, R et al. 2014). Jujube is gaining traction for cultivation on marginal agricultural land, due to its exceptional resistance and adaptability to drought, as well as barren and saline soil, and needs to be called a potential superfruit due to its biochemical composition (Adeli & Samavati 2015).

The Chinese Jujube thrives in climates with a long, dry summer following sufficient rain early on in the season and a cool temperature throughout its dormancy (Maraghni, Gorai & Neffati 2010). For over 15 years, trees have been developed successfully in South Australia, Western Australia, and Victoria (Dongheng Liu, Xingqian Ye & Jiang 2016). Jujubes are grown mainly in Western Australia's Perth plains, northern goldfields, and south-west area, and are sold at small markets and a few Asian markets in Perth. Western Australia's proximity to Southeast Asia and its counter-season productivity to the northern hemisphere offer an incentive to sell commodity in response to growing demand (Dongheng Liu, Xingqian Ye & Jiang 2016). Thus, a Jujube sector has the ability to be a new productive agriculture sector for Australia, supplying both domestic and international markets (Maraghni, Gorai & Neffati 2010). The establishment of a Jujube industry would aid in the battle against salinity, which is a problem for organic farming in Western Australia and South Australia (Dongheng Liu, Xingqian Ye & Jiang 2016).

A potential superfruit species should address the varied needs of farmers, investors, advertisers, states, and society as a whole (Dongheng Liu, Xingqian Ye & Jiang 2016). Generally, farmers favour fruit trees that produce early, achieve good and reliable yields easily, and are resistant to pests, simple to control, have low production costs, and have significant economic benefits (Dongheng Liu, Xingqian Ye & Jiang 2016). The government and community place a higher premium on ecological stewardship, productive land usage, and economic and social benefits for rural people in marginal regions (Maraghni, Gorai & Neffati 2010). Jujube qualifies to be called a potential superfruit based on the following fundamental characteristics. To begin, Jujubes will satisfactorily satisfy a variety of grower needs. It can flower and bear fruit a year after grafting or planting and can achieve large yields 3–5 years after an orchard is established using a high-density planting scheme (Dongheng Liu, Xingqian Ye & Jiang 2016). Jujube is very drought resistant, infertile, salinity tolerant, and needs little water and fertiliser (Richardson et al. 2004). As a result, Jujube planting and maintenance costs are considerably lower than those associated with other traditional fruit trees (Richardson et al. 2004). It is a drug/food homologue and a well-known and widely used conventional Chinese medication that accounts for 50% of Chinese herbal remedies formulations (Dongheng Liu, Xingqian Ye & Jiang 2016). Additionally, the Jujube fruit has extremely positive connotations in Chinese culture, including sweetness, a prosperous market, fertility, peace, and happiness (Soundharrajan et al. 2015).

2.2. Commercially available products of Jujube

Jujube fruits are consumed fresh, dried, or manufactured as "Chinese dates" and have been incorporated into recipes for confectionery such as cake, Graham bread, compote, candy (Song, J et al. 2022). Jujube fruits processed in various ways including cloying with sugar, preserving the fruits in sweet sour infused vinegar, preservation in sweet infusions such as compote (cultivars and seedlings), and dried Jujube fruits.

Jujubes have a long and illustrious reputation as a historically nutritious meal. However, Jujube products have a low shelf life and should be consumed within ten days if not kept under stable conditions (Wojdyło et al. 2016). Thus, processing Jujube into a commodity is among the safest methods for storing it for an extended period. Candied fruit (CJ), also known as crystallised fruit or glacé fruit, a well-known Chinese food, is made by immersing entire fruit or smaller parts of fruit/peel in a heated icing sugar, which retains the fruit's moisture and gradually preserves it. Fresh Jujube brandy (50 percent ethanol by volume) is a common alcoholic drink in the Hebei province, especially in the Taihang Mountain districts (Dongheng Liu, Xingqian Ye & Jiang 2016). It is made by distilling fermented broth obtained by persistent Jujube fermentation with Saccharomyces cerevisiae. The occurrence of organic compounds during the distillation, storage, and fermentation stages distinguishes this kind of alcoholic drink (Wojdyło et al. 2016). Jujube fruit drinks have risen in popularity worldwide in recent years owing to their high nutritional content. As with other plants, Jujube drinks such as juice, tea, and wine are also available in the market owing to the fruit's high concentration of bioactive compounds.

2.3. Major elements and nutrients in Jujube

Jujube is a richly nutritional food that is filled with carbohydrates, dietary fibre, and proteins. This healthy food is also associated with unsaturated fatty acids and minerals and vitamins. The major nutrients are described below:

2.3.1. Carbohydrates and Proteins

Protein and carbohydrates have been related primarily to support muscle tone and function and Jujube fruit are said to support same. Therefore, carbohydrates and protein contents of various Chinese Jujube cultivars have been determined. Reported carbohydrate contents of different Jujube varieties range from 80.86% to 85.63%, protein contents vary from 4.75% to 6.86% (Abdoul-Azize 2016). Carbohydrate contents of Jujube cultivars grown in Hupingzao, Xiaozao, Huizar, and Junzao in the Chinese Northwest region ranged from 82% to 89% per unit dry weight (Liu et al. 2020), while protein contents were between 4.5% and 6% (Rahman et al. 2018b).

2.3.2. Dietaty Fibre

Lignin, cellulose, hemicellulose, etc. are polysaccharides that are considered as dietary fibre (DF), which represent the water-insoluble fibres (Nguyen et al. 2019). Water-insoluble fibres such as pectin, mucilage, and gums are known to support the growth of beneficial bacteria whose metabolic activities enhance the nutrient and energy count within the human body (Chen, K et al. 2019). In biotechnology, consideration is given to crude separation, membrane separation, and various enzymatic methodologies to expedite the DF extraction process (Lu et al. 2021). Jujube Zj2 contents of soluble and insoluble fibres were 3.8 and 6%, respectively, after enzymatic treatment Qiao et al. (2014), and the content of total fibre was 0.7% to 1.1% (Kou et al. 2015). Acid-based treatment yielded 5.1% of DF, when 5 g of Jujube were hydrolysed with 150 mL H_2SO_4 for 40 min (Wojdyło et al. 2016). Base-treatment with 100 mL KOH for 30 min has also been processed for Jujube DF extraction (Rajaei, A. et al. 2021b), but the optimal condition using the cellulose enzymatic method would provide a yield of more than 6%.

2.3.3. Vitamins and Minerals

Jujube is rich in Vitamin C and various minerals. Seventeen minerals have been identified in Jujube along with 6 macro elements like Ca, K, Mg, P, S, and Na. The remainder are trace elements such as Zn, Mn, Mo, Fe, Ni, Rb, Pb, Se, Br, Cu, and SR (Song, J et al. 2022). According to published data, Jujube has the highest content of Potassium (~1.73% of the entire fruit) (Abdoul-Azize 2016), but contents can be affected by cultivar. Major Vitamins such as A, B complex, Riboflavin, Ascorbic Acid (Vitamin C), Thiamine, and the pigment Carotene have all been identified in Jujube fruit (Chen, J & Tsim 2020).

Ascorbic Acid is highly active in this fruit and processes numerous biological functions. Extensive studies regarding the contents of Jujube have shown the abundance of Vitamin C, ranging from 1.67 to 4.25 mg/g of Vitamin C within the fruits (Liu et al. 2020).

Jujube fruits are highly regarded for their good nutritional value, containing a healthy dose of vitamins C and A, as well as minerals and vitamin B complexes (Rajaei, A. et al. 2021b). Jujube bark, nuts, leaves, and root extract are all used in herbal medicine to cure a variety of diseases around the world.

2.3.4. Fatty acids

Fatty acid content of Jujube fruit is low but can still contribute necessary nourishment (Lu et al. 2021). Fatty acids present are myristic acid, palmitic acid, myristoleic acid, trans-palmitoleic acid, stearic acid, cis-palmitoleic acid, oleic acid, elaidic acid, octadecenoic acid, linolenic acid, and particular emphasis is placed on linoleic acid. When the fruit start to ripen, capric acid (C10:0), myristoleic acid (C14:1n5), lauric acid (C12:0), palmitic acid (C16:1n7), and linoleic acid (C18:2n6c) and oleic acid (C18:1n9c) have been identified (Qiao et al. 2014) with predominant acids being oleic acid (C18:1) and linoleic acid (C18:2) (Lu et al. 2021).

2.4. Bioactive compounds in Jujube

Various bioactive compounds were identified in Jujube fruit triggering extensive research. Essential amino acids, polyphenols, and polysaccharides were characterised together with other bioactive compounds that are beneficial for the body (Kou et al. 2015). The fruit of *Z. jujuba* includes significant quantities of mineral matter, glutamic acid, sterols, antioxidants, tocopherols, fibres, amino acids, fatty acids, triacylglycerol, and starch that are thought to be responsible for the majority of its health benefits, including immunomodulatory, gastroprotective, hypoglycemic, and antioxidant effects (Naik et al. 2013). In this regard, *Z. jujuba* fruit is an important supply of nutrients and antimicrobial, antioxidant, antifungal, anti-inflammatory, immune-suppressive, and antiulcerogenic substances. *Z. jujuba* root includes saponins, a significant amount of essential fatty acids, polyphenol, and vitamin C, as well as many cyclopeptide alkaloids called jujubaines that exhibit a variety of pharmacological activities, including antiproliferative, antioxidant, and antidiabetic properties (Elaloui et al. 2016).

2.4.1. Polyphenols

Polyphenols are a class of organic molecules obtained from plants (Ivanišová et al. 2017). Polyphenols can scavenge reactive free radicals and preventing peroxidative reactions due to the presence of several phenolic groups (Zhang, L et al. 2017). Polyphenols can scavenge reactive free radicals and preventing peroxidative reactions due to the presence of several phenolic groups (Zhang, L et al. 2017). Polyphenols are a diverse group of compounds of phenolic acids, flavonoids, tannins, lignans, and stilbenes. Jujube polyphenols have been effectively used to treat human diseases (Kou et al. 2015). Polyphenol members of the family such as phenolic acids, flavonoids, and other active ingredients are abundant in all parts of *Z. jujuba*.

The identification, extraction, and purification of polyphenols within the fruits is tedious. The Folin-Ciocalteu colorimetric process is done to assess the total polyphenol content (Abdoul-Azize 2016). The range of bound polyphenols ranged from 0.043 to 0.558-milligram gallic acid (Wojdyło et al. 2016). Ultra-performance liquid chromatography-photodiode array-fluorescence detector determined 89% to 94% of total polyphenol content (Rajaei, A. et al. 2021b). 25 phenolic compounds were identified in Spanish Jujube, with contents ranging from 1442 to 3432 mg/100-gram dry matter (Wojdyło et al. 2016). 16 Jujube cultivars had polyphenol contents ranging from 2.53 to 4.95 mg per gram fruit. Some of the components were proto-catechuic acid, p-hydroxybenzoic acid, rutin, and chlorogenic acid (Chen, J & Tsim 2020).

2.4.2. Flavonoids

Jujube fruit contains different types of flavonoids such as flavan 3-ols (Dongheng Liu, Xingqian Ye & Jiang 2016). Nutritional health benefits of Jujube are said to reduce cancer risk and may treat insomnia, enhance gastrointestinal health, boost immunity, reduce inflammation, and reduce stress (Song, L et al. 2019). Specific carbohydrates and dammarane saponins are found in *Z. jujuba* leaves, including three jujubogenin glycosides, jujuboside B, and jujubasaponine IV (Rostami & Gharibzahedi 2016). The seeds of *Z. jujuba* are used to make jujuba oil, which is high in important liposoluble antioxidants, fatty acids, and several sterols. Plant-derived sterols were shown to lower serum LDL cholesterol levels. Seven sterols were identified in *Z. jujuba* seed oil which affects the oils consistency (Zhang, R et al. 2014).

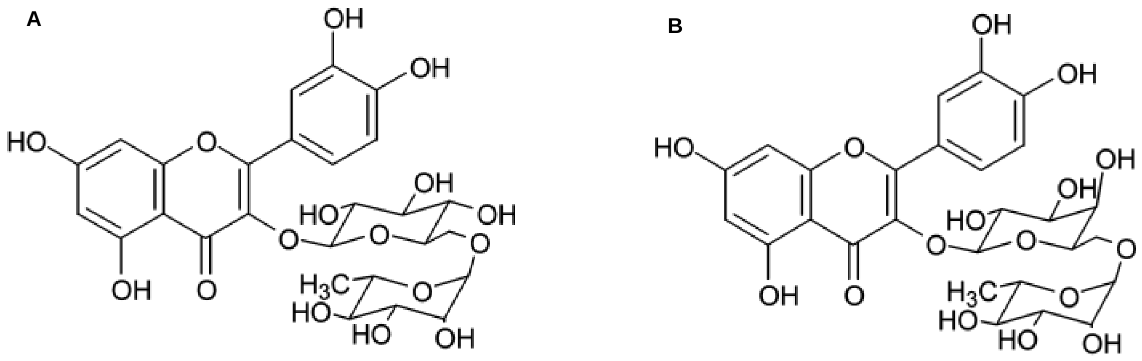

Figure 2.1. Chemical structures of flavonoids. A: Quercetin-3-rutinoside, B. Quercetin-3-rutinobioside (source: (Choi et al. 2012)). Reprinted with permission from ACS Publications.

2.4.3. Triterpenic acids

Triterpenoid content is impacted by cultivation and fruit processing. The triterpenoid content of 15 Jujubes cultivars (Song, L et al. 2020) ranged from 7.5 mg per gram to 16.57 mg per gram, based on gallic acid equivalent (Abdoul-Azize 2016). Total triterpenes found in processed fruit of 99 Jujube cultivars ranged from 1.08 to 7.92 mg per gram of dry weight (Lu et al. 2021). Betulinic acid, maslinic acid, apostolic acid, ursolic acid, and oleanolic acid were the major triterpenoid acids, 16 terterpenic acids and their isomers were in total detected within Jujubes (Chen, J & Tsim 2020). Ursonic and pentacyclic are being considered for the treatment of tumours, skin aging, and other health related issues (Abdoul-Azize 2016). Research on the anti- hyperglycemia effect of Jujube fruits was conducted in Japan (Rajaei, Ahmad et al. 2021a). Oleanonic acid, betulinic acid, and ursolic acid can glucose levels in mammals. Triterpenes, which are members of the phytosterol complex, are naturally occurring bioactive compounds present in grains and vegetables. Triterpenes and triterpenic acids, which are forms of pentacyclic triterpenes, have been shown to have a range of biological benefits, including hepatoprotective, antioxidative, anticancer, anti-inflammatory, and antimicrobial properties, all with low toxicity (Yue et al. 2014). In macrophages, 3-O-trans-coumaroyl alphitolic acid and alphitolic acid in fruit will substantially inhibit nitric oxide (NO) release and inducible nitric oxide synthase (iNOS) expression (Song, L et al. 2020). Additionally, betulinic acid derived from Jujube has been shown to induce apoptosis in the human breast cancer cell line MCF-7 via the mitochondrial transduction pathway (Yue et al. 2014). Guo et al claimed that there are 10 triterpenic acids present in dried jujube fruit and identified two new terpenoids. Jujube fruit contains different types of flavonoids such as flavonols and flavan 3-ols (Dongheng Liu, Xingqian Ye & Jiang 2016).

2.4.4. Alkaloids

Alkaloids are organic compounds containing complex nitrogen containing heterocyclic ring, mostly found Jujube fruit, roots, leaves, seeds and stems. Research on alkaloids has been rarely reported as they are difficult to extract and separate (Senchina et al. 2014). A study by Zhang, H et al. (2010) extracted the alkaloids using 70% ethanol by ultrasound-assisted extraction and confirmed its antioxidant activity by DPPH assay. from Goutou cultivar compounds that are related to the consideration of organic association (Zhang, H et al. 2010).

2.4.5. Polysaccharides

Polysaccharides are an important component of human diets and can be water-soluble, neutral, or acidic for operations. The use of hot-water extraction (Liu et al. 2020), alkali purification (Song, J et al. 2022), and microwave-based extraction (Abdoul-Azize 2016) are the main extraction procedures. Gas Chromatography-mass spectrometry (GC-MS) is used to identify polysaccharides (Chen, J & Tsim 2020). High-performance liquid chromatography (HPLC) separates polysaccharides on the basis of molecular weight, while Infrared (IR) spectroscopy, gas chromatography-mass spectrometry (GC-MS), nuclear magnetic resonance (NMR), methylation analysis, and acid hydrolysis are proposed for the identification of complicated structures within the fruit (Kou et al. 2015). The last half-decade was dedicated to the research of understanding the Jujube monosaccharide and polysaccharide components and their complicated association. PZMP1, SAZMP3, ZMP, PZMP3-2, and PZMP2-2 are the five fragments of polysaccharides that were isolated from Jujube fruits (Lu et al. 2021). Ultrasound-assisted water extraction is also often used for processing. Rhamnose, xylose, arabinose, mannose, and GalA are common sugars, with 93.48% contributed to four-linked GalA (Qiao et al. 2014).

2.4.6. Amino Acids

Jujube fruits contain 12 major amino acids (Abdoul-Azize 2016). The amino acid content is cultivar-dependent and depends on fruit maturity. More than a dozen amino acids were identified in Jujube cultivars from Hupingzao, Huizao, Xiaozao, and Junzao located within the region of north-west China. Of these, Glutamic Acid, Proline, Aspartic Acid are the three main amino acids, present with ~70% of the total amino acid content (Lu et al. 2021). Anti-inflammatory and antioxidant properties are influenced by amino acid composition. Essential amino acids such as lysine (Lys), tryptophan (Trp), threonine (Thr), valine (Val), isoleucine (Ile), histidine (His), leucine (Leu), phenylalanine (Phe), methionine (Met) tyrosine (Tyr), and cysteine (Cys) were identified in four Jujube cultivars (Wojdyło et al. 2016). Regarding fruit maturation, research showed that the amino acid content decreased with the gradual ripening of the Jujubes. True effects are however difficult to establish, as various environmental conditions lead to increase or decrease of amino acid content within Jujubes.

2.5. Research advances in Jujube health functions

Modern research on bioactive compounds present in Jujube showed anticancer, antioxidant, anti-inflammatory, antiviral properties, and other healthcare effects ((Gao et al. 2012), (Ji, X. et al. 2018))

2.5.1. Anticancer activity

A study by Abedini et al. (2016) found that the aqueous extract of Jujube significantly inhibited cell growth of cervical cancer cells (OV2008) and breast cancer cells (MCF-7) in dose-time dependent manner (Abedini et al. 2016). A recent study proved that ursonic acid (triterpenic acid) in Jujube extract inhibited extracellular signal-regulated kinase (ERK) and cyclic adenosine monophosphate response element binding (CREB) signalling pathways in non-small cell lung cancer cells by reducing MMP-2 and MMP-9 (gelatinases) expression (Son & Lee 2020). A study by Ji, X. et al. (2018) extracted a neutral polysaccharide (PZMP1) by ultrasound assisted aqueous extraction, separated and quantified. It showed effective dose-dependent hypolipidemic activity (Ji, X. et al. 2018). They also reported in other study that, Jujube polysaccharides have potential to show prebiotic activity on intestinal microbiota, thus, it could prevent and treat colorectal cancer (Ji, Xiaolong et al. 2020). A study by Plastina (2016)stated that triterpenic acid extracts inhibited the growth of selected cancer cell lines and showed that even malignant breast cancer cells were killed. Huang et al. (2007) found that extracts of Jujube decreased the viability of human hepatoma cells (HepG2) and Choi et al. (2012) found that extracts of Jujube inhibited HeLa cervical cancer cells.

2.5.2. Antioxidant activity

Jujube is a potential source of natural antioxidant for food industry. Oxidative stress may result in oxidative damage to broad biomolecules such as proteins, lipids, and DNA, increasing the risk of tumour and cardiovascular disease, as well as age-related functional decline. As a result, it was thought that antioxidants have the ability to reduce the chance of contracting chronic diseases (Rajaei, A. et al. 2021b). The triterpenoids demonstrated significant free radical scavenging behaviour, which can contribute to the antioxidant activity of sour jujube. Z. jujuba contains a variety of antioxidants, including alkaloids, flavonoids, phenolic acids, and saponins (Abdeddaim et al. 2014). By reducing reactive oxygen species, these materials have been shown to protect against inflammation and oxidative stress. Interestingly, multiple in vitro experiments have shown the ability of various sections of Z. jujuba to scavenge free radicals, such as those generated during lipid peroxidation, thus preventing cell harm. Furthermore, an aqueous leaf extract of Z. jujuba leaves and roots significantly improves haemolysis and glutathione reductase activity in diabetic rats while decreasing glutathione peroxidase activity, catalase activity, and antioxidant capacity, implying that this plant reverses diabetes-induced antioxidant deficiency (Benammar 2011). Additionally, glutathione has been implicated in protein, cellular detoxification, and inflammation. As a result, Z. jujuba extract can be beneficial for cellular defence. In vitro results on human T cells indicate that the fruits of Z. jujuba have the highest antioxidant activity, accompanied by the branches, root, and seed (Lu et al. 2021). Additionally, Z. jujuba secondary metabolites administered orally in carrageenan-induced rat paw edoema demonstrated dose-dependent anti-inflammatory effects by inhibiting paw edoema and nitrite synthesis in lipopolysaccharide-activated RAW 264.7 macrophages lacking cytotoxicity (Ghazghazi et al. 2014).

CHAPTER 3. MATERIALS AND METHODS

3.1. Materials and Methods

3.1.1. Plant Materials and Processing

The five Jujube (*Ziziphus jujuba mill*) cultivars, Li2, Chico, Shanxi Li, Sihong and Honeyjar, were harvested from the Riverland, South Australia. The Jujube fruit was harvested in February 2021 and brought to Flinders on March 27th, 2021. The plant material was stored at 4°C until further processing. The processing of plant material which started four weeks after receipt and storage at 4°C therefore dealt with over-ripe fruit, while the leaves and seeds were not adversely affected. Decayed fruit were excluded from processing. The germplasm was introduced by CSIRO from the United States of America in 1992. Jujube leaves and fruits were donated by the South Australia Jujube Growers Association (SAJGA). The schematic overview of the experimental design and biomass processing approach is described in Fig. 3.1. The standard level of independent replication was n = 3, except for total flavonoids and triterpenoids which had a replication of n = 2 and n = 1, respectively. Also for cell growth inhibition studies for crude ethanolic leaf extracts the level of independent replication was n = 2.

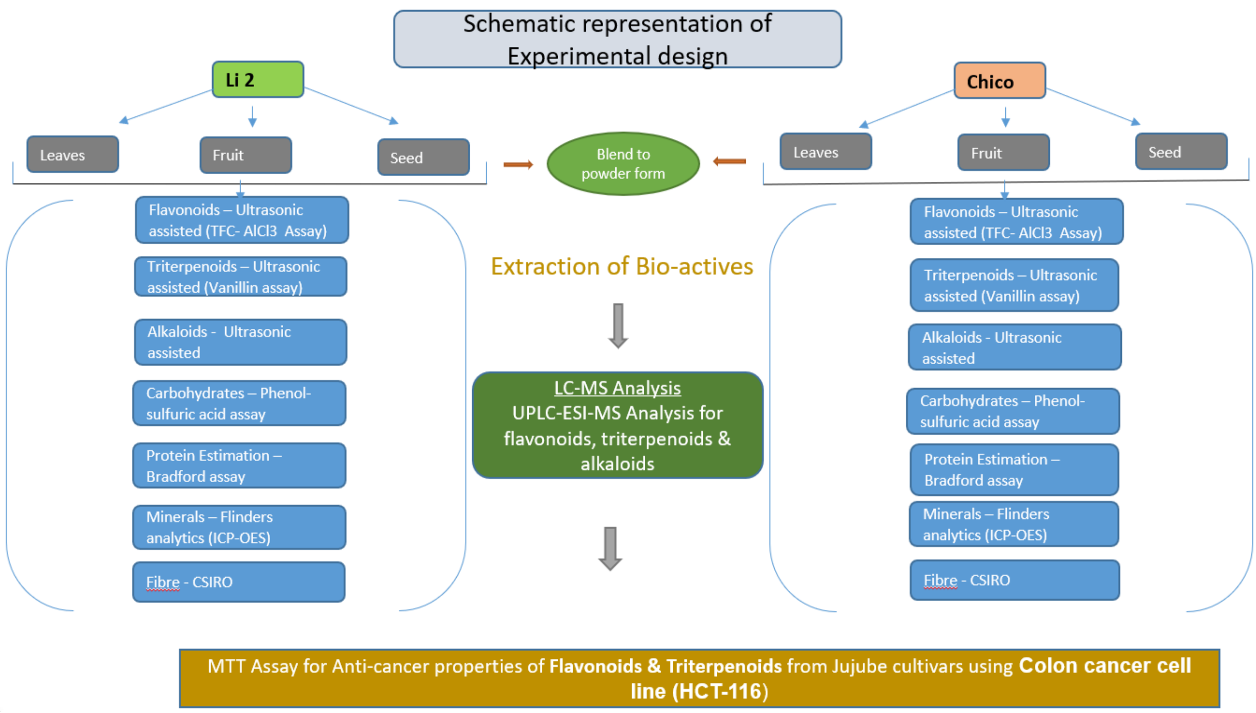

Figure 3.1. Schematic overview of the experimental design and biomass processing approach

Five kilograms of fruit of the cultivars were washed with tap water, finely chopped, using a knife and separated from the seeds. The finely chopped fruits was blended (Blendtec blender), frozen at -80°C overnight, and lyophilized (VirTis benchtop K, BTEKEL, Quantum Scientific). The lyophilized fruit samples were then ground with a mortar & pestle to fine powders and milled to a define particle size, using a 250 µm sieve. The fruit samples were stored at -20°C until use (Fig. 3.2).

Five hundred grams of the Jujube cultivars leaves were frozen at -80°C overnight and lyophilized, ground using a mortar and pestle to fine powders, and milled to a define particle size, using 250 µm sieve. Similarly, seeds of the five cultivars were frozen, lyophilized, blended (Blendtec blender), and milled using a 250 µm sieve. Processed leave and seed samples were stored at -20°C until use (Fig. 3.2).

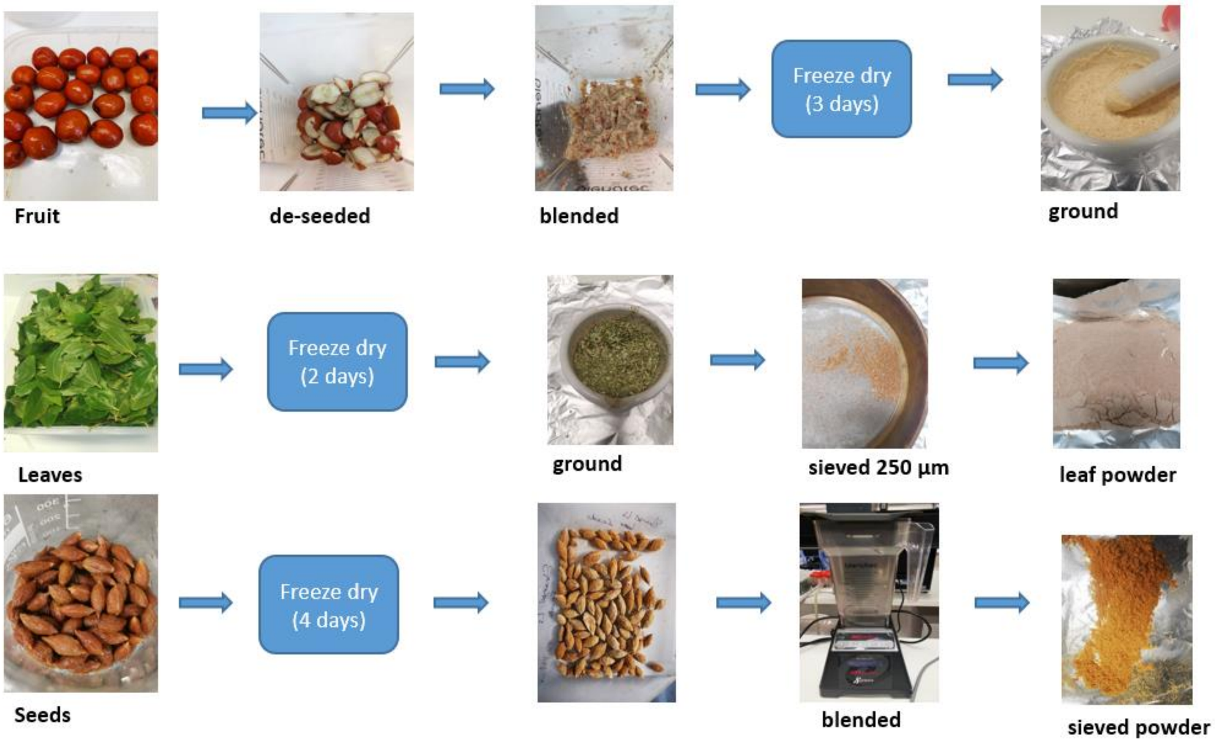

Figure 3.2. Processing of fruits, leaves and seeds of Jujube cultivars

3.1.2. Ultrasonic-assisted extraction

One gram of milled processed Jujube leaves, fruits, and seeds of all five cultivars were extracted with 15 mL of 80% ethanol (Chem supply, Australia) in an ice-bath by ultrasound, using a three mm probe at 20 kHz and 130 W (Sonic Vibra Cell VCX 130 PB) for 30 min (pulse on/off 30:5 sec) using 40% amplitude. After extraction, the samples were centrifuged (Eppendorf5804) at 2,823 rcf for 10 min and the volume of the aspirated supernatant was determined. Then, the solvent was evaporated (Labconco centrivap), frozen at -80°C overnight (Forma™ FDE series, FDE60086FA, Thermo Scientific), and lyophilized, the dry weights were obtained (AB-204S; Mettler Toledo), and the percent dry weight of the total material extracted was calculate as per equation 3.1 (Eq. 3.1). The extracts were stored at -20°C until further use.

$$DE\ (\%) = \frac{DWExtract}{DWSample} * 100 \qquad \text{(Eq. 3.1)}$$

DE (%) is the dry weight of extract in percent, DWExtract is the dry weight of the extract, DWSample is the dry weight of the sample. (Appendix: Table A.1.1)

To determine carbohydrate and protein contents, 1 g of processed samples were reconstituted in 30 mL Milli-Q water (Millipore Milli-Q Academic water purification system) and extracted with ultrasound for 10 min as detailed above. Samples were centrifuged at 2,823 rcf for 10 min, the supernatants were aspirated, the volumes determined, before storage at -20°C until further use.

3.1.3. Total Protein content

The total protein content was determined using the Bradford assay (Bradford 1976). To obtain a working dilution of the Bio-Rad dye reagent one part of the concentrated dye reagent was diluted with four parts of MilliQ water. Bovine serum albumin (BSA) (Sigma-Aldrich, United States) was used as a standard. Briefly, 5 µL of the ultrasonic-assisted Jujube water extract or BSA-standards (0-1 mg/mL) were transferred to a Costar 96 well microplate (Corning Costar®) and 250 µL of the working solution of the dye reagent was added to the samples. After shaking the microplate gently for 60 s, samples were allowed to incubate at room temperature for 30 min. After incubation, absorbance was measured at 595 nm (BMG FLUOstar OMEGA plate reader). Total protein contents of samples were calculated using the linear regression equation derived from the standard curve. The results were expressed as g BSA eq/ 100 g DW.

3.1.4. Total Carbohydrate content

Ultrasound-assisted water extracts were thawed and centrifuged at 2,823 rcf for 5 min and diluted 80 times. Total carbohydrates contents were determined using the phenol-sulphuric acid calorimetric assay method. Glucose (Sigma-Aldrich, United States) was used as a standard. Briefly, 50 µL of sample or glucose standard (0-400 µg/mL) were transferred to Costar assay microtitre plate and 30 µL of phenol reagent (5 % w/w in milli-Q water) was added. 150 µL of concentrated sulfuric acid was immediately added to the samples using a multichannel pipette. The microtitre plate was incubated at 60°C for 5 min. After cooling for 5 min in an ice bath, absorbance was measured at 470 nm against milliQ water as a blank on a BMG FLUOstar OMEGA plate reader. Total carbohydrate contents of Jujube extracts were calculated using the linear regression equation obtained from the glucose standard curve (R^2=0.9998). The results were expressed in g Glucose equivalents / 100 g DW.

3.1.5. Total Dietary Fibre

Total dietary fibre analysis was outsourced to CSIRO, SA, Australia. The freeze-dried Jujube powder was analyzed using ANKOM technology automation and the AOAC 991.43 method.

3.1.6. Total Mineral content

The mineral analysis was assessed at Flinders Analytical, Flinders University, Bedford Park, South Australia. The samples were analyzed on a Perkin Elmer ICP-OES Optima 8000 in radical mode. Prior to the analysis, samples were acid digested in a DigiPREP block digestion system in the following way. Approximately, 100 mg of Jujube extract was weighed into 50 mL digestion tubes and 5% HNO_3 was added (diluted up to 50 mL). A 5 mL aliquot of each sample was transferred to 15 mL ICP tubes and diluted to 10 mL with Milli-Q water giving a HNO_3 concentration of 5%. Shanxi Li leaves and Li 2 leaves were diluted 20-fold, as mineral contents were higher in those two samples. Yttrium was used as an external standard to correct for drift during the run. Results were reported in µg/L and are given as mg of element per gram of dried sample (mg/g DW Jujube sample).

$$Mineral\ content\ (mg/gDW) = (M(\mu g/L))(Vsample(mL)/\ 1000\ \times DW(g) \quad \text{(Eq. 3.2)}$$

DW is the Dry weight of sample; M is Mass of mineral; V is volume of sample

3.1.7. Determination of total polyphenolics content (TPC)

Total phenolic contents of ultrasound-assisted ethanol extracts of Jujube cultivars were determined using the Folin-Ciocalteau method (Benzie & Strain 1996). Briefly, 20 µL of samples (1 mg/mL) were added to a 96-well microtitre plate (Corning Costar®). Then 100 µL of 10% Folin-Ciocalteau reagent (Sigma-Aldrich, United States) was added. After incubation for 5 min, 80 µL of 7.5% NaCO3 (Sigma-Aldrich, United States) was added and incubated for 2 h at room temperature in the dark. The absorbance was measured at 725nm (BMG FLUOstar OMEGA plate reader). A standard curve was obtained by a serial dilution of Gallic acid (20-100 µg/mL) (Sigma-Aldrich, United States) for calculating total phenolic contents. Results are expressed in gram of Gallic acid equivalents (GAeq) per 100 g dry extracts (DE) of Jujube cultivars.

3.1.8. Determination of total flavonoid content (TFC)

To determine the total flavonoid content in the ultrasound-assisted ethanol Jujube extracts, the aluminum chloride calorimetric assay method was used (Bhaskar & Nagella

2021). Freeze-dried Jujube extracts (1 mg/mL) were reconstituted in 80% ethanol (Chem supply, Australia). Briefly, 100 µL of Jujube extracts (1 mg/mL) of leaves, fruits, and seeds of the five cultivars were transferred to 2 mL Eppendorf tubes and 100 µL of $AlCl_3$ (Sigma-Aldrich, United States) (10% w/v) was added to the samples, mixed well (Ratek vortex mixer, Adelab Scientific) before adding 100 µL of 1 M sodium acetate anhydrous (Sigma-Aldrich, United States). The samples were mixed well using ratek vortex mixer. The mixture was incubated for 45 min in the dark at room temperature. Finally, 100 µL of the mixture was transferred to a 96-well flat bottom microtitre plate (Corning Costar®) and absorbance was recorded at 415 nm (BMG FLUOstar OMEGA plate reader). MilliQ water was used as a blank. Total flavonoid content is expressed as Quercetin equivalents (QE_{eq}) obtained from a Quercetin standard (Sigma-Aldrich, United States) calibration curve from 0 to 1 mg/mL (R^2 = 0.9991). Total flavonoid content is expressed as QE_{eq} g/ 100 g DE of Jujube cultivars.

3.1.9. Determination of total triterpenes content (TTC)

Total triterpenoid content was determined using the vanillin-sulfuric acid assay method with some modifications (Pedrosa et al. 2020). Briefly, 20 µL of the Jujube extracts (0.5, 1 mg/mL) or the standard ursolic acid (0.0125 – 0.25 mg/mL) prepared in methanol (Sigma-Aldrich, United States) were transferred to Eppendorf tubes and lyophilized, Then, 125 µL of vanillin-acetic acid solution (5:95 w/v) was added. 250 µL of sulfuric acid (Sigma-Aldrich, United States) solution was added to each tube, vortexed for 10 s (Ratek vortex mixer) and incubated for 30 min at 60°C. 1250 µL of acetic acid was added to each tube and vortexed for 5 s. After 40 min incubation at room temperature, 100 µL of standard or sample was transferred into a microtiter plate in triplicate and absorbance was measured at 548 nm. Results were expressed as ursolic acid (Sigma-Aldrich, United States) equivalents in grams per 100 g of the dry weight (DW) of the dry extract (DE) of Jujube cultivars (UA_{eq} µg/ g DW).

3.1.10. Antioxidant activity

The Ferric Reducing Antioxidant Power (FRAP) assay assesses reducing power of a compound based on the reduction of the ferric tripyridyltriazine complex (Fe^{3+}-TPTZ) to the ferrous complex (Fe^{2+}-TPTZ); the latter forms a blue complex at low pH (Benzie & Strain 1996). The FRAP assay was performed based on the method of (Benzie & Strain 1996). The FRAP reagent was prepared by mixing 25 mL acetate buffer (300 mM, pH 3.6, 10 mL TPTZ solution + 2.5 mL $FeCl_3.6H_2O$). One mM ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) at concentrations of 0-1 mM was used as a standard. Similarly, samples were prepared to a concentration of 1 mg/mL. Briefly, 6 µL of sample or standards were transferred to a Costar assay microplate, 18 µL of MilliQ water and 180 µL warmed FRAP reagent (37ºC) were added and incubated for 5 min at room temperature. The absorbance of the samples was measured at 593 nm on a BMG FLUOstar OMEGA plate reader.

3.1.11. Analysis of bio actives by ultra-performance liquid chromatography-mass spectrometry (UPLC-ESI-MS)

UPLC-ESI-MS spectrometry conditions:

Ethanolic extracts of leaves, fruit, and seeds of the 5 Jujube cultivars were analyzed using a Waters ACQUITY UPLC system coupled with a Waters Synapt HDMS qTOF Mass spectrometer. Rutin (Quercetin-3-O-rutinobioside) (609 Da), Quercetin served as a standard for flavonoids (500 ng/mL-10 µg/mL), while ursolic acid (10 ng/mL-10 µg/mL) was used as a standard for triterpenic acids, and Quinine (500 ng/mL-10 µg/mL) was used as standard for alkaloids. A Phenomenex Kinetex X-B C18 100A (50×2.1 mm, 2.6 µm) column was used with injection volume of 5.0 µL. The mobile phases consisted of 1 % formic acid (solution A) & acetonitrile (solution B). A gradient elution was conducted with a flow rate of 0.3 mL/min of solution A (volume ratio) in mobile phase as follows: 0-0.1 min, 95% A; 0-2 min, 95% A; 2-20 min, 70% A; 20-30 min, 10% A; 30-35 min, 10% A; 35-37 min, 95% A; 37-40 min, 95% A.

Mass spectra parameters were in negative (3.0 kV) ionization or positive (3.5 kV) ionization mode with the capillary voltage of 40V, source temperature (4V), desolvation temperature of 350ºC & desolvation gas (N_2), desolvation flowrate was 500 L/h, trap collision energy (6V), transfer collision energy (2V) and the scan range was m/z 100-1000.

3.2. Statistical analysis

Data were statistically analysed via one way analysis of variance (One-way ANOVA) using IBM SPSS STATISTICS 27. Significance was set to 0.05, and data were inspected for normality and homogeneity of variances using q-q plots and the Levene's test, respectively. To determine the source of significance, Benjamini Hochberg post hoc tests were performed.

3.3. Cell viability assay (MTT Assay)

3.3.1. Cell Culture

The colorectal cancer cell line HCT116 (**ATCC** CCL-247) was obtained from the American Tissue Culture Collection. Under aseptic conditions, HCT116 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Sigma-Aldrich, United States) supplemented with 10% fetal bovine serum (FBS) (Sigma-Aldrich, United States), 1% Pen Strep (penicillin & streptomycin) (Sigma-Aldrich, United States) in an incubator at 37ºC, supplemented with 5% CO_2.

3.3.2. MTT assay

The MTT assay works on principle of reducing the yellow tetrazole (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide) to form purple formazan in living cells. (Choi et al. 2012). Prior to the experiment, the MTT dye (Sigma-Aldrich, United States) was prepared at 5 mg/mL using phosphate buffer and stored at -20°C. HCT116 cells were added to the Costar 96 well cell culture plate (5×10^5 cells/well) and incubated for 24 h. The cells were treated with Jujube leaf -, fruit -, and seed extracts at 5 concentrations (0.3125, 0.625, 1.25, 2.5, 5 mg/mL) in DMEM medium. HCT116 cells were treated with 5-fluorouracil (Sigma-Aldrich, United States) as a positive control at five concentrations (200, 400, 800,1600, 3200 µM). After 48 h incubation, the DMEM medium was decanted and the MTT solution (10 µL of MTT dye + 90 µL of DMEM) was added to each well, followed by incubation at 37°C for 4 h. Then, the medium was decanted and 50 µL of DMSO (Sigma-Aldrich, United States) was added to each well, followed by incubation at 37°C for 10 min. Finally, absorbances were measured on a BMG FLUOstar OMEGA plate reader at 570_{nm}.

3.3.3. Statistical Analysis

The reported results represent two biological replications (n = 2). Graph pad prism was used to determine the dose response curves for cytotoxicity of Jujube extracts. A One-way ANOVA with Tukey post hoc test (p=<0.05) using IBM SPSS STATISTICS 27.

CHAPTER 4. RESULTS

4.1. Biochemical composition and Mineral contents of Jujube cultivars

4.1.1. Total Protein content

Irrespective of Jujube cultivar, total protein contents were highest in leaves, followed by fruit and lowest in seeds (Fig. 4.1A). Total protein content of Jujube leaves was significantly higher (0.60-2,02 g/ 100 g DW), than in fruits (0.33-0.588 g/100 g DW) and seeds (0.05-0.354 g/100 g DW) (Fig. 4.1A). Total protein content was highest in Honeyjar leaves, followed by Sihong which was statistically significant (Benjamini Hochberg test: $p < 0.05$), but total protein content of leaves was not significantly different in Li 2, Chico and Shanxi Li cultivars (Fig. 4.1A). Total protein content in fruit were highest and not significantly different in Li 2, Chico, Shanxi Li, and Honeyjar, but significantly lower in Sihong (Benjamin hoc test: $p < 0.05$). Total protein contents of seeds were not significantly different in Chico, Shanxi Li, and Sihong cultivars but significantly higher in the Honeyjar cultivar. (Benjamini Hochberg test: $p < 0.05$), while protein was below the detection limit in Li 2 seeds.

4.1.2. Total Carbohydrate content

Irrespective of cultivar, total carbohydrate contents were highest in fruits, followed by seeds and lowest in leaves (Fig. 4.1B). Total carbohydrate content of the fruits of the five Jujube cultivar's showed significant differences ranging from 33.4 – 58.45 g/ 100 g DW (Fig. 4.1B). Total carbohydrate content was significantly higher in fruits of Li 2 (58.45±4.6), Chico (47.3±3.32), and Shanxi Li (54.64±0.89) compared to Sihong (33.44±6.07) and Honeyjar (39.16±3.07). The 5 Jujube cultivar's leaves showed less carbohydrate content. Total carbohydrate content of leaves was not significantly different in leaves of Li 2 (5.94±1.17), Chico (9.11±0.69), Shanxi Li (13.05±1.56), and Sihong (4.95±0.56) cultivars but significantly lower in the Honeyjar cultivar (1.69±0.49). Significantly higher total carbohydrate content was observed for Chico seeds (30.46±5.86) followed by Shanxi Li (20.87±1.95) and Sihong (16.07±1.97) which were not significantly different to each other, while seed total carbohydrate content was significantly lower in Honeyjar seeds (3.80±1.34).

4.1.3. Total dietary fibre

Total dietary fibre (TDF) was highest in seeds, followed by leaves and fruit in declining order (Fig. 4.1C). The TDF in seeds varied from 56.4 to 85.5 g per 100 g dry weight. Highest TDF content was present in Shanxi Li seeds (85.5 g/100 g dry weight), whereas Sihong seeds had the lowest (56.4 g/100 g dry weight). TDF content of Jujube cultivar fruit ranged from 6.7

to 10.5 g per 100 g DW. TDF content was highest in Li 2 fruits (10.5 g/100g DW), while fruits of Honeyjar had the lowest (6.7 g/100 g DW). There was no significant difference in TDF content for leaves for the five Jujube cultivars, ranging from 33.3 to 33.9 g per 100 g of dry weight Jujube leaves.

4.1.4. Total mineral content

Analysis for iron (Fe), magnesium (Mg), calcium (Ca), and potassium (K) is presented as total mineral content (Fig. 4.1D), In general, Ca contents were highest, followed by K, Mg, and Fe in declining order and contents of these minerals were highest in leaves. Jujube leaf Ca content ranged from 39.84 to 46.61 mg/ g dry weight. (Table 5.3), with Honeyjar leaves showing the highest and lowest content in Shanxi Li. Jujube leaf K content ranged from 13.71 to 17.33 mg/ g dry weight, with Li 2 leaves showing highest and Honeyjar the lowest content. Jujube leaf Mg content ranged from 3.51 to 4.13 mg/ g dry weight, while Fe contents were much lower, ranging from 0.14 to 0.488 mg/ g dry weight and differences were not significantly different for cultivars for both minerals.

Jujube fruit had a high potassium content in all cultivars, and mineral content declined in the following order K>Ca>Mg>Fe (Fig. 4.1D). K content ranged from 8.42 to 10.26 mg/ g dry weight of Jujube, with Shanxi Li having the highest and Sihong the lowest K content. Calcium content ranged from 0.62 to 1.01 mg/g dry weight, with Honeyjar having the highest and Sihong the lowest Ca content. Magnesium content in fruit varied from 0.36 to 0.44 mg/ g dry weight, while fruit Fe contents were below the detection limit for Li 2, Shanxi Li, and Honeyjar cultivars. Fe content in Chico and Sihong were 0.0088, 0.0076 mg/ g dry weight of Jujube, respectively.

Mineral contents of Jujube seeds declined in the same order as for fruit (K>Ca>Mg>Fe) (Fig. 4.1D). K content ranged from 1.91 to 8.81 mg/ g dry weight, with Shanxi Li having the highest and Honeyjar the lowest. Seed Ca content differed significantly, ranging from 0.55 to 5.89 mg/ g dry weight, with Li 2 having the highest and Honeyjar the lowest content. Seed Mg content ranged from 0.143 to 1.625 mg/ g dry weight, while Fe contents was much lower, ranging from 0.031 to 0.057 mg/ g dry weight. Seed Fe content of Honeyjar was below the detection limit.

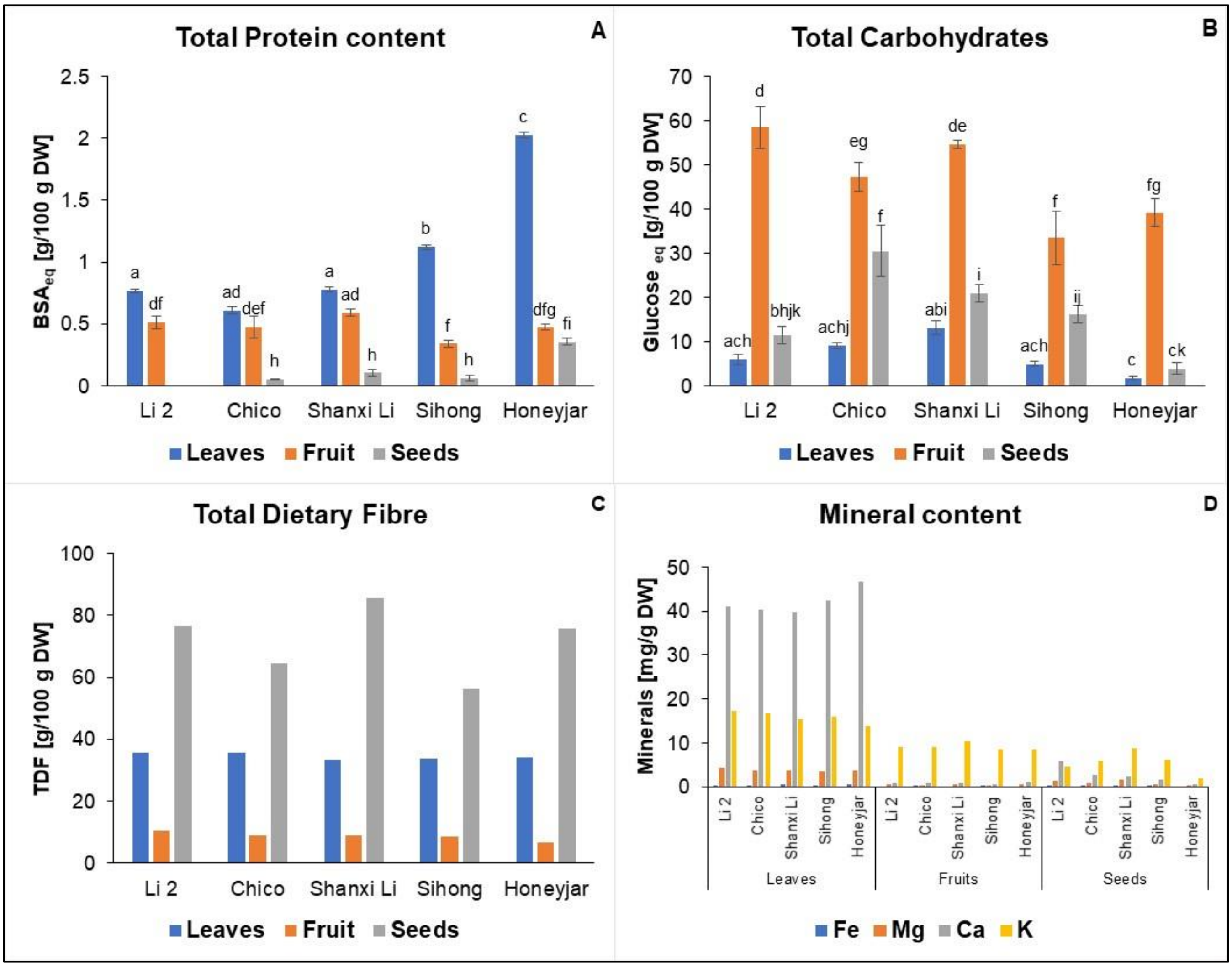

Figure 4.1. The biochemical composition of the Jujube cultivars. A: the total proteincontent (n = 3); B: total carbohydrates content (n = 3); C: total dietary fibre content (n = 1); D: total mineral content (Fe, Mg, Ca, and K) (n = 1).

4.2. Quantification of Bioactive compounds and antioxidant capacity

4.2.1. Total Polyphenolics content

The total phenolics content (TPC) content, reported as Gallic acid equivalents (GAE), was highest in leaves (14.7 to 18.57 g/ 100 g DE), followed by seeds (2.18 to 4.43 g/ 100 g DE), and lowest in fruit (0.98 to 1.54 g/ 100 g DE) of Jujube cultivars (Fig. 4.2A). Jujube leaf TPC content was significantly higher in Sihong leaves (18.57±0.10 g/ 100 g DE) ($p = <0.05$) (Table 5.5), followed by Chico, Shanxi Li, Honeyjar which were not significantly different to each other (16.77 to 17.11 g/ 100 g DE), and a significantly lower (14.707±0.77 g/ 100 g DE) in Li 2 (Fig. 4.2A). An analysis of variance (ANOVA) showed that fruit TPC contents were not significantly different amongst the cultivars (0.982 to 1.54 g/100 g DE) (Benjamini Hochberg test: $p < 0.05$). (Appendix: Table A.2.1). Of the five cultivars, Sihong seeds showed highest TPC (4.433±0.60

g/ 100 g DE), but there is no significant difference in the other cultivars (2.18 to 3.28 g/ 100 g DE).

4.2.2. Total Flavonoids content

Similarly, total flavonoid content (TFC), reported in Quercetin equivalents (QE), was highest in Jujube leaves (4.2 to 6.2 g/ 100 g DE), followed by seeds (0.288 to 1.16 g/ 100 g DE), and lowest in fruit (0.432 to 0.829 g/ 100 g DE) (Fig. 4.2B, Appendix: Table A.2.3). Jujube leaf TFC was significantly higher in Sihong cultivars (6.269±0.2 g/ 100 g DE) (Benjamini Hochberg test: $p < 0.05$), but not significantly different in Li 2 and Shanxi Li cultivars (5.13±0.06, 5.218±0.13 g/ 100 g DW), but significantly lower in Chico (4.538±0.14 g/ 100 g DE) and Honeyjar (4.284±0.26 g/ 100 g DE) cultivars (Fig 4.2B). In contrast, there was no significant difference in the TFC of Jujube fruit extracts across all cultivars. Seed TFC were not significantly different in Chico (4.538±0.14 g / 100 g DE), Shanxi Li (0.962±0.12 g/100 g DE), and Honeyjar (0.912±0.30 g/ 100 g DE) extracts but significantly lower in Li 2 (0.912±0.3 g/ 100 g DE) and Sihong (0.707±0.12 g/ 100 g DE) cultivars.

4.2.3. Total antioxidant capacity

The antioxidant capacity of leaf, fruit and seed extracts of the five Jujube cultivars were determined using the FRAP assay (Fig. 4.2C, Appendix: Table A.2.5). Jujube leaf extracts had the highest antioxidant capacity (90.87 to 229.83 mmol FeSO4/ 100 g DE), followed by extracts of seeds (12.6 to 32.99 mmol $FeSO_4$/ 100 g DE) and fruit (8.47 to 16.4 mmol $FeSO_4$/ 100 g DE). Jujube antioxidant capacity of leaf extracts declined in the following order Shanxi Li (229.534±5.95) > Sihong (173.458±23.07) > Honeyjar (162.405±38.35) > Chico (144.608±11.17), and Li 2 (90.87±8.29). Total FRAP activity was significantly higher in leaf extracts of the Shanxi Li cultivar (Benjamini Hochberg test: $p < 0.05$), while FRAP activity was not significantly different in Chico, Sihong and Honeyjar cultivars, but significantly lower in Li 2 cultivar. (Fig. 4.2C). Total FRAP activity in the fruit (8.47 to 16.4 mmol $FeSO_4$/ 100 g DE) and seed (12.62 to 28.84 mmol $FeSO_4$/ 100 g DE) extracts were not significantly different across all cultivars and to each other (Benjamini Hochberg test: $p < 0.05$) (Fig 4.2C).

4.2.4. Total Triterpenes content

Total triterpenoid content (TTC) was significantly higher in extracts of leaves (190.73 to 365.75 µg/ g DE) (Appendix: Table A.2.6), followed by seeds (61.31 to 141.79 µg/ g DE), and lowest in fruits (36.63 to 69.75 µg/ g DE). In contrast, TTC of leaf extracts were not significantly different in Shanxi Li (365.75±9.63 µg/ g DE) and Honeyjar (339.56±6.63 µg/ g DE), but significantly lower in Li 2 (209.35±9.53 µg/ g DE), Chico (190.73±3.58 µg/ g DE) and Sihong

(218.48±4.97 µg/ g DE) cultivars (Benjamini Hochberg test: $p < 0.05$) (Fig. 4.2D). In contrast, there was no significant difference in the TTC of Jujube fruit extracts across all cultivars. Seed TTC were not significantly different in Chico (103.08±4.44 µg/ g DE), Shanxi Li (122.12±6.49 µg/ g DE), Sihong (141.79±1.7 µg/ g DE) and Honeyjar (114.40±5.43 µg/ g DE) extracts but significantly lower in Li 2 (61.31±13.99 µg/ g DE).

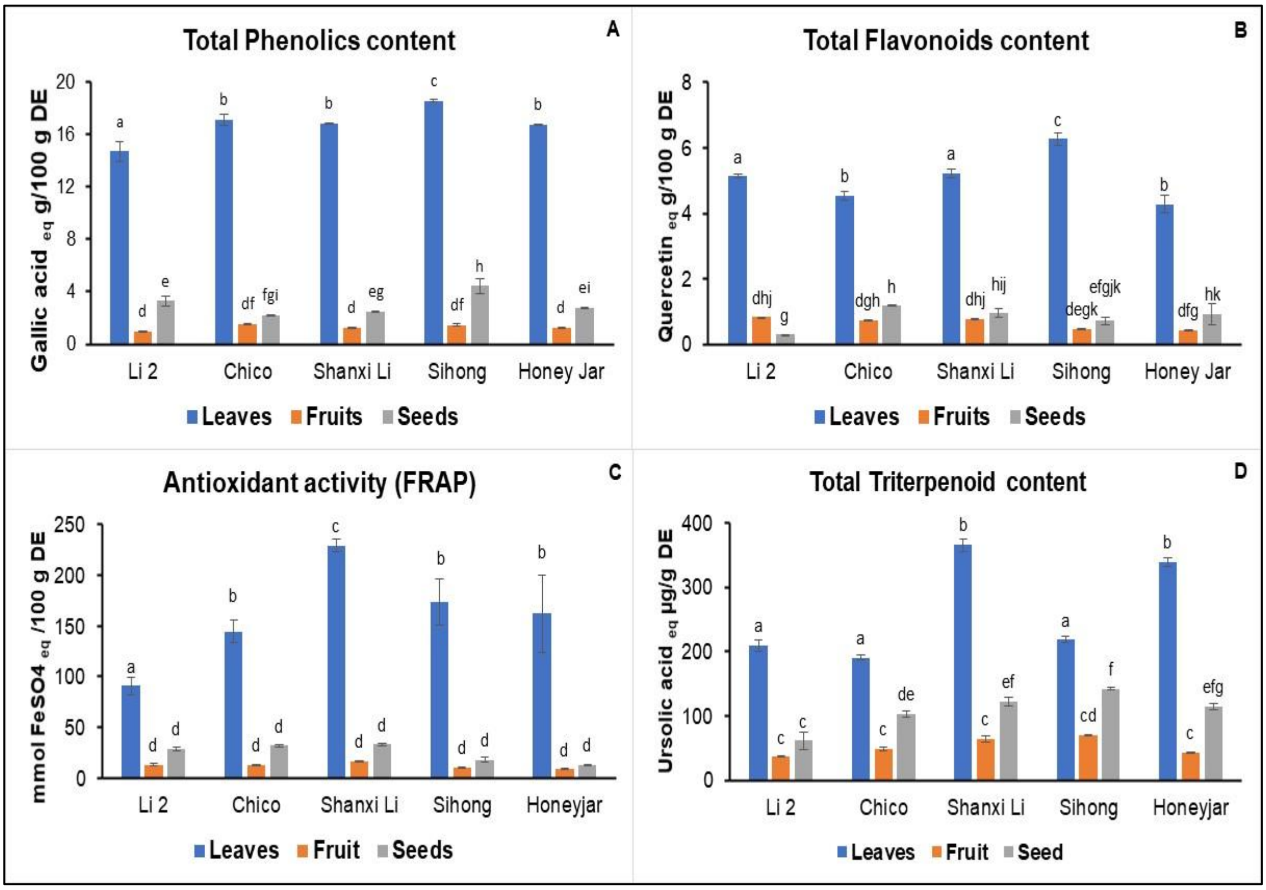

Figure 4.2. Bioactive compounds of Jujube cultivar extracts. A: total polyphenolics content (TPC) (n = 3); B: total flavonoids content (TFC) (n = 2); C: antioxidant capacity (FRAP) (n = 3); D: total triterpenoid content (TTC) (n =1)

4.3. UPLC-ESI-MS analysis of bioactive compounds in Jujube cultivars

To confirm bioactivity of ethanolic extracts of Jujube cultivar leaves, seeds and fruit, UPLC-ESI-MS analysis was performed. Used the UPLC-ESI-MS chromatogram of flavonoids of Sihong leaf extract confirmed the presence of Rutin (Quercetin-3-O-rutinobioside) (Fig. 4.3) and identified nine other flavonoid compounds present (Table 4.1) based on the elution order in another study (Song, L et al. 2019). These results were compared with the study by Song, L et al. (2019) to confirm the identity of those specific compounds based on their molecular weights. The two peaks eluting at 9.57 and 9.83 min had a molecular weight (MW) of 609 Da and were identified as Quercetin-3-O-robinobioside and Rutin (Quercetin-3-O-rutinobioside), the latter also identified by the standard used. The peaks at 9.72, 10.05 min with a MW of 463 Da were identified as Hyperoside (Quercetin-3-O-ß-d-galactoside) and Quercetin-3-O-robinoside. Peaks at 10.51, 11.19 identified as isomers, known to be Kaempferol-3-O-robinobioside and Kaempferol-3-O-rutinoside (Song, L et al. 2019). A peak at 11.39 min with a MW of 447.1 Da was identified as Kaempferol-3-O-glucoside. The two peaks (8 and 9) at 11.51, 11.73 were identified as Quercetin-3-O-ß-1-arabinosyl-(1-2)-α-1-rhamnoside, and Quercetin-3-O-ß-d-xylosyl-(1-2)-α-1-rhamnoside.

The UPLC-ESI-MS chromatograms of Li 2, Shanxi Li and Honeyjar cultivars did not contain peaks 8 and 9 suggesting that Quercetin-3-O-ß-1-arabinosyl-(1-2)-α-1-rhamnoside and Quercetin-3-O-ß-d-xylosyl-(1-2)-α-1-rhamnoside were not present in the leaves of these cultivars (Appendix Fig. A.2.1). Among the five cultivars, highest contents of Rutin (Quercetin-3-O-rutinobioside) were present in ethanolic extracts of Jujube leaves, ranging from 16.07 (Chico) to 50.98 (Shanxi Li) mg/ g dried extract weight, (Table 4.4). Ethanolic extracts of Jujube fruit extracts showed detectable contents of Rutin (0.05, 0.06 mg/ g dry extract) in Li 2 and Honeyjar cultivars. Rutin contents in fruit extracts of Chico, Shanxi Li and Sihong cultivars are below the detection limit. (Table 4.4). Ethanolic extracts of Jujube seeds of Chico, Honeyjar, and Sihong contained small amounts of Rutin (0.07, 0.15, 0.16 mg/ g dry extract, respectively) and contents were below the detection limit for Li 2 and Shanxi Li cultivars.

Of the five cultivars, content of Quercetin was highest in leaf extracts, ranging from 0.42 (Honeyjar) to 1.01 (Sihong) mg/ g dry extract. Of all the five cultivars, ethanolic extracts of Shanxi Li fruit had the lowest content of Quercetin (0.07 mg/g dry extract), while contents were below the detection limit for the other cultivars. Ethanolic extracts of Jujube seeds had a higher content of Quercetin compared to fruit extracts. The Quercetin content in the seed extracts ranged from 0.10 (Sihong) to 0.16 (Chico) mg/ g dried extract. (Table 4.4)

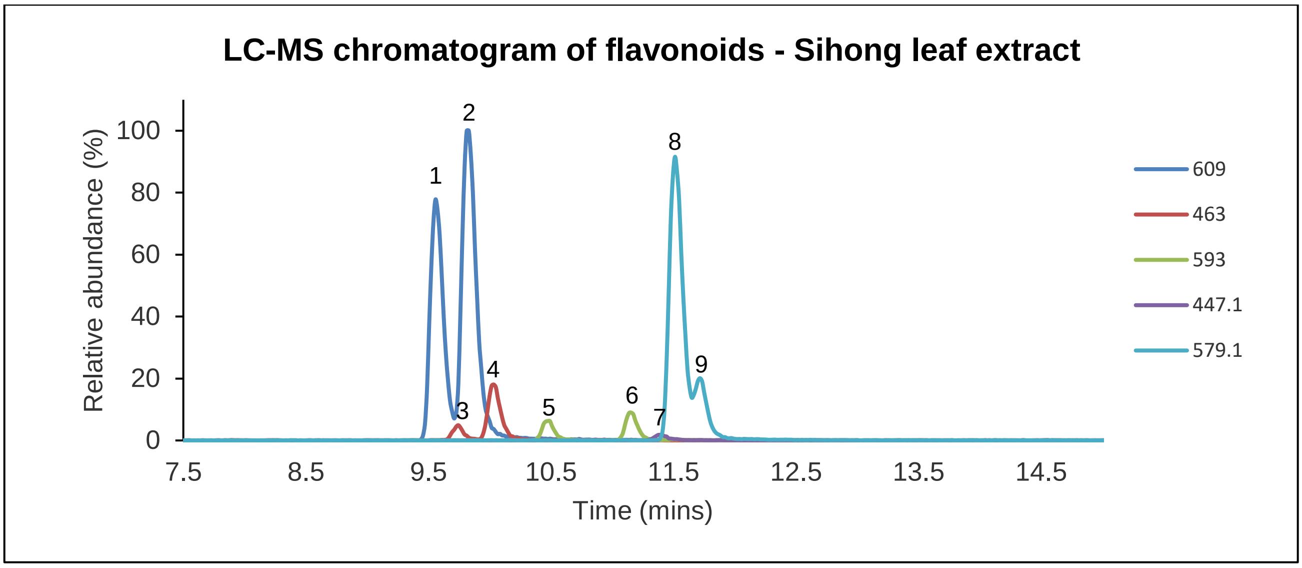

Figure 4.3. UPLC-ESI-MS chromatogram showing peaks of flavonoid compounds in ethanolic leaf extracts of the Sihong cultivar.

Table 4.1. Flavonoid composition of Sihong ethanolic leaf extracts identified and quantified (Rutin – Quercetin-3-O-rutinoside) by UPLC-ESI-MS obtained using positive ion mode ($R^2 = 0.999$)

Peak	Rt (Min)	Compounds	MW (Da)
1	9.57	Quercetin-3-O-robinobioside	609
2	9.83	Rutin (Quercetin-3-O-rutinoside)	609
3	9.72	Hyperoside (Quercetin-3-O-ß-d-galactoside)	463
4	10.05	Quercetin-3-O-robinoside	463
5	10.51	Kaempferol-3-O-robinobioside	593
6	11.19	Kaempferol-3-O-rutinoside	593
7	11.39	Kaempferol-3-O-glucoside	447
8	11.51	Quercetin-3-O-ß-1-arabinosyl-(1-2)-α-1-rhamnoside	579.1
9	11.73	Quercetin-3-O-ß-d-xylosyl-(1-2)-α-1-rhamnoside	579.1

Triterpenic acids of ethanolic extracts of Jujube leaf, fruit and seed were also analysed using UPLC-ESI-MS with Ursolic as a standard. The LC-MS chromatogram of Jujube leaf extract of Shanxi Li cultivar is shown in Figure 4.4.

Ten peaks were observed: two peaks at 27.74 and 27.92 min with a MW of 455.35 Da were identified as Ursolic acid and Betulonic acid (an Ursolic acid isomer) (Fig 4.4; Table 4.2).

The other peaks were tentatively identified as Maslinic acid isomers with a MW 471.3 Da. Maslinic acid standards are required to confirm the identification.

The quantitative analysis of the Ursolic acid is shown in the (Table 4.4). In general, ethanolic leaf extracts of the Jujube cultivars had the highest content of Ursolic acid, ranging from 0.24 to 3.15 mg/ g dry extract weight, followed by seed and fruit extracts. Ursolic acid content declined in the following order Sihong (3.15) > Shanxi Li (1.83) > Li 2 (1.46) > Chico (0.38) > Honeyjar (0.24). For ethanolic fruit extracts, Shanxi Li and Sihong contained low amounts of Ursolic acid (0.04 and 0.05 mg/ g dry extract, respectively). Amounts were below detection limit for the other fruit extracts of the Jujube cultivars. Ethanolic extracts of Jujube seeds had quantifiable amounts for Li 2, Chico, Shanxi Li, containing 0.09, 0.10, 0.23 mg/ g of Ursolic acid, respectively. Sihong and Honeyjar seed extracts also contained Ursolic acid, but amounts were below the limit of quantification (Table 4.4)

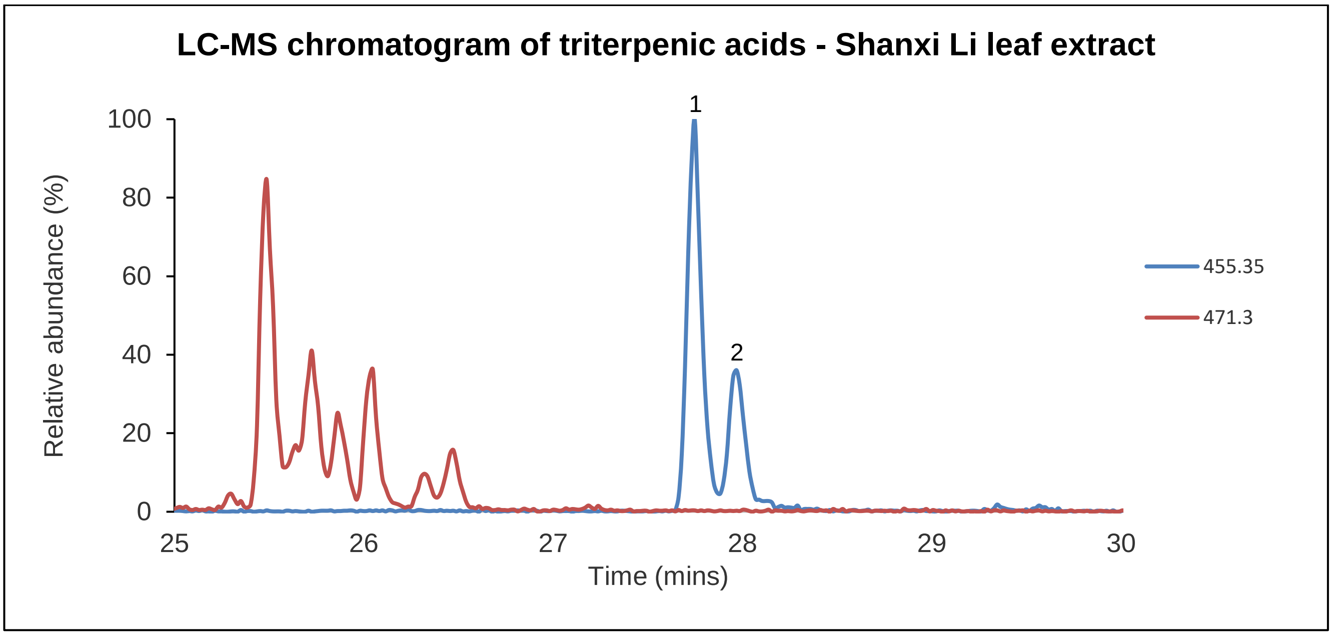

Figure 4.4. UPLC-ESI-MS chromatogram of triterpenic acids of Shanxi Li ethanolic leaf extracts, standard Ursolic acid (MW – 455.35 Da)

Table 4.2. Triterpenoid composition of Shanxi Li ethanolic leaf extracts identified and quantified (Ursolic acid) by UPLC-ESI-MS obtained using positive ion mode

Peak No	**Rt (min)**	**Compound**	**MW (Da)**	**Regression equation**	**R^2**
1	27.748	Ursolic acid	455.35	y=0.0854424x+3.25694	0.990817
2	27.952	Betulonic acid	455.35	-	-

To analyse the alkaloid content in the Jujube leaf, fruit and seed extracts, UPLC-ESI-MS analyses were performed. Quinine was used as standard for the analysis (Table 4.3). Across all cultivars, the Quinine peak was observed for leaf, fruit and seed extracts but amounts were not quantifiable. For all the Jujube extracts, Peak 1 at 4.64 min was identified as Quinine (Fig 4.5) in the Sihong ethanolic leaf extract and no other peaks were observed in the chromatogram.

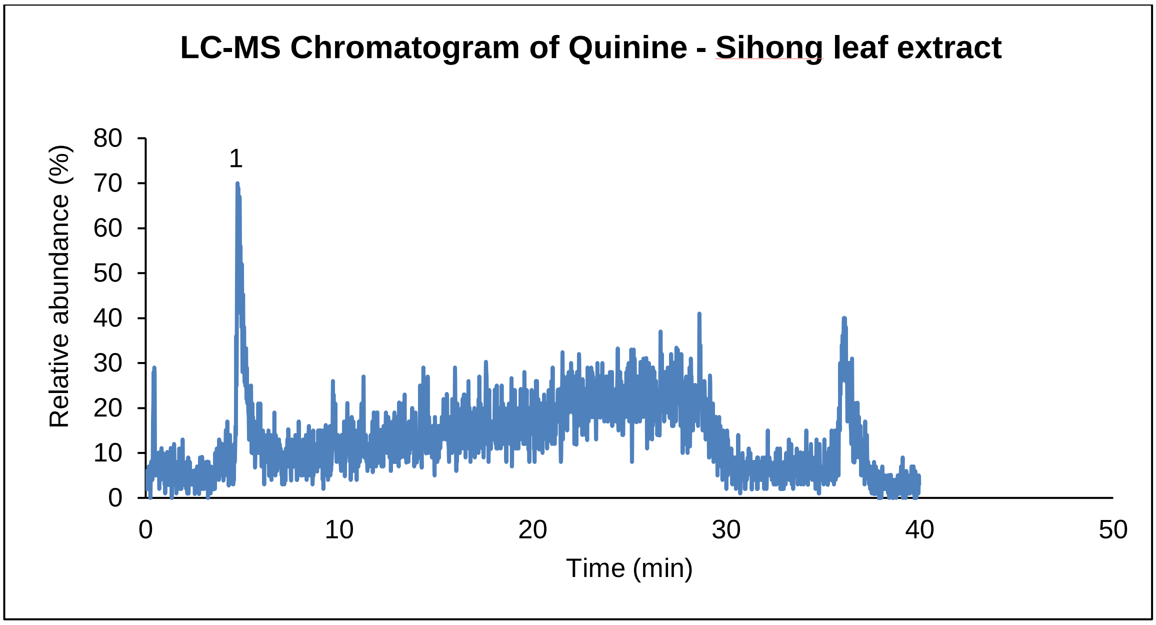

Figure 4.5. UPLC-ESI-MS chromatogram showing Quinine in the Sihong leaf extract

Table 4.3. Quinine of Sihong ethanolic leaf extracts identified and quantified (Quinine) by UPLC-ESI-MS obtained using positive ion mode (R^2 = 0.89)

Peak No	Rt (min)	Compound	MW (Da)	Regression equation	R^2
1	4.64	Quinine	325.14	y=0.0141984x-17.1088	0.890003

Table 4.4. Quantification of the bioactive compounds Rutin, Quercetin, Ursolic acid and Quinine in ethanolic extracts of leaves, fruit and seeds of the five Jujube cultivars. The results are shown in mg/g dried extract.

	Cultivar	Rutin	Quercetin	Ursolic acid	Quinine
Leaves	**Li 2**	28.29	0.69	1.46	<23.02
	Chico	16.07	0.55	0.38	<26.13
	Shanxi Li	50.98	0.53	1.83	<27.44
	Sihong	34.27	1.01	3.15	<21.03
	Honeyjar	30.47	0.42	0.24	<24.58
Fruit	**Li 2**	0.05	<0.05	<0.03	<0.05
	Chico	<0.05	<0.05	<0.04	<0.05
	Shanxi Li	<0.05	0.07	0.04	<0.05
	Sihong	<0.05	<0.05	0.05	<0.05
	Honeyjar	0.06	<0.05	<0.04	<0.05
Seed	**Li 2**	<0.05	0.12	0.09	<0.05
	Chico	0.07	0.15	0.10	<0.06
	Shanxi Li	<0.05	0.11	0.23	<0.05
	Sihong	0.15	0.10	<0.03	<0.05
	Honeyjar	0.16	0.11	<0.03	<0.06

4.4. Potential anticancer property of Jujube cultivars (Leaves/Fruits/Seeds)

Ethanolic leaf extracts of the Jujube cultivars (Li2, Chico, and Sihong) were cytotoxic to HCT116, a colon cancer cell line (Fig. 4.6). All leaf extracts showed close to 100% cytotoxicity in 48-h exposure treatments at a concentration of 5 mg mL^{-1}, which exceeded the cytotoxicity of 5-fluoro uracil. Li 2 cultivar showed significantly high cytotoxicity among three cultivars, but there was no significant difference between Chico and Sihong cultivars in the cytotoxicity of the cultivar extracts at the highest concentration used (p = <0.05); Appendix: Table A.3.1). In contrast ethanolic extracts of fruit and seed of these cultivars showed no cytotoxicity to HCT116 cell lines at these concentrations (Appendix: Figures A.3.2, A.3.3).

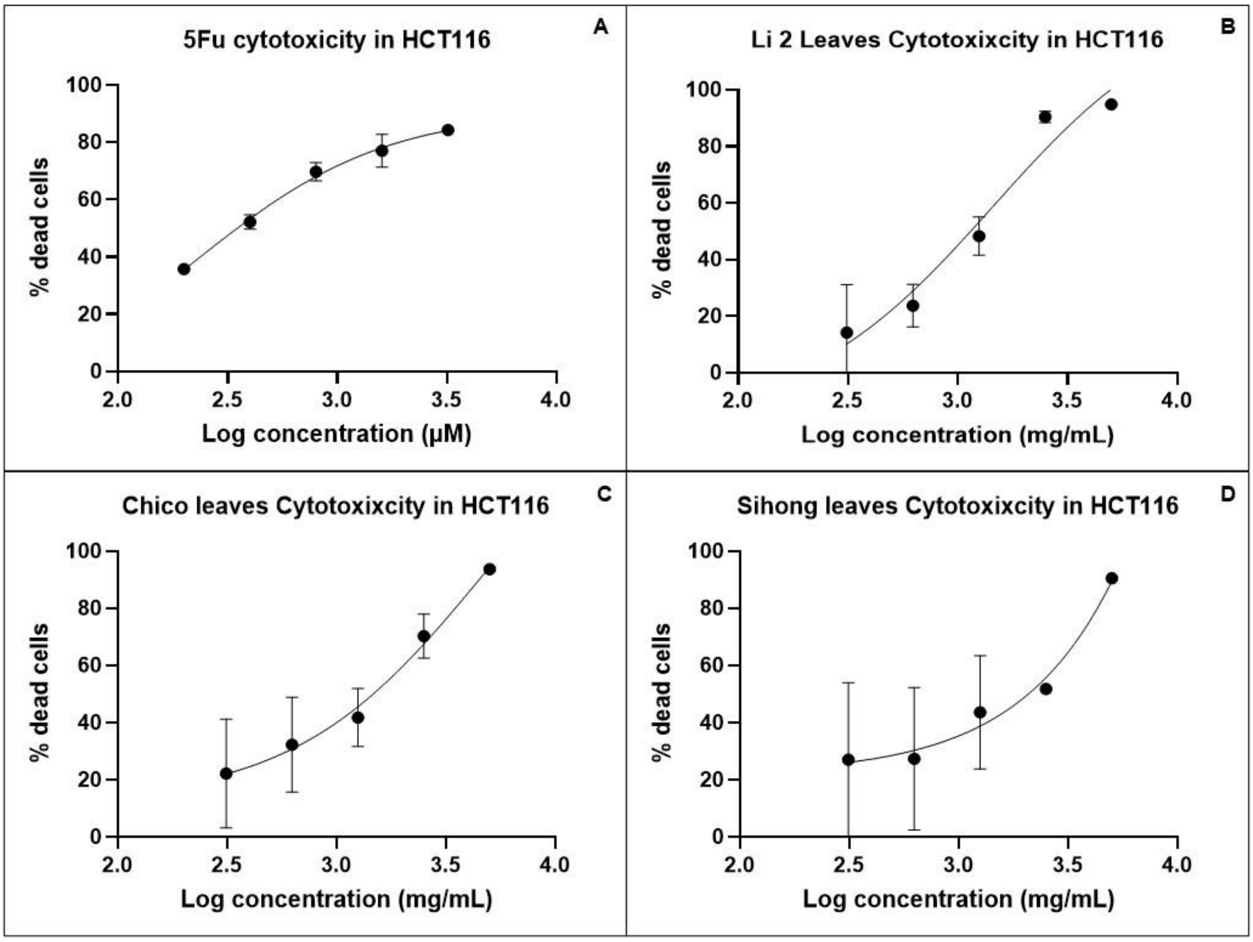

Figure 4.6. 48-h dose response curves for cytotoxicity of Jujube ethanolic leaf extracts on HCT116 cells (A) 5 fluoro uracil. (B) Li 2 leaf extracts. (C) Chico leaf extracts. (D) Sihong leaf extracts. n = 2.

CHAPTER 5. GENERAL DISCUSSION

5.1. Biochemical composition

The biochemical composition of leaves, fruit and seeds of SA Jujube cultivars (*Ziziphus jujuba* Mill) was determined in this research. This study is the first conducted on Jujube cultivars grown in South Australia and is the first to document the biochemical composition of Australian grown cultivars in general. Various studies have been conducted on leaves and seeds of the *Ziziphus* family, but not on *Ziziphus jujuba* Mill. Therefore, detailed knowledge on the biochemical composition of Jujube leaves and seeds, including bioactive compounds, is very limited. A detailed literature search using Google Scholar and Web of Science found that research was carried out to investigate specific active compound groups only. Therefore, a comparison with research results obtained here will be limited to those compounds.

The protein content of fruit of Jujube cultivars grown in Iran, Syria, and various Chinese provinces is generally low, only up to 7.1 g/ 100 g DW (Table 5.1). The protein content of SA jujube fruits was, however, 10 to 12 times lower. Previous research showed that fruit protein content is inversely correlated with fruit maturity, specifically, senescence-induced reduction in water and protein contents per unit fruit weight (Choi et al. 2012). As fruit used in this research was very ripe bordering on senescence, this could be the primary reason for the low protein content. It is, however, likely that differing climatic, soil, species and genotypes additionally affected protein content, as levels vary widely (Table 5.1). Future analyses on the effect of fruit maturity of SA Jujube cultivars would assist to quantitate the impact fruit maturity has by itself on protein content.

The protein content of Jujube leaves and seeds are not published; therefore, this discussion will compare contents with some edible crops (Table. 5.2). Leaves and most seeds of the edible crops had a 5-50 times higher protein content, and even date seeds, which had the lowest protein content for leaves and seeds were 5-10 times higher compared to the levels found in SA jujube seeds.

In general, total carbohydrate contents of the five cultivars analysed here were lower than those reported for fruit of Jujube cultivars harvested in the Minqin county Gansu province, China (89.73 g/ 100 g DW) & eastern China (85.63 g/ 100 g DW), Shandong province, China (84.85 g/ 100 g DW), Chinese Xinjiang Hotan red dates (73.6 g/100 g DW), Korea. ((Choi et al. 2012); (Rahman et al. 2018a)).

Table 5.1. Comparison of water (%), total protein (g/ 100 g DW), total carbohydrates (g/ 100 g DW), and total dietary fibre (TDF) (g/ 100 g DW) of SA Jujube cultivars with other cultivars fruits.

Source/ Reference	*Ziziphus jujuba cv*	Water %	Total Protein	Total Carbohydrates	TDF
Leaves (this study)	**Li 2**	71.31	0.764±0.01	5.948±1.17	35.5
	Chico	68.36	0.607±0.02	9.110±0.69	35.5
	Shanxi Li	65	0.775±0.01	13.059±1.56	33.3
	Sihong	74.19	1.122±0.01	4.955±0.56	33.7
	Honeyjar	66.8	2.027±0.02	1.695±0.49	33.9
Fruit (this study)	**Li 2**	74.8	0.510±0.05	58.454±4.64	10.5
	Chico	68.36	0.475±0.09	47.304±3.32	8.9
	Shanxi Li	73.57	0.588±0.02	54.646±0.89	8.9
	Sihong	65.11	0.3391±0.03	33.443±6.07	8.5
	Honeyjar	70.6	0.470±0.02	39.169±3.07	6.7
Seeds (this study)	**Li 2**	50.39	-	11.369±1.92	76.7
	Chico	39.25	0.05305	30.464±5.86	64.4
	Shanxi Li	53.56	0.102±0.02	20.876±1.95	85.5
	Sihong	73.34	0.058±0.02	16.077±1.97	56.4
	Honeyjar	65.11	0.354±0.02	3.806±1.34	75.8
(Li et al. 2007) (fruit)	**Jianzao, Yazao, Junzao**	-	5.762±0.9	82.375±1.5	2.79
(Rahman et al. 2018b) (fruit)	**Hupingzao, Huizao, Junzao, Xianzao**	36.53±8.17 (moisture)	5.219±0.59	87.485±3.02	-
(Chen, K et al. 2019) (fruit)	**Dazao, Junzao, Huizao**	68.5, 67.5, 63.6	3.97, 1.87, 2.5	-	7.32, 5.0, 5.6
(Saja, Manal & Francois 2021) (fruit)	**Junzao, Yazao (Syria)**	-	4.8, 7.2	60.7, 51.0	6.0, 9.6
(Hernandez et al. 2015) (fruit)	**Spain**	-	3.7-5.0	-	0.7-1.0
(Hoshyar et al. 2015) (fruit)	**Iran**		5.1 to 7.1		
± Standard error of the mean					

Similarly, carbohydrate contents were generally higher in leaves and seeds of edible crops (Table 5.2), but comparable for spinach and Shanxi Li leaves (Tables 5.1 and 5.2), while Li 2 and Honeyjar were the only cultivars that had a lower seed carbohydrate content compared to seeds of Pisum sativum, with the remaining cultivars being up to 2-fold higher. Date pits had comparable carbohydrate content compared to Honeyjar, while the other cultivars exceeded that content 2-6 times (Tables 5.1 and 5.2).

Table: 5.2. Comparison of total protein, taal carbohydrates, and total fibre contents in Ziziphus jujuba leaves, seeds, spinach, lettuce, date pits, barley, and legumes (*pisum sativum*)

Source	Total protein %	Total carbohydrates %	TDF%	References
Recommended daily intake (g)	46-64	310	25-30	(National Health and Mediacal Research Council 2017)
Ziziphus jujuba leaves (this study	0.607-2.02	1.69-13.05	33-35.5	-
Ziziphus jujuba seeds (this study)	0.05-0.35	3.80-30.46	56.4-85.5	-
Spinach (*Spinacia oleracea*)	31.15	16.4	24.26	(El-Sayed 2020)
Lettuce (*Lactuca sativa* L.)	24-26.42	22-26.13	26-28.74	(Sularz et al. 2020)
Date pits	4.8-6.9	2.4-4.7	67.6-74.2	(Ahmad & Imtiaz 2019)
Barley	8-13.8	78-83.9	11.6	(Henry 1988)
Legume seed (*Pisum sativum*)	23-31	14	15-21	(Gatehouse, Croy & Boulter 1980), (Guillon & Champ 2002)

Total dietary fibre (TDF) is defined as the indigestible carbohydrates and lignins (Nguyen et al. 2019). As the carbohydrates are insoluble, these are not captured in total carbohydrate contents reported above. In general, total fibre contents of the five cultivars analysed here were higher than those reported for fruit of Jujube cultivars harvested in China, Syria as reported by Chen, K et al. (2019) and Hernandez et al. (2015). The total fibre contents in SA Jujube leaves were higher than in spinach (*Spinacia oleracea*) and lettuce (*Lactuca sativa*). (Table 5.2). The total fibre in the Jujube seeds of all cultivars was 5-8 times higher than in barley, and 3-4 times higher than in legume seeds (*Pisum sativum*), while date pits had similar amounts (Table 5.2).

Potassium levels of fruit of the five Jujube cultivars were 2-10 times higher than in Chinese cultivars reported by Li et al. (2007), and 3-4 times higher than for Spanish cultivars reported by Saja, Manal and Francois (2021), while the calcium content was similar to both studies. Iron content was 5-10 times lower than for cultivars reported by Li et al. (2007) and Saja, Manal and Francois (2021). The mineral content investigated in the four cultivars of Jujube leaves from Turkey by San et al. (2009) were similar for Ca, but higher for Mg, K, and Fe for leaves of SA jujube cultivars (Table 5.3). Similarly, the Mg and K contents in SA Jujube leaves were higher than reported for *Ziziphus mauritiana* from the Republic of Niger (Table 5.3). For fruit, Mg and K contents were 100 to 120% higher for Mg and Ca in the cultivars investigated here than reported by San et al. (2009) (Table 5.2).

It is evident that, the potassium content in SA Jujube is 5 to 10 times higher than those reported by Li et al. (2007). While Mg and Ca contents of SA Jujube fruits were similar to the results produced Li et al., iron content in SA Jujube was 6 to 8 times lower than reported for Chinese cultivars. (Li et al. 2007). This could be explained by differing climatic conditions, harvesting time and cultivars. (Li et al. 2007). In general, the mineral contents of Jujube leaves of different cultivars are notably higher than in Jujube fruits. It is evident from this study that SA Jujube leaves and fruits are a good source of K, Mg and Ca. This study showed that, the seeds of SA jujube are also a good source of minerals since the seeds showed higher contents of minerals than fruits. (Table 5.3). However, evaluation of other minerals, like Na contents in SA Jujube cultivars is needed and it might be the promising future direction of this study.

Table 5.3. Mineral content (Fe, Mg, Ca, K) in the Jujube leaves, fruit and seeds in mg/ 100 g DW.

Source/ Reference	*Ziziphus jujuba cv*	Fe	Mg	Ca	K
Leaves	**Li 2**	14.08	413.13	4117.32	1733.7
	Chico	23.33	385.83	4018.08	1676.4
	Shanxi Li	48.86	377.4	3984.11	1536.9
	Sihong	41.83	351.91	4248.42	1588.7
	Honeyjar	44.19	360.95	4661.04	1371.3
Fruits	**Li 2**	<LOQ	46.28	77.63	911.76
	Chico	0.88	39.03	94.55	900.18
	Shanxi Li	<LOQ	44.86	69.76	1026.43
	Sihong	0.76	36.62	62.94	842.12
	Honeyjar	<LOQ	46.24	101.74	843.78
Seeds	**Li 2**	5.71	147.81	589.78	445.89
	Chico	4	69.52	277.17	590.02
	Shanxi Li	4.76	162.51	227.71	881.62
	Sihong	3.16	54.49	158.34	622.42
	Honeyjar	<LOQ	14.38	55.98	191.84
(Wang, L et al. 2018)	**Shanxi province, China (15 cultivars fruit)**	5.27-12.5	51.2-70	16.2-30.2	-
(Hernandez et al. 2015)	**Spain (4 jujube fruit cultivars)**	10.2-17.1 mg/kg	0.40-0.77 g/kg	0.23-0.72 g/kg	11.9-17.3 g/kg
(Li et al. 2007)	**China (five fruit cultivars)**	4.7-7.9	24.6-51.2	45.6-118	79.2-458
(Saja, Manal & Francois 2021)	**Junzao, yazao fruit (Spain)**	9.2, 7.7	-	65.8, 91.9	201, 179.1
(Sena et al. 1998)	**Jujube cultivars (leaves)**	14.08-48.86	239.67-271.33	3612.70 - 4961.30	751-1078
(San et al. 2009)	**Four Jujube cultivars leaves (Turkey)**	16.3-21.33	239.6-271.33	3612-4961.3	751-1078.3
(San et al. 2009)	**Four Jujube cultivars fruit (Turkey)**	0.67-1.43	18.1-20.87	79.33-121.33	314.67-420

Table 5.4. Comparison of minerals (Fe, Mg, Ca, K) in Jujube cultivars (mg/ 100 g DW) and estimated human daily requirement (mg)

Estimated daily requirement/ Source	Fe	Mg	Ca	K	Reference
♂ (male)	6	350	840	3800	(National Health and Mediacal Research Council 2017)
♀ (female)	8	265	840	2800	
Leaves	14.08-48.86	351.91-413.13	3984.11-4661.04	1371.3-1733.7	-
Fruit	0.76-0.88	36.62-46.24	62.94-101.74	842.12-1026.43	-
Seeds	3.16-5.71	14.38-162.51	55.98-589.78	191.84-881.62	-

5.2. Quantification of Bioactive compounds and antioxidant capacity

The content of bioactive compounds in the SA Jujube fruit were similar to previous studies (Ref to Table 5.5), but levels are strongly influenced by fruit maturity, origin, environmental -, geographical -, and climatic conditions, as well as the nature of the soil Choi et al. (2012), which are often not completely documented. Leaf extracts of SA Jujube cultivars contained significantly higher amounts of polyphenols, flavonoids and triterpenic acids compared to seed and fruit extracts. The contents of Jujube secondary metabolites: total phenolics, total flavonoids, total triterpenoids and antioxidant capacity (FRAP) are summarised in Table 5.5.

Flavonoids are the major group of phenolics. Rutin (Quercetin-3-O-rutinoside) is the highest flavonoid present in the Jujube leaves, fruit and seeds of all cultivars. Rutin is a common dietary flavonoid with many pharmacological activities such as antioxidant, anticancer, anti-inflammatory activities (Song, L et al. 2019). Among identified peaks in the LC-MS analysis of Jujube leaf extracts, Rutin (Quercetin-3-O-rutinoside) is the highest flavonoid present in all cultivars studied here, which is consistent with other reports on Jujube fruit (Gao et al. 2012). In general, rutin has been reported to be present in more than 70 plant species (Gao et al. 2012). Consistent with reports of previous studies, Rutin is major the flavonoid in jujube fruits (Gao et al. 2012). The higher phenolic compound contents reported in this study could be explained using ultrasound-assisted extraction, but the impact of solvent used and/or the combination of extraction technology and solvent could also impact these results. Recovery of polyphenols from Jujube was influenced by solvent used for extraction (Cadi et al. 2020), but the impact of different green extraction technologies was not investigated. Hence, future studies should examine the impact of solvent in combination with a range of green extraction technologies for the development of processing pathways for the emerging industry.

In general, Jujube leaves had a higher phenolic compound content than Jujube fruits (San & Yildirim 2010). For leaves, Rutin content was 10 to 15-times higher in SA Jujubes compared to Xinjiang Jujube leaves extract, while the total flavonoid content (TFC) was only 2-times higher (Table 5.5). Song, L et al. (2019)'s study showed that Kaempferol-3-O-rutinoside (593 Da) was not present in Xinjiang Jujube leaves, but it was detected in SA Jujube extracts. Catechin (flavan-3-ol) content of Jujube leaves were reported by San and Yildirim (2010), whereas it was not detected in this study. This proves that the flavan-3-ols group of flavonoids is not present in SA jujube cultivars. The rutin content of Turkey Jujube cultivar leaf extracts reported by San and Yildirim (2010) were 10 to 15-times lower, ranging from 0.269 to 0.367 g/ 100 g DW), compared to SA jujube leaves (1.6 to 5.1 g/ 100 g DW).As extraction conditions were similar (they also used ultrasound-assisted ethanol extraction and optimised supercritical CO_2 extraction), Extraction conditions are unlikely the reason for the observed differences. It appears to be more likely that cultivar and/or growth conditions also impact flavonoid yields.

The TFC in SA jujube fruit (0.43 to 0.82 g/100 DW) was much lower than reported for immature Korean Jujube fruits (26.52 g/100 g DW) but higher than in mature Korean Jujube fruits (0.35 g/100 g DW) (Choi et al. 2012). The study by Choi et al. (2012) also showed that Jujube fruits cultivated in Korea contained epicatechin (flavan-3-ol) (Choi, S-H et al. 2012a), whereas no peak was observed for epicatechin in SA jujube cultivars. The same study showed that the water, protein, total flavonoid contents, and antioxidant capacity were 20%, 10, 26, and 3-times lower, respectively, in fully mature Jujube fruits (reddish brown colour) compared to the immature green stage (Choi et al. 2012).

No flavonoids and saponins were identified in Jujube seed extracts from eight cultivars grown in Thailand (Taechakulwanijya et al. 2016), but rutin and quercetin were detected in seeds of the SA cultivars.

The anti-oxidant capacity generally correlates with amounts of phenolic compounds (Li et al. 2007). Many studies observed significant correlations between total phenolic, flavonoid and total triterpenoid contents and anti-oxidant activity (Yang et al. 2009). Similarly, a positive correlation was observed for SA Jujube leaf, fruit and seed extracts. Leaf antioxidant activity ranged from 90.87 to 229.534, 8.47 to 16.4 for fruit, and 12.62 to 32.99 mmol $FeSO_4$ eq/ 100 g DE) for seeds extract. The antioxidant capacity of SA Jujube fruit extract is lower than reported for Spanish Jujube (17.6 to 34.82 mmol $FeSO_{4\ eq}$/ 100 g DW) (Wojdyło et al. 2016). A study by Choi et al. (2012) concluded that the antioxidant capacity of Jujube fruits decreased with the maturity of fruits, which could be a reason for the low activity of SA Jujube fruit.

In contrast anti-oxidant activity of leaf extracts were higher for SA Jujube cultivars compared to Chinese Jujube leaf extracts of *Zizyphus jujuba* Mill cv. Junzao, cultivated in Xinjiang province, China at optimal extraction conditions (12.68±0.211 mmol $FeSO_{4\ eq}$/ 100 g

DW (Song, L et al. 2019). The results of the FRAP assay should be interpreted with caution, as typically studies use a number of assays to determine full antioxidant capacity. Thus, to confirm the antioxidant capacity of the Jujube extracts, future research should include using the DPPH radical scavenging assay, as it quantifies antioxidant capacity, which is different to antioxidant activity FRAP assay, determining the content of functional antioxidants.

Table 5.5. Estimation of total polyphenols (g/100 g DW), total flavonoids (g/100 g DW), total triterpenes (µg/ g DW) and FRAP (mmol $FeSO_4$/100 g DW) activity of SA Jujube cultivars

Source	Cultivar	Total Polyphenols	Total Flavonoids	Total Triterpenes	FRAP activity
Leaves (This study)	**Li 2**	14.707±0.77	5.130±0.06	209.35±9.53	90.876±8.29
	Chico	17.114±0.38	4.538±0.14	190.73±3.58	144.60±11.17
	Shanxi Li	16.840±0.08	5.218±0.13	365.75±9.63	229.53±5.95
	Sihong	18.570±0.10	6.269±0.2	218.48±4.97	173.45±23.07
	Honeyjar	16.771±0.04	4.284±0.26	339.56±6.63	162.40±38.35
Fruits (This study)	**Li 2**	0.982±0.02	0.829	36.63±0.79	13.162±0.90
	Chico	1.541±0.01	0.720±0.02	48.70±2.96	13.162±0.36
	Shanxi Li	1.227±0.04	0.762±0.03	64.19±4.44	16.408±0.18
	Sihong	1.452±0.09	0.468	69.75±1.39	10.097±1.08
	Honeyjar	1.247	0.432	42.99±0.66	8.474±0.54
Seeds (This study)	**Li 2**	3.286±0.38	0.288±0.01	61.31±13.99	28.849±1.44
	Chico	2.183±0.03	1.167±0.01	103.08±4.44	31.734±1.80
	Shanxi Li	2.521±0.01	0.962±0.12	122.12±6.49	32.996±1.26
	Sihong	4.433±0.60	0.707±0.12	141.79±1.7	17.850±2.34
	Honeyjar	2.742±0.07	0.912±0.30	114.40±5.43	12.621±1.08
(Wojdyło et al. 2016)	**Four Spanish Jujube cultivars (fruit)**	1.44-3.43	-	-	17.66-34.82
(Zhang, H et al. 2010)	**Dongzao, Muzao, Hamidazao (fruit pulp)**	0.813, 0.593, 0.557	0.39, 0.224, 0.217	-	-
(Zhang, H et al. 2010)	**Dongzao, Muzao, Hamidazao (seed)**	0.416, 0.289, 0.228	0.328, 0.160, 0.158		
(Zhang, Y et al. 2021)	**Chinese Jujube fruits (37 cultivars)**	0.845-1.633	0.70-4.11	24.97-78.33 (mg/g DW)	25-62.13 mg Trolox/g DW)
(Siriamornpun, Weerapreeyakul & Barusrux 2015)	**Six cultivars ripe fruit**	1.04-1.51	1.10-1.62	-	134-148 (mmol/g DW)
(Wang, B et al. 2016)	**Jishanbanzao fruit (north china)**	0.07-0.864.73	0.167-1.0		
(Choi et al. 2012)	**Jujube fruit (Korea)**	2.36	1.794		
(Al-Saeedi, Al-Ghafri & Hossain 2016)	**Fruit (Oman)**	64 mg/g	0.29µg/g		
(Lin et al. 2020)	**Miaoli fruit (Taiwan)**	3..83	4.38	-	-
(Song, L et al. 2019)	**Xinjiang Jujube leaves**		2.905		12.68
(Song, L et al. 2020)	**99 cultivars fruit (Xinjiang province)**	-	-	19.21 mg/g	0.968-5.529
± Standard error of the mean					

Triterpenes are secondary metabolites, widely distributed in plants leaves, fruits, stems, and barks etc. Jujube also contain triterpenes and more than 15 triterpenic acids were identified (Song, L et al. 2020). In this study, total triterpene contents were analysed using vanillin-sulfuric acid assay using ursolic acid as a standard. Total triterpene content in the SA jujubes were significantly higher in leaves (190.73 to 365.75 µg/ g DE) compared to fruit and seeds. Total triterpenic acid content of 99 Jujube cultivars was 3730.970 µg/g DW (mean value) under optimal conditions of extraction and total triterpenoid yield was 19.21 mg/ g DW under the optimal conditions. (Song, L et al. 2020), which was significantly higher than results obtained for the SA Jujube cultivars. It is very likely that the assay was not optimal for quantitative analysis of triterpenes (see Section 5.5).

The UPLC-MS results showed that Ursolic acid content is higher in leaves (240 to 3150 µg/ g DE) compared to fruit (<30 to 50 µg/ g DE) and seeds (<30 to 230 µg/ g DE), which, except for leaves, were significantly lower compared to values reported for fruit of the 99 Jujube cultivars (5.267 to 685.32 µg/ g DW) (Song, L et al. 2020) and fruit peel of *Malus domestica* (14300 µg/ g DW). Higher ursolic acid levels were reported for leaves rosemary leaves (15800 µg/ g DW) compared to contents in SA Jujube leaves, with highest amounts found in Sihong (3150 µg/ g DE) and Shanxi Li (1830 µg/ g DE).

Ursolic acid, betulinic acid and oleanolic acid have the cytotoxic effects. To identify the contents of these triterpenic acids, LC-MS analysis has to be performed with specific standards. LC-MS analysis identified maslinic acid isomers, but the detailed structure needs to be analysed. Triterpenes have not been studied in detail for Jujube cultivars, despite proven important pharmacological activities such as anti-cancer, antioxidant, anti-inflammatory activities of maslinic acid, ursolic acid, betulinic acid, and oleanolic acids (Song, L et al. 2020). The lack of published triterpene details limits comparisons of results obtained here.

In summary, the results for SA Jujube fruit characterisation should be revisited, as the semi-red maturity stage of Jujube fruit is most appropriate stage for preserving its bioactive compounds (Zhang, Q et al. 2020) and several studies reported that the bioactive compounds in Jujube fruits is negatively corelated with the fruit maturity (Wang, B et al. 2016). In addition, future studies need to examine extraction processes applied, as factors like extraction conditions, solvent concentrations, liquid-to-solid ratios, and technique of extractions can significantly affect extraction efficiencies (Song, L et al. 2020). Such studies need to be accompanied by comparisons of cultivar, climatic conditions, geographical factor, and harvesting conditions, as these additionally affect the bioactive compounds profile (Song, L et al. 2020). While the extraction process applied in this study is likely more advanced compared to other published data and yielded higher contents for leaves in particular, the low triterpenoid content of fruit may be primarily driven by the advanced fruit age, as large variations have been observed at different stages of fruit maturity (Song, L et al. 2020). Furthermore, anti-oxidant activity studies need to examine the impact of extraction methods, as these can positively or negatively impact on antioxidant activities (Zhang, H et al. 2010).

5.3. Anticancer activity of Jujube extracts

The cytotoxic activity of ethanolic extract of Jujube leaves, fruit and seeds are presented in Fig 4.6. and the photographs for cytotoxic activity (MTT assay) of 96 well plate culture for HCT116 cells in Figure A.3.1. This study simply aimed to see whether or not the crude extract had any effects on a cancer cell line, which it did, but to determine if it could be used in cancer treatment, the inclusion of a positive control (healthy cell line) was needed to show the no general cytotoxic effects. All five concentration of the ethanolic extracts of SA Jujube leaves (0.3125, 0.625, 1.25, 2.5, and 5 mg/ mL) and 5 Fluoro uracil drug (200, 400, 800, 1600, 3200 µM) – used as a positive control – induced cell death in dose-dependent manner, while the no treatment control (cells and media) confirmed the viability of the cancer cells. In contrast, the results of fruit and seed extract cannot be interpreted, as it reacted with the cells causing precipitation. In contrast to SA Jujube leaf effectivity, hot water extracts and ethanol extracts of Jujube leaves showed potent inhibition of growth rate of a human lung cancer cells (A549) at much lower concentrations (50, 100, 500 µg/mL) and the hot water extract of Jujube leaves inhibited 61.3% of the growth of AGS cells (stomach cancer cells) at 1,000 µg/mL concentration (Kim & Son 2011). The extracts concentrations used in that study though were less effective against breast cancer cells (MCF-7), with hot water extracts achieving only 5-15% and ethanol extracts only 16-17% of growth inhibition (Kim & Son 2011). This indicates that effective dosage is cell line dependent, which might explain the difference in effective dosage concentration seen here.

Although the absorbance of the MTT assay could not be quantified for crude extracts of SA Jujube fruit and seeds, likely due to the age of the extract, a colour change in the MTT assay was noted, which could indicate that they are not cytotoxic to HCT116 cells, at least not at the concentrations tested (Appendix: Fig A.3.2, A.3.3). Similarly, aqueous extracts of jujube fruit (500, 1000, 1,800 µg/ mL) were not cytotoxic to breast cancer cells (MDA-MB-468) (Hoshyar et al. 2015) and methanolic extracts of seed and pulp at concentrations of 25, 100, 200 mg/L showed no cytotoxicity in MCF-7, PC3, DU-145, HepG2, C26, HTC, Hella, PCL12, and A2780 cells (Rajaei, Ahmad et al. 2021a). Likewise, aqueous, ethyl acetate and hexane extracts of Jujube seeds showed no significant cytotoxic effect on Jurkat leukemia T cells

(Taechakulwanijya et al. 2016). In summary, SA Jujube leaf ethanol extracts were cytotoxicity to HCT116 cells at high concentrations, but the fruit and seed extracts were not, which is likely corelated with the high contents of bioactive contents present in the Jujube leaf extracts.

5.4. Commercial implications

The nutraceutical industry is a rapidly growing industry due to high potential therapeutic effects, stated to be useful for avoiding the onset of some chronic diseases. Jujube could be of interest the production of nutraceutical products, especially the leaves due to high mineral, flavonoid, dietary fibre, triterpenoid contents, which could be extracted and encapsulated or pressed into tablet form. The key findings of this study are that, leaves of all Jujube cultivars are the great natural sources of Rutin content followed by Quercetin. Rutin is a bioflavonoid with numerous pharmacological properties. Rutin has great commercial value, is sold as an herbal supplement, and has been used in alternative medicine due to strong antioxidant properties. Quercetin acts as antioxidant and scavenges free radicals. Hence, flavonoids such as Rutin, Quercetin, and triterpenoids could be purified for the production of dosage-controlled nutraceutical products. The high phenolic contents of Jujube leaves could be exploited for skin care products (Gallic acid).

Nutraceuticals in form of minerals, vitamins, dietary fibres, antioxidants are recommended for treatments of cardiovascular diseases. Jujube exhibits a high content of total dietary fibre and could be used to produce fibre supplements. However, further investigation is required to define soluble and insoluble fibres in Jujube samples.

5.5. Project limitations

This study is limited in replication for some analytical procedures, and it also explored only a single extraction method - ultrasound-assisted extraction with 80% ethanol as a solvent –due to time and financial constraints of a Master research project. There may be an advantage to explore other extraction techniques such as supercritical CO_2 extraction for flavonoids, microwave-intensified extraction with ethanol as a solvent, which could not be done in the microwave available, and exploring the effects of different ultrasound extraction conditions on yields. Also, investigations into bioactive compound contents by UPLC-MS and mineral contents only had one replicate due to financial limitations, hence data are pre-liminary and should be interpreted with caution. Ursolic acid, used as a standard, was too expensive ($140 per 5 mg) made triplicate analysis financially impossible for total triterpenoid content, hence there was no replication possible. The assay used for the quantification of total triterpenoid contents may have underestimated the true contents, because of the inexperience with conducting the assay, although due diligence was applied to follow the SWP in every detail. Likewise, the FRAP assay is likely not the best analytical procedure to assess antioxidant activity, but time constraints did not allow to apply other antioxidant assays, such as the DPPH radical scavenging assay, ABTS radical scavenging assay, which are more commonly used (Moniruzzaman et al. 2011). For the anti-cancer activity, time constraints did only allow for the testing of the crude extracts on a single cancer cell line (HCT116). Clearly, future studies need to broaden the investigations to include a normal cell line and other cancer cell lines and testing of isolated defined bioactive extracts (separated and quantified polyphenols and flavonoids), in addition to refining cell cytotoxicity assays.

5.6. Future directions

To translate biochemical composition into nutritional health applications requires to define safe dosages for some of the bio actives. Similarly, to validate bioactive compounds perform similarly in-vivo, animal studies and more cell line studies are needed. To validate the potential anti-cancer activity, future studies need to demonstrate that crude extracts or purified compounds are not cytotoxic to healthy cell lines at effective concentrations. Based on the bioactive profile of Jujube cultivar leaves, the industry should evaluate if non-fruit-bearing cultivars have the same biochemical profile. If bioactivities are the same, the Jujube industry could plant these for producing high-value health supplements, rather than leaves being a waste due to the deciduous nature.

SA Jujube cultivars contained significant amounts of flavonoids such as rutin and quercetin. The best future direction is to work on purification of crude extract, isolation of different flavonoid compounds, and to assess the antioxidant capacities of extracts on healthy cell lines. Another research focus could be to optimize the extraction conditions of flavonoids (rutin and quercetin) and triterpenoids (ursolic acid, maslinic acid isomers, and betulinic acid) from Jujube leaves and translate into Jujube by-products. Additionally, vitamin content was not investigated in this study. Thus, future research could analyse the ascorbic acid (vitamin C) content in Jujube fruits, since Jujube fruit have been shown to contain high amounts.

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APPENDICES

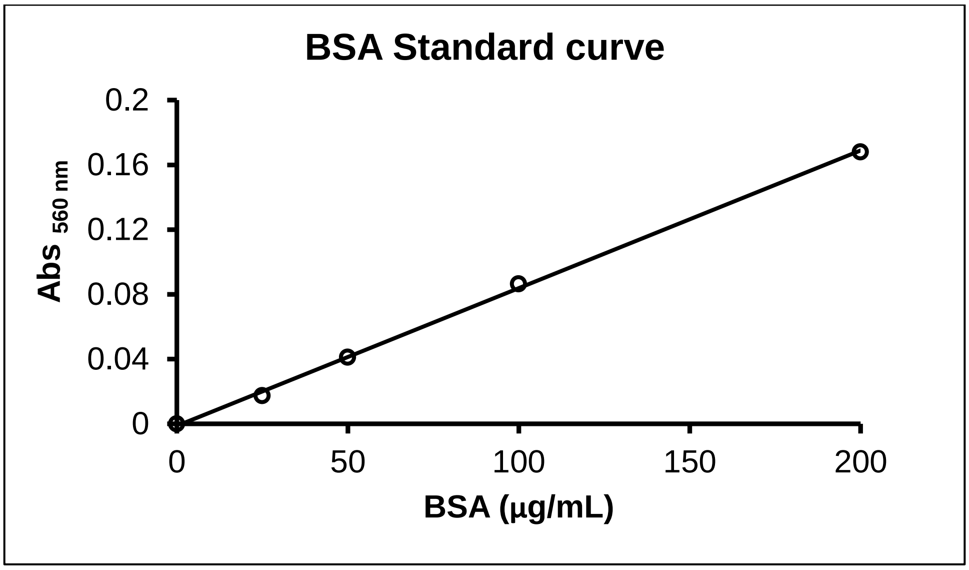

Figure A.1.1. BSA standard curve for estimation of total protein content (y = 0.0009x, r^2=0.999)

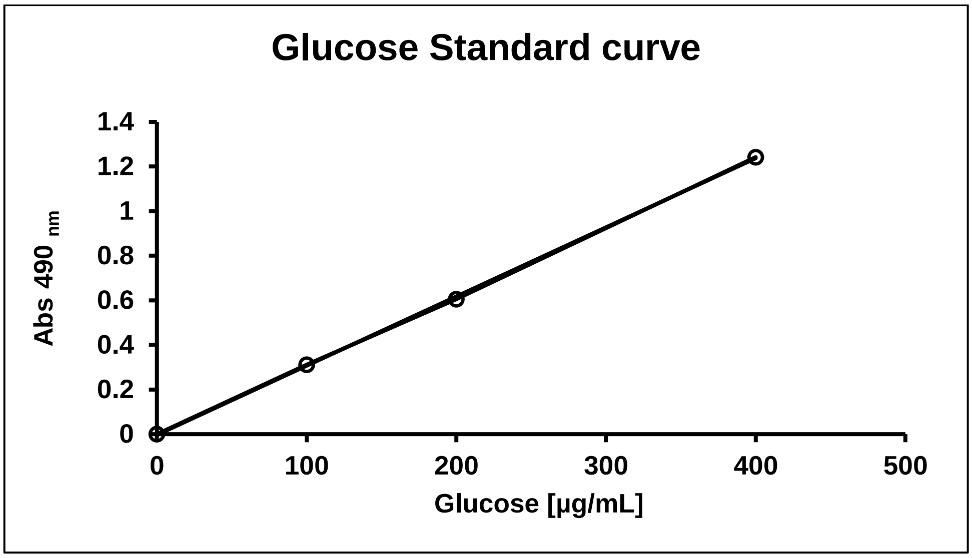

Figure A.1.2. Glucose standard curve for estimation of total protein content (y = 0.0031x, r^2=0.999)

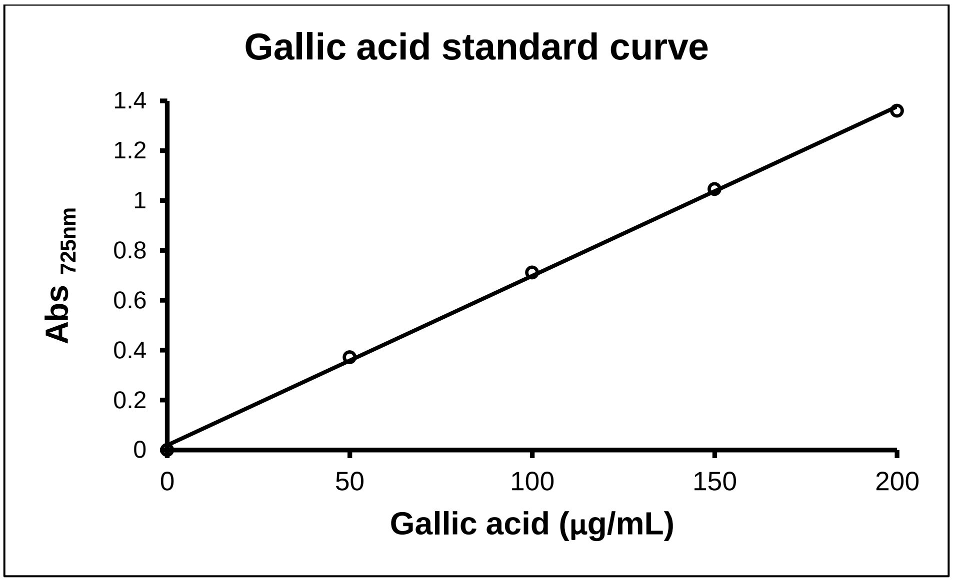

Figure A.1.3. Gallic acid standard curve for estimation of total phenolics content (y = 0.0068x, r^2=0.999)

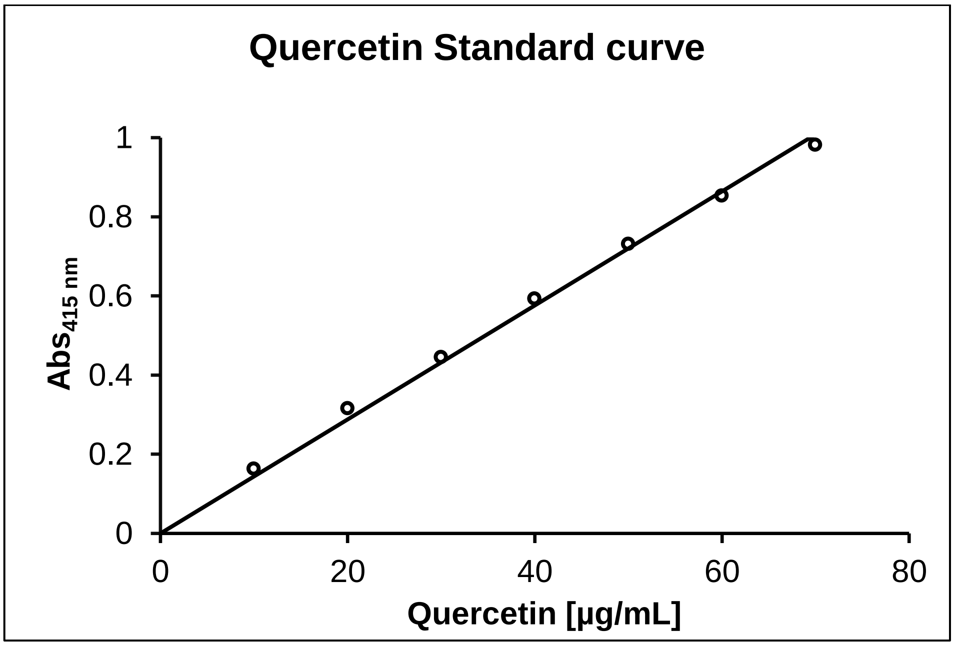

Figure A.1.4. Quercetin standard curve for estimation of total flavonoid content (y = 0.0136x, r^2=0.999)

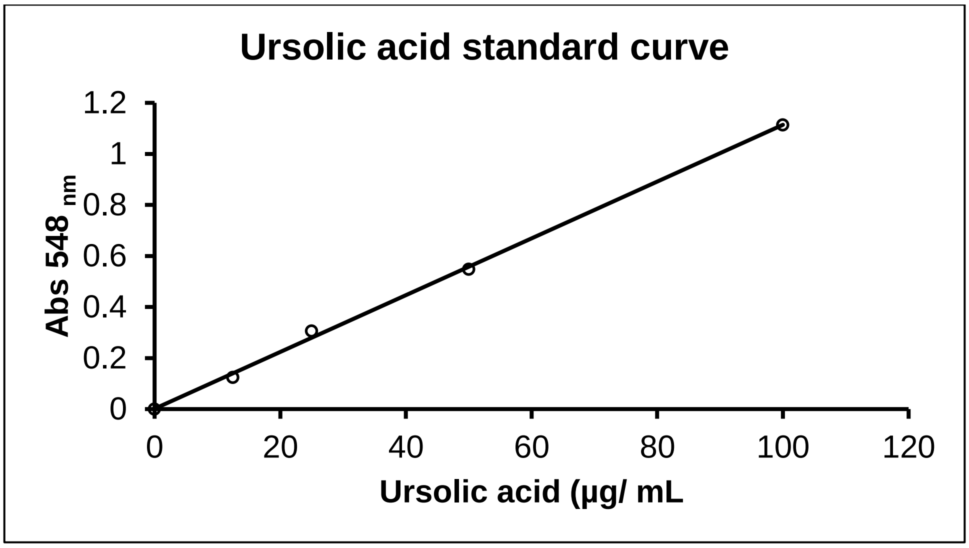

Figure A.1.5. Ursolic acid standard curve for estimation of total triterpenoids content (y = 0.0111x, r^2=0.998)

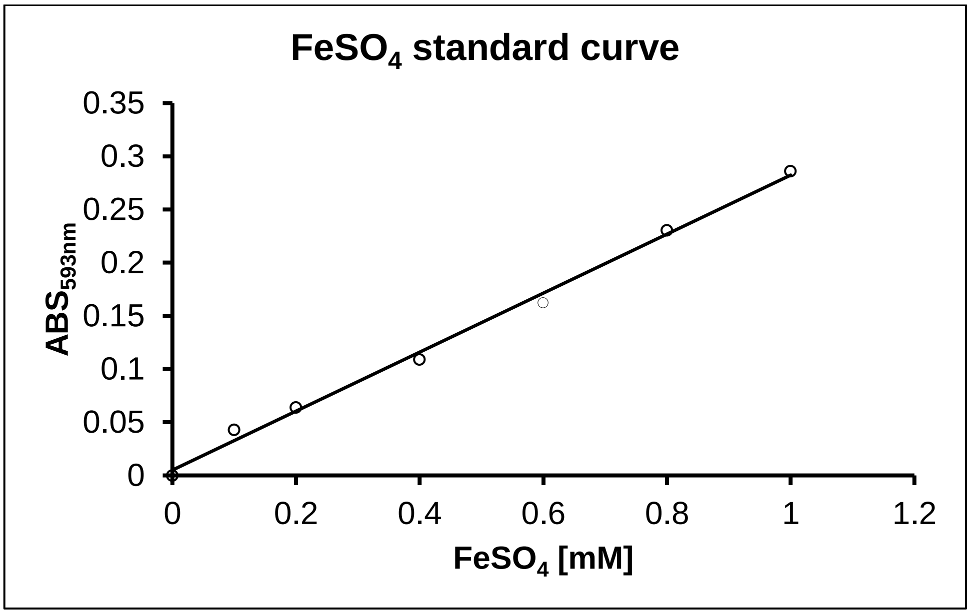

Figure A.1.6. $FeSO_4$ standard curve for estimation of total flavonoid content (y = 0.2773x, r^2=0.995)

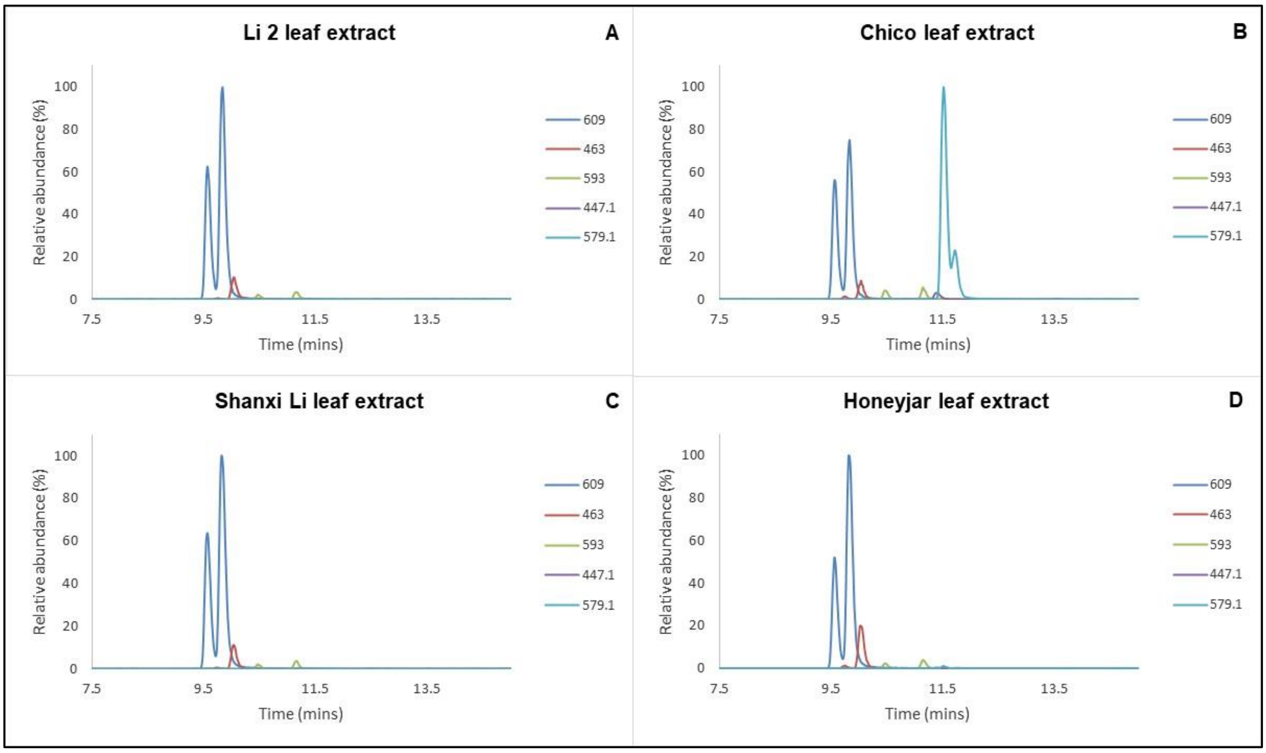

Figure A.2.1. UPLC-ESI-MS chromatograms showing flavonoid compounds in ethanolic leaf extracts of (A) Li 2 leaf extract, (B) Chico leaf extract, (C) Shanxi Li leaf extract, (D) Honeyjar leaf extract.

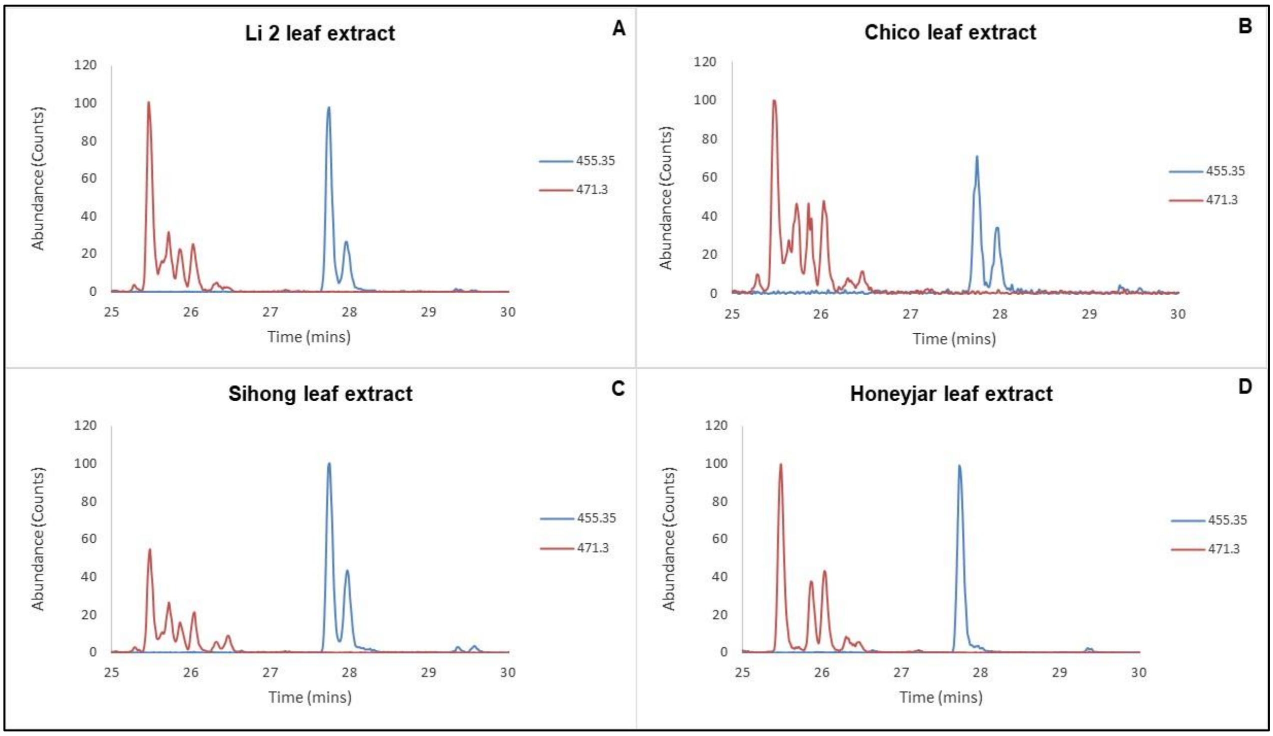

Figure A.2.2. UPLC-ESI-MS chromatograms showing triterpenic acids in ethanolic leaf extracts of (A) Li 2 leaf extract, (B) Chico leaf extract, (C) Sihong leaf extract, (D) Honeyjar leaf extract.

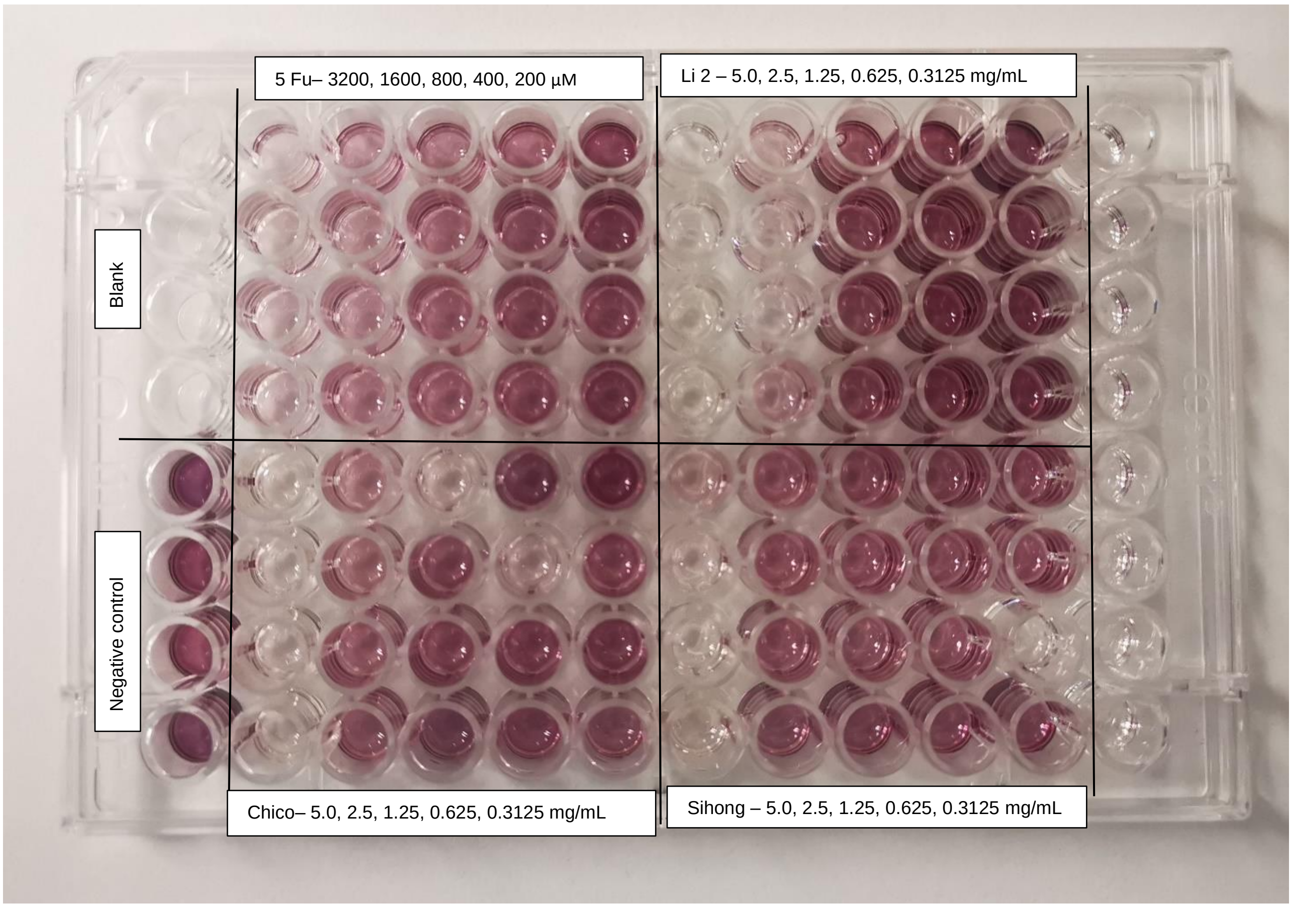

Figure A.3.1. MTT assay plate showing HCT116 cells treatment with 5-Fu, Li 2, Chico, Sihong leaf extracts.

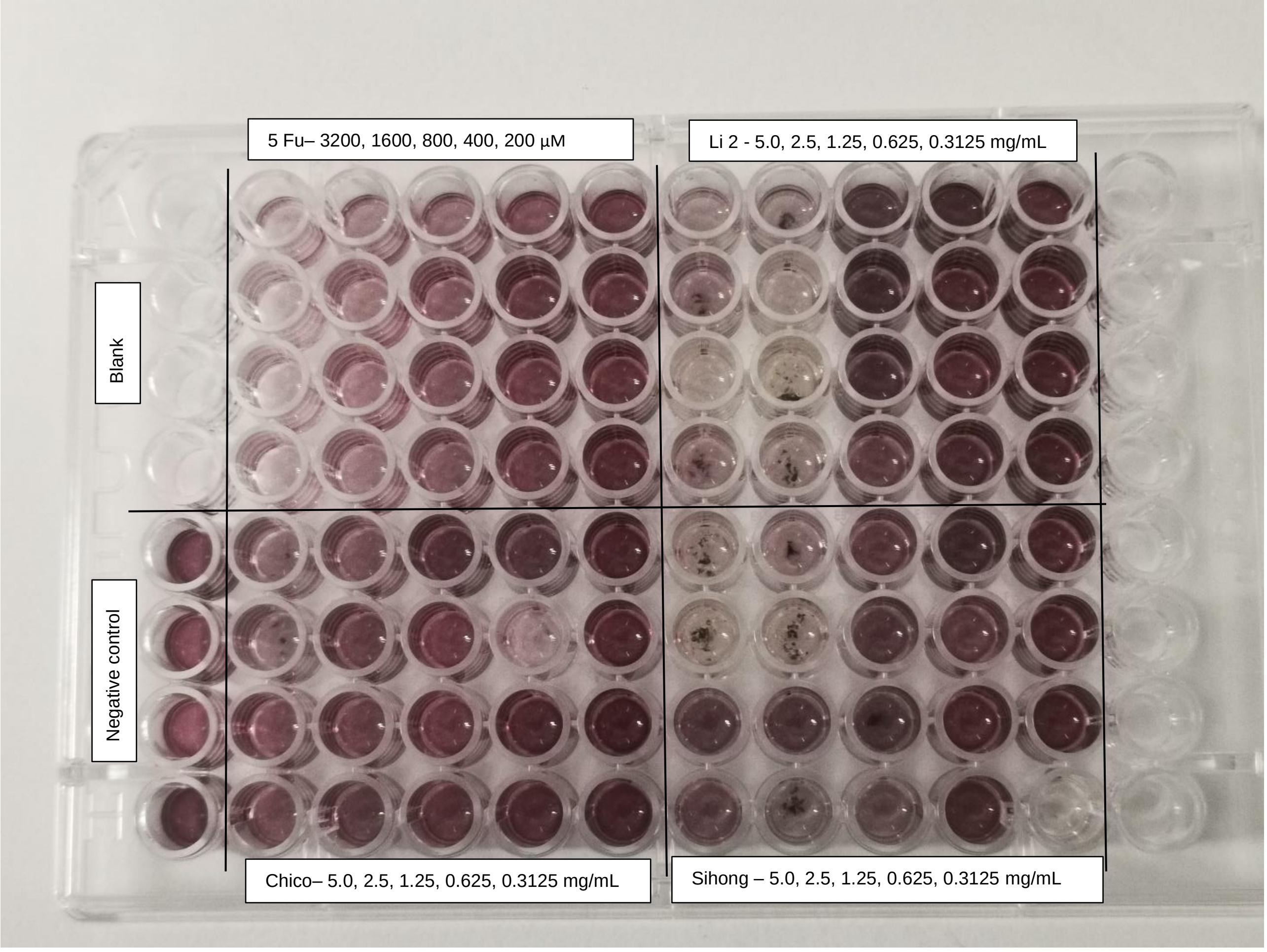

Figure A.3.2. MTT assay plate showing HCT116 cells treatment with 5-Fu, Li 2, Chico, Sihong fruit extracts.

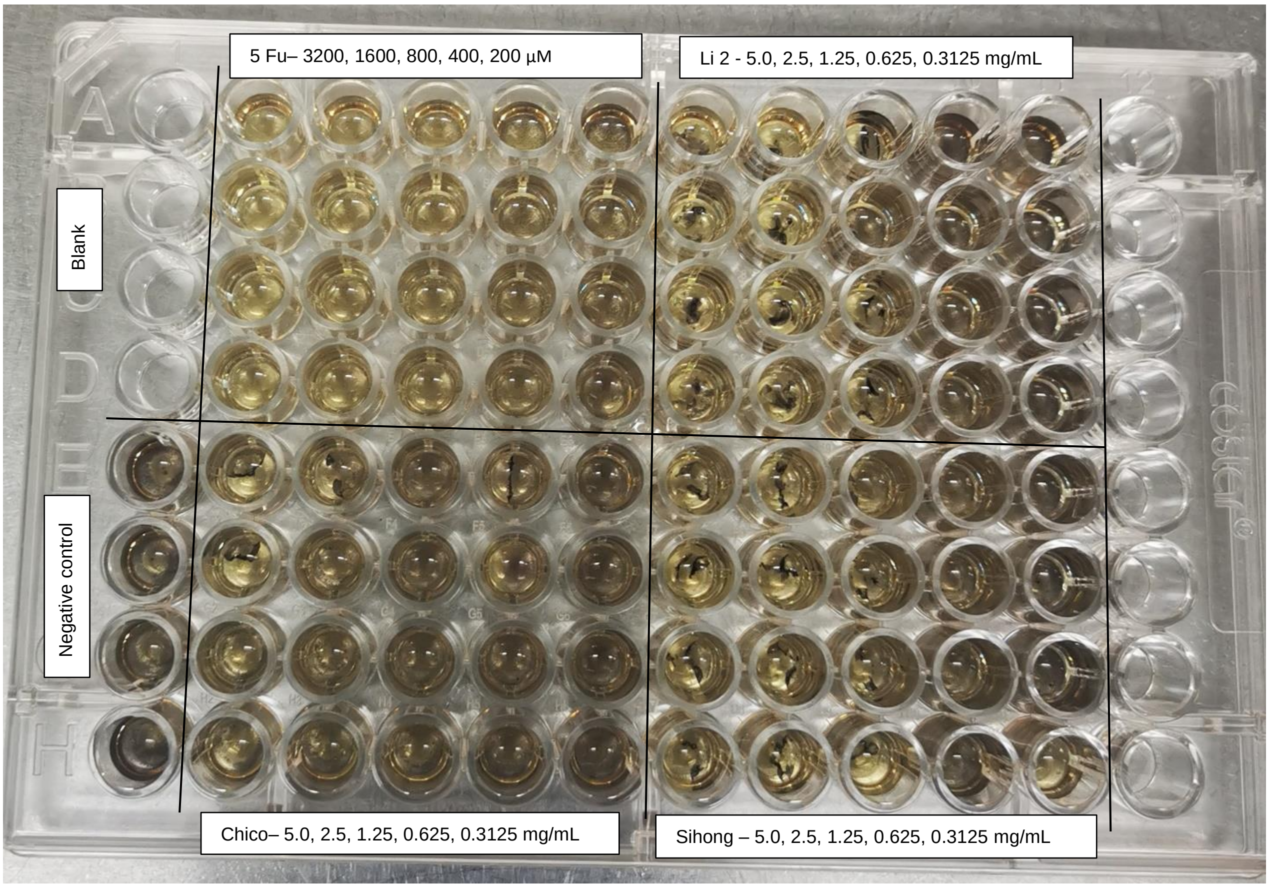

Figure A.3.3. MTT assay plate showing HCT116 cells treatment with 5-Fu, Li 2, Chico, Sihong seed extracts.

Table. A.1.1. The dried extract % from ultrasound-assisted ethanolic extraction of Jujube powder.

Ziziphus jujuba cv	Leaves	Fruit	Seeds
Li 2	31.24	86.3	12.9
Chico	36.1	77.9	16.7
Shanxi Li	40.23	81.5	21.2
Sihong	38.09	81.8	15.8
Honeyjar	13.08	81.6	16.4

Figure A.4.1. Ethanolic extracts of Jujube samples using ultrasound-assisted extraction.

Table. A.2.1. Statistical analysis of total protein contents in Jujube samples (n=3), comparison between leaves, fruit, and seed contents of 5 cultivars.

Tests of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Total_protein	Based on Mean	2.783	13	28	.011
	Based on Median	.798	13	28	.657
	Based on Median and with adjusted df	.798	13	10.274	.655
	Based on trimmed mean	2.584	13	28	.017

Tests of Normality[b]

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Part_of_tree_cultivar	Statistic	df	Sig.	Statistic	df	Sig.
Total_protein	11	.253	3	.	.964	3	.637
	12	.248	3	.	.968	3	.657
	13	.292	3	.	.923	3	.463
	14	.334	3	.	.859	3	.265
	15	.204	3	.	.993	3	.843
	21	.264	3	.	.954	3	.588
	22	.256	3	.	.962	3	.623
	23	.337	3	.	.855	3	.253
	24	.181	3	.	.999	3	.942
	25	.349	3	.	.832	3	.194
	32	.328	3	.	.871	3	.298
	33	.309	3	.	.900	3	.387
	34	.347	3	.	.835	3	.202
	35	.232	3	.	.980	3	.726

a. Lilliefors Significance Correction

ANOVA

Total_protein

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10.125	13	.779	176.333	.000
Within Groups	.124	28	.004		
Total	10.248	41			

Multiple Comparisons

Dependent Variable: Total_protein

Tukey HSD

(I) Part_of_tree_cultivar	(J) Part_of_tree_cultivar	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
11	12	.15650926	.05426312	.242	-.0421156	.3551341
	13	-.01120370	.05426312	1.000	-.2098285	.1874211
	14	-.35830556*	.05426312	.000	-.5569304	-.1596807
	15	-1.26310556*	.05426312	.000	-1.4617304	-1.0644807
	21	.25366111*	.05426312	.004	.0550363	.4522860
	22	.28824074*	.05426312	.001	.0896159	.4868656
	23	.17588333	.05426312	.123	-.0227415	.3745082
	24	.42502778*	.05426312	.000	.2264029	.6236526
	25	.29354630*	.05426312	.001	.0949215	.4921711
	32	.71113889*	.05426312	.000	.5125140	.9097637
	33	.66206481*	.05426312	.000	.4634400	.8606897
	34	.70558333*	.05426312	.000	.5069585	.9042082
	35	.40928704*	.05426312	.000	.2106622	.6079119
12	11	-.15650926	.05426312	.242	-.3551341	.0421156
	13	-.16771296	.05426312	.166	-.3663378	.0309119
	14	-.51481482*	.05426312	.000	-.7134397	-.3161900
	15	-1.41961481*	.05426312	.000	-1.6182397	-1.2209900
	21	.09715185	.05426312	.863	-.1014730	.2957767
	22	.13173148	.05426312	.486	-.0668934	.3303563
	23	.01937407	.05426312	1.000	-.1792508	.2179989
	24	.26851852*	.05426312	.002	.0698937	.4671434
	25	.13703704	.05426312	.427	-.0615878	.3356619
	32	.55462963*	.05426312	.000	.3560048	.7532545
	33	.50555556*	.05426312	.000	.3069307	.7041804
	34	.54907407*	.05426312	.000	.3504492	.7476989
	35	.25277778*	.05426312	.004	.0541529	.4514026
13	11	.01120370	.05426312	1.000	-.1874211	.2098285
	12	.16771296	.05426312	.166	-.0309119	.3663378

	14	-.34710185*	.05426312	.000	-.5457267	-.1484770
	15	-1.25190185*	.05426312	.000	-1.4505267	-1.0532770
	21	.26486481*	.05426312	.002	.0662400	.4634897
	22	.29944444*	.05426312	.000	.1008196	.4980693
	23	.18708704	.05426312	.080	-.0115378	.3857119
	24	.43623148*	.05426312	.000	.2376066	.6348563
	25	.30475000*	.05426312	.000	.1061252	.5033748
	32	.72234259*	.05426312	.000	.5237177	.9209674
	33	.67326852*	.05426312	.000	.4746437	.8718934
	34	.71678704*	.05426312	.000	.5181622	.9154119
	35	.42049074*	.05426312	.000	.2218659	.6191156
14	11	.35830556*	.05426312	.000	.1596807	.5569304
	12	.51481482*	.05426312	.000	.3161900	.7134397
	13	.34710185*	.05426312	.000	.1484770	.5457267
	15	-.90480000*	.05426312	.000	-1.1034248	-.7061752
	21	.61196667*	.05426312	.000	.4133418	.8105915
	22	.64654630*	.05426312	.000	.4479215	.8451711
	23	.53418889*	.05426312	.000	.3355640	.7328137
	24	.78333333*	.05426312	.000	.5847085	.9819582
	25	.65185185*	.05426312	.000	.4532270	.8504767
	32	1.06944444*	.05426312	.000	.8708196	1.2680693
	33	1.02037037*	.05426312	.000	.8217455	1.2189952
	34	1.06388889*	.05426312	.000	.8652640	1.2625137
	35	.76759259*	.05426312	.000	.5689677	.9662174
15	11	1.26310556*	.05426312	.000	1.0644807	1.4617304
	12	1.41961482*	.05426312	.000	1.2209900	1.6182397
	13	1.25190185*	.05426312	.000	1.0532770	1.4505267
	14	.90480000*	.05426312	.000	.7061752	1.1034248
	21	1.51676667*	.05426312	.000	1.3181418	1.7153915
	22	1.55134630*	.05426312	.000	1.3527215	1.7499711
	23	1.43898889*	.05426312	.000	1.2403640	1.6376137
	24	1.68813333*	.05426312	.000	1.4895085	1.8867582
	25	1.55665185*	.05426312	.000	1.3580270	1.7552767
	32	1.97424444*	.05426312	.000	1.7756196	2.1728693

	33	1.92517037*	.05426312	.000	1.7265455	2.1237952
	34	1.96868889*	.05426312	.000	1.7700640	2.1673137
	35	1.67239259*	.05426312	.000	1.4737677	1.8710174
21	11	-.25366111*	.05426312	.004	-.4522860	-.0550363
	12	-.09715185	.05426312	.863	-.2957767	.1014730
	13	-.26486481*	.05426312	.002	-.4634897	-.0662400
	14	-.61196667*	.05426312	.000	-.8105915	-.4133418
	15	-1.51676667*	.05426312	.000	-1.7153915	-1.3181418
	22	.03457963	.05426312	1.000	-.1640452	.2332045
	23	-.07777778	.05426312	.969	-.2764026	.1208471
	24	.17136667	.05426312	.145	-.0272582	.3699915
	25	.03988519	.05426312	1.000	-.1587397	.2385100
	32	.45747778*	.05426312	.000	.2588529	.6561026
	33	.40840370*	.05426312	.000	.2097789	.6070285
	34	.45192222*	.05426312	.000	.2532974	.6505471
	35	.15562593	.05426312	.249	-.0429989	.3542508
22	11	-.28824074*	.05426312	.001	-.4868656	-.0896159
	12	-.13173148	.05426312	.486	-.3303563	.0668934
	13	-.29944444*	.05426312	.000	-.4980693	-.1008196
	14	-.64654630*	.05426312	.000	-.8451711	-.4479215
	15	-1.55134630*	.05426312	.000	-1.7499711	-1.3527215
	21	-.03457963	.05426312	1.000	-.2332045	.1640452
	23	-.11235741	.05426312	.713	-.3109823	.0862674
	24	.13678704	.05426312	.429	-.0618378	.3354119
	25	.00530556	.05426312	1.000	-.1933193	.2039304
	32	.42289815*	.05426312	.000	.2242733	.6215230
	33	.37382407*	.05426312	.000	.1751992	.5724489
	34	.41734259*	.05426312	.000	.2187177	.6159674
	35	.12104630	.05426312	.612	-.0775785	.3196711
23	11	-.17588333	.05426312	.123	-.3745082	.0227415
	12	-.01937407	.05426312	1.000	-.2179989	.1792508
	13	-.18708704	.05426312	.080	-.3857119	.0115378
	14	-.53418889*	.05426312	.000	-.7328137	-.3355640
	15	-1.43898889*	.05426312	.000	-1.6376137	-1.2403640

	21	.07777778	.05426312	.969	-.1208471	.2764026
	22	.11235741	.05426312	.713	-.0862674	.3109823
	24	.24914444*	.05426312	.005	.0505196	.4477693
	25	.11766296	.05426312	.652	-.0809619	.3162878
	32	.53525556*	.05426312	.000	.3366307	.7338804
	33	.48618148*	.05426312	.000	.2875566	.6848063
	34	.52970000*	.05426312	.000	.3310752	.7283248
	35	.23340370*	.05426312	.011	.0347789	.4320285
24	11	-.42502778*	.05426312	.000	-.6236526	-.2264029
	12	-.26851852*	.05426312	.002	-.4671434	-.0698937
	13	-.43623148*	.05426312	.000	-.6348563	-.2376066
	14	-.78333333*	.05426312	.000	-.9819582	-.5847085
	15	-1.68813333*	.05426312	.000	-1.8867582	-1.4895085
	21	-.17136667	.05426312	.145	-.3699915	.0272582
	22	-.13678704	.05426312	.429	-.3354119	.0618378
	23	-.24914444*	.05426312	.005	-.4477693	-.0505196
	25	-.13148148	.05426312	.489	-.3301063	.0671434
	32	.28611111*	.05426312	.001	.0874863	.4847360
	33	.23703704*	.05426312	.009	.0384122	.4356619
	34	.28055556*	.05426312	.001	.0819307	.4791804
	35	-.01574074	.05426312	1.000	-.2143656	.1828841
25	11	-.29354630*	.05426312	.001	-.4921711	-.0949215
	12	-.13703704	.05426312	.427	-.3356619	.0615878
	13	-.30475000*	.05426312	.000	-.5033748	-.1061252
	14	-.65185185*	.05426312	.000	-.8504767	-.4532270
	15	-1.55665185*	.05426312	.000	-1.7552767	-1.3580270
	21	-.03988519	.05426312	1.000	-.2385100	.1587397
	22	-.00530556	.05426312	1.000	-.2039304	.1933193
	23	-.11766296	.05426312	.652	-.3162878	.0809619
	24	.13148148	.05426312	.489	-.0671434	.3301063
	32	.41759259*	.05426312	.000	.2189677	.6162174
	33	.36851852*	.05426312	.000	.1698937	.5671434
	34	.41203704*	.05426312	.000	.2134122	.6106619
	35	.11574074	.05426312	.675	-.0828841	.3143656

32	11	-.71113889*	.05426312	.000	-.9097637	-.5125140
	12	-.55462963*	.05426312	.000	-.7532545	-.3560048
	13	-.72234259*	.05426312	.000	-.9209674	-.5237177
	14	-1.06944444*	.05426312	.000	-1.2680693	-.8708196
	15	-1.97424444*	.05426312	.000	-2.1728693	-1.7756196
	21	-.45747778*	.05426312	.000	-.6561026	-.2588529
	22	-.42289815*	.05426312	.000	-.6215230	-.2242733
	23	-.53525556*	.05426312	.000	-.7338804	-.3366307
	24	-.28611111*	.05426312	.001	-.4847360	-.0874863
	25	-.41759259*	.05426312	.000	-.6162174	-.2189677
	33	-.04907407	.05426312	1.000	-.2476989	.1495508
	34	-.00555556	.05426312	1.000	-.2041804	.1930693
	35	-.30185185*	.05426312	.000	-.5004767	-.1032270
33	11	-.66206481*	.05426312	.000	-.8606897	-.4634400
	12	-.50555556*	.05426312	.000	-.7041804	-.3069307
	13	-.67326852*	.05426312	.000	-.8718934	-.4746437
	14	-1.02037037*	.05426312	.000	-1.2189952	-.8217455
	15	-1.92517037*	.05426312	.000	-2.1237952	-1.7265455
	21	-.40840370*	.05426312	.000	-.6070285	-.2097789
	22	-.37382407*	.05426312	.000	-.5724489	-.1751992
	23	-.48618148*	.05426312	.000	-.6848063	-.2875566
	24	-.23703704*	.05426312	.009	-.4356619	-.0384122
	25	-.36851852*	.05426312	.000	-.5671434	-.1698937
	32	.04907407	.05426312	1.000	-.1495508	.2476989
	34	.04351852	.05426312	1.000	-.1551063	.2421434
	35	-.25277778*	.05426312	.004	-.4514026	-.0541529
34	11	-.70558333*	.05426312	.000	-.9042082	-.5069585
	12	-.54907407*	.05426312	.000	-.7476989	-.3504492
	13	-.71678704*	.05426312	.000	-.9154119	-.5181622
	14	-1.06388889*	.05426312	.000	-1.2625137	-.8652640
	15	-1.96868889*	.05426312	.000	-2.1673137	-1.7700640
	21	-.45192222*	.05426312	.000	-.6505471	-.2532974
	22	-.41734259*	.05426312	.000	-.6159674	-.2187177
	23	-.52970000*	.05426312	.000	-.7283248	-.3310752

	24	-.28055556*	.05426312	.001	-.4791804	-.0819307
	25	-.41203704*	.05426312	.000	-.6106619	-.2134122
	32	.00555556	.05426312	1.000	-.1930693	.2041804
	33	-.04351852	.05426312	1.000	-.2421434	.1551063
	35	-.29629630*	.05426312	.001	-.4949211	-.0976715
35	11	-.40928704*	.05426312	.000	-.6079119	-.2106622
	12	-.25277778*	.05426312	.004	-.4514026	-.0541529
	13	-.42049074*	.05426312	.000	-.6191156	-.2218659
	14	-.76759259*	.05426312	.000	-.9662174	-.5689677
	15	-1.67239259*	.05426312	.000	-1.8710174	-1.4737677
	21	-.15562593	.05426312	.249	-.3542508	.0429989
	22	-.12104630	.05426312	.612	-.3196711	.0775785
	23	-.23340370*	.05426312	.011	-.4320285	-.0347789
	24	.01574074	.05426312	1.000	-.1828841	.2143656
	25	-.11574074	.05426312	.675	-.3143656	.0828841
	32	.30185185*	.05426312	.000	.1032270	.5004767
	33	.25277778*	.05426312	.004	.0541529	.4514026
	34	.29629630*	.05426312	.001	.0976715	.4949211

*. The mean difference is significant at the 0.05 level.

Table. A.2.2. Statistical analysis of total carbohydrates contents in Jujube samples (n=3), comparison between leaves, fruit, and seed contents of 5 cultivars.

Tests of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Total_carbohyrates	Based on Mean	2.457	14	30	.019
	Based on Median	1.214	14	30	.316
	Based on Median and with adjusted df	1.214	14	10.416	.383
	Based on trimmed mean	2.367	14	30	.023

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Part_of_tree_cultivar	Statistic	df	Sig.	Statistic	df	Sig.
Total_carbohyrates	11	.243	3	.	.972	3	.681
	12	.280	3	.	.938	3	.518
	13	.182	3	.	.999	3	.935
	14	.225	3	.	.984	3	.755
	15	.236	3	.	.977	3	.710
	21	.261	3	.	.957	3	.601
	22	.190	3	.	.998	3	.905
	23	.334	3	.	.860	3	.266
	24	.274	3	.	.945	3	.546
	25	.224	3	.	.984	3	.759
	31	.313	3	.	.894	3	.368
	32	.177	3	.	1.000	3	.961
	33	.287	3	.	.929	3	.485
	34	.192	3	.	.997	3	.896
	35	.238	3	.	.976	3	.702

a. Lilliefors Significance Correction

ANOVA

Total_carbohyrates

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15574.771	14	1112.484	42.009	.000
Within Groups	794.466	30	26.482		
Total	16369.237	44			

Multiple Comparisons

Dependent Variable: Total_carbohyrates

Tukey HSD

(I) Part_of_tree_cultivar	(J) Part_of_tree_cultivar	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
11	12	-3.16247312	4.20176027	1.000	-18.6459977	12.3210515
	13	-7.11096774	4.20176027	.919	-22.5944923	8.3725569
	14	.99236559	4.20176027	1.000	-14.4911590	16.4758902
	15	4.25279570	4.20176027	.999	-11.2307289	19.7363203
	21	-52.50608602*	4.20176027	.000	-67.9896106	-37.0225614
	22	-41.35645161*	4.20176027	.000	-56.8399762	-25.8729270
	23	-48.69808602*	4.20176027	.000	-64.1816106	-33.2145614
	24	-27.49473118*	4.20176027	.000	-42.9782558	-12.0112066
	25	-33.22161290*	4.20176027	.000	-48.7051375	-17.7380883
	31	-5.42075269	4.20176027	.991	-20.9042773	10.0627719
	32	-24.51623656*	4.20176027	.000	-39.9997612	-9.0327120
	33	-14.92806452	4.20176027	.068	-30.4115891	.5554601
	34	-10.12913978	4.20176027	.526	-25.6126644	5.3543848
	35	2.14156989	4.20176027	1.000	-13.3419547	17.6250945
12	11	3.16247312	4.20176027	1.000	-12.3210515	18.6459977
	13	-3.94849462	4.20176027	1.000	-19.4320192	11.5350300
	14	4.15483871	4.20176027	.999	-11.3286859	19.6383633
	15	7.41526882	4.20176027	.894	-8.0682558	22.8987934
	21	-49.34361290*	4.20176027	.000	-64.8271375	-33.8600883
	22	-38.19397850*	4.20176027	.000	-53.6775031	-22.7104539
	23	-45.53561290*	4.20176027	.000	-61.0191375	-30.0520883
	24	-24.33225807*	4.20176027	.000	-39.8157827	-8.8487335
	25	-30.05913979*	4.20176027	.000	-45.5426644	-14.5756152
	31	-2.25827957	4.20176027	1.000	-17.7418042	13.2252450
	32	-21.35376344*	4.20176027	.001	-36.8372880	-5.8702388
	33	-11.76559140	4.20176027	.299	-27.2491160	3.7179332
	34	-6.96666667	4.20176027	.930	-22.4501913	8.5168579
	35	5.30404301	4.20176027	.992	-10.1794816	20.7875676
13	11	7.11096774	4.20176027	.919	-8.3725569	22.5944923
	12	3.94849462	4.20176027	1.000	-11.5350300	19.4320192

	14	8.10333333	4.20176027	.821	-7.3801913	23.5868579
	15	11.36376344	4.20176027	.349	-4.1197612	26.8472880
	21	-45.39511828*	4.20176027	.000	-60.8786429	-29.9115937
	22	-34.24548387*	4.20176027	.000	-49.7290085	-18.7619593
	23	-41.58711828*	4.20176027	.000	-57.0706429	-26.1035937
	24	-20.38376344*	4.20176027	.003	-35.8672880	-4.9002388
	25	-26.11064516*	4.20176027	.000	-41.5941698	-10.6271206
	31	1.69021505	4.20176027	1.000	-13.7933095	17.1737397
	32	-17.40526882*	4.20176027	.017	-32.8887934	-1.9217442
	33	-7.81709678	4.20176027	.854	-23.3006214	7.6664278
	34	-3.01817204	4.20176027	1.000	-18.5016966	12.4653526
	35	9.25253763	4.20176027	.661	-6.2309870	24.7360622
14	11	-.99236559	4.20176027	1.000	-16.4758902	14.4911590
	12	-4.15483871	4.20176027	.999	-19.6383633	11.3286859
	13	-8.10333333	4.20176027	.821	-23.5868579	7.3801913
	15	3.26043011	4.20176027	1.000	-12.2230945	18.7439547
	21	-53.49845161*	4.20176027	.000	-68.9819762	-38.0149270
	22	-42.34881720*	4.20176027	.000	-57.8323418	-26.8652926
	23	-49.69045161*	4.20176027	.000	-65.1739762	-34.2069270
	24	-28.48709677*	4.20176027	.000	-43.9706214	-13.0035722
	25	-34.21397849*	4.20176027	.000	-49.6975031	-18.7304539
	31	-6.41311828	4.20176027	.962	-21.8966429	9.0704063
	32	-25.50860215*	4.20176027	.000	-40.9921268	-10.0250775
	33	-15.92043011*	4.20176027	.039	-31.4039547	-.4369055
	34	-11.12150537	4.20176027	.381	-26.6050300	4.3620192
	35	1.14920430	4.20176027	1.000	-14.3343203	16.6327289
15	11	-4.25279570	4.20176027	.999	-19.7363203	11.2307289
	12	-7.41526882	4.20176027	.894	-22.8987934	8.0682558
	13	-11.36376344	4.20176027	.349	-26.8472880	4.1197612
	14	-3.26043011	4.20176027	1.000	-18.7439547	12.2230945
	21	-56.75888172*	4.20176027	.000	-72.2424063	-41.2753571
	22	-45.60924731*	4.20176027	.000	-61.0927719	-30.1257227
	23	-52.95088172*	4.20176027	.000	-68.4344063	-37.4673571
	24	-31.74752688*	4.20176027	.000	-47.2310515	-16.2640023

	25	-37.47440860*	4.20176027	.000	-52.9579332	-21.9908840
	31	-9.67354839	4.20176027	.596	-25.1570730	5.8099762
	32	-28.76903226*	4.20176027	.000	-44.2525569	-13.2855077
	33	-19.18086022*	4.20176027	.006	-34.6643848	-3.6973356
	34	-14.38193548	4.20176027	.090	-29.8654601	1.1015891
	35	-2.11122581	4.20176027	1.000	-17.5947504	13.3722988
21	11	52.50608602*	4.20176027	.000	37.0225614	67.9896106
	12	49.34361290*	4.20176027	.000	33.8600883	64.8271375
	13	45.39511828*	4.20176027	.000	29.9115937	60.8786429
	14	53.49845161*	4.20176027	.000	38.0149270	68.9819762
	15	56.75888172*	4.20176027	.000	41.2753571	72.2424063
	22	11.14963441	4.20176027	.377	-4.3338902	26.6331590
	23	3.80800000	4.20176027	1.000	-11.6755246	19.2915246
	24	25.01135484*	4.20176027	.000	9.5278302	40.4948794
	25	19.28447312*	4.20176027	.005	3.8009485	34.7679977
	31	47.08533333*	4.20176027	.000	31.6018087	62.5688579
	32	27.98984946*	4.20176027	.000	12.5063249	43.4733741
	33	37.57802150*	4.20176027	.000	22.0944969	53.0615461
	34	42.37694624*	4.20176027	.000	26.8934216	57.8604708
	35	54.64765591*	4.20176027	.000	39.1641313	70.1311805
22	11	41.35645161*	4.20176027	.000	25.8729270	56.8399762
	12	38.19397850*	4.20176027	.000	22.7104539	53.6775031
	13	34.24548387*	4.20176027	.000	18.7619593	49.7290085
	14	42.34881720*	4.20176027	.000	26.8652926	57.8323418
	15	45.60924731*	4.20176027	.000	30.1257227	61.0927719
	21	-11.14963441	4.20176027	.377	-26.6331590	4.3338902
	23	-7.34163441	4.20176027	.901	-22.8251590	8.1418902
	24	13.86172043	4.20176027	.117	-1.6218042	29.3452450
	25	8.13483871	4.20176027	.817	-7.3486859	23.6183633
	31	35.93569893*	4.20176027	.000	20.4521743	51.4192235
	32	16.84021505*	4.20176027	.023	1.3566905	32.3237397
	33	26.42838710*	4.20176027	.000	10.9448625	41.9119117
	34	31.22731183*	4.20176027	.000	15.7437872	46.7108364
	35	43.49802151*	4.20176027	.000	28.0144969	58.9815461

23	11	48.69808602*	4.20176027	.000	33.2145614	64.1816106
	12	45.53561290*	4.20176027	.000	30.0520883	61.0191375
	13	41.58711828*	4.20176027	.000	26.1035937	57.0706429
	14	49.69045161*	4.20176027	.000	34.2069270	65.1739762
	15	52.95088172*	4.20176027	.000	37.4673571	68.4344063
	21	-3.80800000	4.20176027	1.000	-19.2915246	11.6755246
	22	7.34163441	4.20176027	.901	-8.1418902	22.8251590
	24	21.20335484*	4.20176027	.002	5.7198302	36.6868794
	25	15.47647312	4.20176027	.050	-.0070515	30.9599977
	31	43.27733333*	4.20176027	.000	27.7938087	58.7608579
	32	24.18184946*	4.20176027	.000	8.6983249	39.6653741
	33	33.77002150*	4.20176027	.000	18.2864969	49.2535461
	34	38.56894624*	4.20176027	.000	23.0854216	54.0524708
	35	50.83965591*	4.20176027	.000	35.3561313	66.3231805
24	11	27.49473118*	4.20176027	.000	12.0112066	42.9782558
	12	24.33225807*	4.20176027	.000	8.8487335	39.8157827
	13	20.38376344*	4.20176027	.003	4.9002388	35.8672880
	14	28.48709678*	4.20176027	.000	13.0035722	43.9706214
	15	31.74752688*	4.20176027	.000	16.2640023	47.2310515
	21	-25.01135484*	4.20176027	.000	-40.4948794	-9.5278302
	22	-13.86172043	4.20176027	.117	-29.3452450	1.6218042
	23	-21.20335484*	4.20176027	.002	-36.6868794	-5.7198302
	25	-5.72688172	4.20176027	.985	-21.2104063	9.7566429
	31	22.07397850*	4.20176027	.001	6.5904539	37.5575031
	32	2.97849462	4.20176027	1.000	-12.5050300	18.4620192
	33	12.56666667	4.20176027	.214	-2.9168579	28.0501913
	34	17.36559140*	4.20176027	.017	1.8820668	32.8491160
	35	29.63630108*	4.20176027	.000	14.1527765	45.1198257
25	11	33.22161290*	4.20176027	.000	17.7380883	48.7051375
	12	30.05913979*	4.20176027	.000	14.5756152	45.5426644
	13	26.11064516*	4.20176027	.000	10.6271206	41.5941698
	14	34.21397850*	4.20176027	.000	18.7304539	49.6975031
	15	37.47440860*	4.20176027	.000	21.9908840	52.9579332
	21	-19.28447312*	4.20176027	.005	-34.7679977	-3.8009485

	22	-8.13483871	4.20176027	.817	-23.6183633	7.3486859
	23	-15.47647312	4.20176027	.050	-30.9599977	.0070515
	24	5.72688172	4.20176027	.985	-9.7566429	21.2104063
	31	27.80086022*	4.20176027	.000	12.3173356	43.2843848
	32	8.70537634	4.20176027	.742	-6.7781483	24.1889009
	33	18.29354839*	4.20176027	.010	2.8100238	33.7770730
	34	23.09247312*	4.20176027	.000	7.6089485	38.5759977
	35	35.36318280*	4.20176027	.000	19.8796582	50.8467074
31	11	5.42075269	4.20176027	.991	-10.0627719	20.9042773
	12	2.25827957	4.20176027	1.000	-13.2252450	17.7418042
	13	-1.69021505	4.20176027	1.000	-17.1737397	13.7933095
	14	6.41311828	4.20176027	.962	-9.0704063	21.8966429
	15	9.67354839	4.20176027	.596	-5.8099762	25.1570730
	21	-47.08533333*	4.20176027	.000	-62.5688579	-31.6018087
	22	-35.93569893*	4.20176027	.000	-51.4192235	-20.4521743
	23	-43.27733333*	4.20176027	.000	-58.7608579	-27.7938087
	24	-22.07397850*	4.20176027	.001	-37.5575031	-6.5904539
	25	-27.80086022*	4.20176027	.000	-43.2843848	-12.3173356
	32	-19.09548387*	4.20176027	.006	-34.5790085	-3.6119593
	33	-9.50731183	4.20176027	.622	-24.9908364	5.9762128
	34	-4.70838710	4.20176027	.998	-20.1919117	10.7751375
	35	7.56232258	4.20176027	.880	-7.9212020	23.0458472
32	11	24.51623656*	4.20176027	.000	9.0327120	39.9997612
	12	21.35376344*	4.20176027	.001	5.8702388	36.8372880
	13	17.40526882*	4.20176027	.017	1.9217442	32.8887934
	14	25.50860215*	4.20176027	.000	10.0250775	40.9921268
	15	28.76903226*	4.20176027	.000	13.2855077	44.2525569
	21	-27.98984946*	4.20176027	.000	-43.4733741	-12.5063249
	22	-16.84021505*	4.20176027	.023	-32.3237397	-1.3566905
	23	-24.18184946*	4.20176027	.000	-39.6653741	-8.6983249
	24	-2.97849462	4.20176027	1.000	-18.4620192	12.5050300
	25	-8.70537634	4.20176027	.742	-24.1889009	6.7781483
	31	19.09548387*	4.20176027	.006	3.6119593	34.5790085
	33	9.58817204	4.20176027	.610	-5.8953526	25.0716966

	34	14.38709678	4.20176027	.090	-1.0964278	29.8706214
	35	26.65780645*	4.20176027	.000	11.1742819	42.1413311
33	11	14.92806452	4.20176027	.068	-.5554601	30.4115891
	12	11.76559140	4.20176027	.299	-3.7179332	27.2491160
	13	7.81709678	4.20176027	.854	-7.6664278	23.3006214
	14	15.92043011*	4.20176027	.039	.4369055	31.4039547
	15	19.18086022*	4.20176027	.006	3.6973356	34.6643848
	21	-37.57802150*	4.20176027	.000	-53.0615461	-22.0944969
	22	-26.42838710*	4.20176027	.000	-41.9119117	-10.9448625
	23	-33.77002150*	4.20176027	.000	-49.2535461	-18.2864969
	24	-12.56666667	4.20176027	.214	-28.0501913	2.9168579
	25	-18.29354839*	4.20176027	.010	-33.7770730	-2.8100238
	31	9.50731183	4.20176027	.622	-5.9762128	24.9908364
	32	-9.58817204	4.20176027	.610	-25.0716966	5.8953526
	34	4.79892473	4.20176027	.997	-10.6845999	20.2824493
	35	17.06963441*	4.20176027	.020	1.5861098	32.5531590
34	11	10.12913978	4.20176027	.526	-5.3543848	25.6126644
	12	6.96666667	4.20176027	.930	-8.5168579	22.4501913
	13	3.01817204	4.20176027	1.000	-12.4653526	18.5016966
	14	11.12150538	4.20176027	.381	-4.3620192	26.6050300
	15	14.38193548	4.20176027	.090	-1.1015891	29.8654601
	21	-42.37694624*	4.20176027	.000	-57.8604708	-26.8934216
	22	-31.22731183*	4.20176027	.000	-46.7108364	-15.7437872
	23	-38.56894624*	4.20176027	.000	-54.0524708	-23.0854216
	24	-17.36559140*	4.20176027	.017	-32.8491160	-1.8820668
	25	-23.09247312*	4.20176027	.000	-38.5759977	-7.6089485
	31	4.70838710	4.20176027	.998	-10.7751375	20.1919117
	32	-14.38709678	4.20176027	.090	-29.8706214	1.0964278
	33	-4.79892473	4.20176027	.997	-20.2824493	10.6845999
	35	12.27070968	4.20176027	.243	-3.2128149	27.7542343
35	11	-2.14156989	4.20176027	1.000	-17.6250945	13.3419547
	12	-5.30404301	4.20176027	.992	-20.7875676	10.1794816
	13	-9.25253763	4.20176027	.661	-24.7360622	6.2309870
	14	-1.14920430	4.20176027	1.000	-16.6327289	14.3343203

	15	2.11122581	4.20176027	1.000	-13.3722988	17.5947504
	21	-54.64765591*	4.20176027	.000	-70.1311805	-39.1641313
	22	-43.49802151*	4.20176027	.000	-58.9815461	-28.0144969
	23	-50.83965591*	4.20176027	.000	-66.3231805	-35.3561313
	24	-29.63630108*	4.20176027	.000	-45.1198257	-14.1527765
	25	-35.36318280*	4.20176027	.000	-50.8467074	-19.8796582
	31	-7.56232258	4.20176027	.880	-23.0458472	7.9212020
	32	-26.65780645*	4.20176027	.000	-42.1413311	-11.1742819
	33	-17.06963441*	4.20176027	.020	-32.5531590	-1.5861098
	34	-12.27070968	4.20176027	.243	-27.7542343	3.2128149

*. The mean difference is significant at the 0.05 level.

Table. A.2.3. Statistical analysis of total phenolics contents in Jujube samples (n=3), comparison between leaves, fruit, and seed contents of 5 cultivars.

Tests of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Total_polyphenolics	Based on Mean	3.987	14	30	.001
	Based on Median	2.274	14	30	.029
	Based on Median and with adjusted df	2.274	14	6.986	.139
	Based on trimmed mean	3.871	14	30	.001

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Part_of_tree_cultivar	Statistic	df	Sig.	Statistic	df	Sig.
Total_polyphenolics	11	.193	3	.	.997	3	.891
	12	.222	3	.	.986	3	.771
	13	.245	3	.	.971	3	.672
	14	.240	3	.	.975	3	.694
	15	.269	3	.	.949	3	.567
	21	.204	3	.	.993	3	.843
	22	.253	3	.	.964	3	.637
	23	.349	3	.	.832	3	.194
	24	.282	3	.	.936	3	.510
	25	.385	3	.	.750	3	.000
	31	.291	3	.	.925	3	.469
	32	.292	3	.	.923	3	.463
	33	.253	3	.	.964	3	.637
	34	.241	3	.	.974	3	.688
	35	.243	3	.	.972	3	.679

a. Lilliefors Significance Correction

ANOVA

Total_polyphenolics

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2198.546	14	157.039	603.737	.000
Within Groups	7.803	30	.260		
Total	2206.349	44			

Multiple Comparisons

Dependent Variable: Total_polyphenolics

Tukey HSD

(I) Part_of_tree_cultivar	(J) Part_of_tree_cultivar	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
11	12	-2.40686275*	.41642246	.000	-3.9413833	-.8723422
	13	-2.13235294*	.41642246	.001	-3.6668735	-.5978324
	14	-3.86274510*	.41642246	.000	-5.3972656	-2.3282246
	15	-2.06372549*	.41642246	.002	-3.5982460	-.5292050
	21	13.72549020*	.41642246	.000	12.1909697	15.2600107
	22	13.16666667*	.41642246	.000	11.6321461	14.7011872
	23	13.48039216*	.41642246	.000	11.9458716	15.0149127
	24	13.25490196*	.41642246	.000	11.7203814	14.7894225
	25	13.46078431*	.41642246	.000	11.9262638	14.9953048
	31	11.42156863*	.41642246	.000	9.8870481	12.9560891
	32	12.52450980*	.41642246	.000	10.9899893	14.0590303
	33	12.18627451*	.41642246	.000	10.6517540	13.7207950
	34	10.27450980*	.41642246	.000	8.7399893	11.8090303
	35	11.96568627*	.41642246	.000	10.4311658	13.5002068
12	11	2.40686275*	.41642246	.000	.8723422	3.9413833
	13	.27450981	.41642246	1.000	-1.2600107	1.8090303
	14	-1.45588235	.41642246	.077	-2.9904029	.0786382
	15	.34313726	.41642246	1.000	-1.1913833	1.8776578
	21	16.13235294*	.41642246	.000	14.5978324	17.6668735
	22	15.57352941*	.41642246	.000	14.0390089	17.1080499
	23	15.88725490*	.41642246	.000	14.3527344	17.4217754
	24	15.66176471*	.41642246	.000	14.1272442	17.1962852
	25	15.86764706*	.41642246	.000	14.3331265	17.4021676
	31	13.82843137*	.41642246	.000	12.2939109	15.3629519
	32	14.93137255*	.41642246	.000	13.3968520	16.4658931
	33	14.59313726*	.41642246	.000	13.0586167	16.1276578
	34	12.68137255*	.41642246	.000	11.1468520	14.2158931
	35	14.37254902*	.41642246	.000	12.8380285	15.9070695
13	11	2.13235294*	.41642246	.001	.5978324	3.6668735
	12	-.27450981	.41642246	1.000	-1.8090303	1.2600107

	14	-1.73039216*	.41642246	.016	-3.2649127	-.1958716
	15	.06862745	.41642246	1.000	-1.4658931	1.6031480
	21	15.85784314*	.41642246	.000	14.3233226	17.3923637
	22	15.29901961*	.41642246	.000	13.7644991	16.8335401
	23	15.61274510*	.41642246	.000	14.0782246	17.1472656
	24	15.38725490*	.41642246	.000	13.8527344	16.9217754
	25	15.59313725*	.41642246	.000	14.0586167	17.1276578
	31	13.55392157*	.41642246	.000	12.0194010	15.0884421
	32	14.65686274*	.41642246	.000	13.1223422	16.1913833
	33	14.31862745*	.41642246	.000	12.7841069	15.8531480
	34	12.40686274*	.41642246	.000	10.8723422	13.9413833
	35	14.09803921*	.41642246	.000	12.5635187	15.6325597
14	11	3.86274510*	.41642246	.000	2.3282246	5.3972656
	12	1.45588235	.41642246	.077	-.0786382	2.9904029
	13	1.73039216*	.41642246	.016	.1958716	3.2649127
	15	1.79901961*	.41642246	.011	.2644991	3.3335401
	21	17.58823529*	.41642246	.000	16.0537148	19.1227558
	22	17.02941176*	.41642246	.000	15.4948912	18.5639323
	23	17.34313725*	.41642246	.000	15.8086167	18.8776578
	24	17.11764706*	.41642246	.000	15.5831265	18.6521676
	25	17.32352941*	.41642246	.000	15.7890089	18.8580499
	31	15.28431372*	.41642246	.000	13.7497932	16.8188342
	32	16.38725490*	.41642246	.000	14.8527344	17.9217754
	33	16.04901961*	.41642246	.000	14.5144991	17.5835401
	34	14.13725490*	.41642246	.000	12.6027344	15.6717754
	35	15.82843137*	.41642246	.000	14.2939108	17.3629519
15	11	2.06372549*	.41642246	.002	.5292050	3.5982460
	12	-.34313726	.41642246	1.000	-1.8776578	1.1913833
	13	-.06862745	.41642246	1.000	-1.6031480	1.4658931
	14	-1.79901961*	.41642246	.011	-3.3335401	-.2644991
	21	15.78921569*	.41642246	.000	14.2546952	17.3237362
	22	15.23039216*	.41642246	.000	13.6958716	16.7649127
	23	15.54411765*	.41642246	.000	14.0095971	17.0786382
	24	15.31862745*	.41642246	.000	13.7841069	16.8531480

	25	15.52450980*	.41642246	.000	13.9899893	17.0590303
	31	13.48529412*	.41642246	.000	11.9507736	15.0198146
	32	14.58823529*	.41642246	.000	13.0537148	16.1227558
	33	14.25000000*	.41642246	.000	12.7154795	15.7845205
	34	12.33823529*	.41642246	.000	10.8037148	13.8727558
	35	14.02941176*	.41642246	.000	12.4948912	15.5639323
21	11	-13.72549020*	.41642246	.000	-15.2600107	-12.1909697
	12	-16.13235294*	.41642246	.000	-17.6668735	-14.5978324
	13	-15.85784314*	.41642246	.000	-17.3923637	-14.3233226
	14	-17.58823529*	.41642246	.000	-19.1227558	-16.0537148
	15	-15.78921569*	.41642246	.000	-17.3237362	-14.2546952
	22	-.55882353	.41642246	.987	-2.0933441	.9756970
	23	-.24509804	.41642246	1.000	-1.7796186	1.2894225
	24	-.47058824	.41642246	.997	-2.0051088	1.0639323
	25	-.26470588	.41642246	1.000	-1.7992264	1.2698146
	31	-2.30392157*	.41642246	.000	-3.8384421	-.7694010
	32	-1.20098039	.41642246	.259	-2.7355009	.3335401
	33	-1.53921569*	.41642246	.049	-3.0737362	-.0046952
	34	-3.45098039*	.41642246	.000	-4.9855009	-1.9164599
	35	-1.75980392*	.41642246	.013	-3.2943244	-.2252834
22	11	-13.16666667*	.41642246	.000	-14.7011872	-11.6321461
	12	-15.57352941*	.41642246	.000	-17.1080499	-14.0390089
	13	-15.29901961*	.41642246	.000	-16.8335401	-13.7644991
	14	-17.02941176*	.41642246	.000	-18.5639323	-15.4948912
	15	-15.23039216*	.41642246	.000	-16.7649127	-13.6958716
	21	.55882353	.41642246	.987	-.9756970	2.0933441
	23	.31372549	.41642246	1.000	-1.2207950	1.8482460
	24	.08823529	.41642246	1.000	-1.4462852	1.6227558
	25	.29411765	.41642246	1.000	-1.2404029	1.8286382
	31	-1.74509804*	.41642246	.015	-3.2796186	-.2105775
	32	-.64215686	.41642246	.959	-2.1766774	.8923637
	33	-.98039216	.41642246	.562	-2.5149127	.5541284
	34	-2.89215686*	.41642246	.000	-4.4266774	-1.3576363
	35	-1.20098039	.41642246	.259	-2.7355009	.3335401

23	11	-13.48039216*	.41642246	.000	-15.0149127	-11.9458716
	12	-15.88725490*	.41642246	.000	-17.4217754	-14.3527344
	13	-15.61274510*	.41642246	.000	-17.1472656	-14.0782246
	14	-17.34313725*	.41642246	.000	-18.8776578	-15.8086167
	15	-15.54411765*	.41642246	.000	-17.0786382	-14.0095971
	21	.24509804	.41642246	1.000	-1.2894225	1.7796186
	22	-.31372549	.41642246	1.000	-1.8482460	1.2207950
	24	-.22549020	.41642246	1.000	-1.7600107	1.3090303
	25	-.01960784	.41642246	1.000	-1.5541284	1.5149127
	31	-2.05882353*	.41642246	.002	-3.5933441	-.5243030
	32	-.95588235	.41642246	.601	-2.4904029	.5786382
	33	-1.29411765	.41642246	.172	-2.8286382	.2404029
	34	-3.20588235*	.41642246	.000	-4.7404029	-1.6713618
	35	-1.51470588	.41642246	.056	-3.0492264	.0198146
24	11	-13.25490196*	.41642246	.000	-14.7894225	-11.7203814
	12	-15.66176471*	.41642246	.000	-17.1962852	-14.1272442
	13	-15.38725490*	.41642246	.000	-16.9217754	-13.8527344
	14	-17.11764706*	.41642246	.000	-18.6521676	-15.5831265
	15	-15.31862745*	.41642246	.000	-16.8531480	-13.7841069
	21	.47058824	.41642246	.997	-1.0639323	2.0051088
	22	-.08823529	.41642246	1.000	-1.6227558	1.4462852
	23	.22549020	.41642246	1.000	-1.3090303	1.7600107
	25	.20588235	.41642246	1.000	-1.3286382	1.7404029
	31	-1.83333333*	.41642246	.009	-3.3678539	-.2988128
	32	-.73039216	.41642246	.898	-2.2649127	.8041284
	33	-1.06862745	.41642246	.428	-2.6031480	.4658931
	34	-2.98039216*	.41642246	.000	-4.5149127	-1.4458716
	35	-1.28921569	.41642246	.176	-2.8237362	.2453048
25	11	-13.46078431*	.41642246	.000	-14.9953048	-11.9262638
	12	-15.86764706*	.41642246	.000	-17.4021676	-14.3331265
	13	-15.59313725*	.41642246	.000	-17.1276578	-14.0586167
	14	-17.32352941*	.41642246	.000	-18.8580499	-15.7890089
	15	-15.52450980*	.41642246	.000	-17.0590303	-13.9899893
	21	.26470588	.41642246	1.000	-1.2698146	1.7992264

	22	-.29411765	.41642246	1.000	-1.8286382	1.2404029
	23	.01960784	.41642246	1.000	-1.5149127	1.5541284
	24	-.20588235	.41642246	1.000	-1.7404029	1.3286382
	31	-2.03921569*	.41642246	.002	-3.5737362	-.5046952
	32	-.93627451	.41642246	.632	-2.4707950	.5982460
	33	-1.27450980	.41642246	.188	-2.8090303	.2600107
	34	-3.18627451*	.41642246	.000	-4.7207950	-1.6517540
	35	-1.49509804	.41642246	.062	-3.0296186	.0394225
31	11	-11.42156863*	.41642246	.000	-12.9560891	-9.8870481
	12	-13.82843137*	.41642246	.000	-15.3629519	-12.2939109
	13	-13.55392157*	.41642246	.000	-15.0884421	-12.0194010
	14	-15.28431372*	.41642246	.000	-16.8188342	-13.7497932
	15	-13.48529412*	.41642246	.000	-15.0198146	-11.9507736
	21	2.30392157*	.41642246	.000	.7694010	3.8384421
	22	1.74509804*	.41642246	.015	.2105775	3.2796186
	23	2.05882353*	.41642246	.002	.5243030	3.5933441
	24	1.83333333*	.41642246	.009	.2988128	3.3678539
	25	2.03921569*	.41642246	.002	.5046952	3.5737362
	32	1.10294118	.41642246	.380	-.4315793	2.6374617
	33	.76470588	.41642246	.865	-.7698146	2.2992264
	34	-1.14705882	.41642246	.322	-2.6815793	.3874617
	35	.54411765	.41642246	.990	-.9904029	2.0786382
32	11	-12.52450980*	.41642246	.000	-14.0590303	-10.9899893
	12	-14.93137255*	.41642246	.000	-16.4658931	-13.3968520
	13	-14.65686274*	.41642246	.000	-16.1913833	-13.1223422
	14	-16.38725490*	.41642246	.000	-17.9217754	-14.8527344
	15	-14.58823529*	.41642246	.000	-16.1227558	-13.0537148
	21	1.20098039	.41642246	.259	-.3335401	2.7355009
	22	.64215686	.41642246	.959	-.8923637	2.1766774
	23	.95588235	.41642246	.601	-.5786382	2.4904029
	24	.73039216	.41642246	.898	-.8041284	2.2649127
	25	.93627451	.41642246	.632	-.5982460	2.4707950
	31	-1.10294118	.41642246	.380	-2.6374617	.4315793
	33	-.33823529	.41642246	1.000	-1.8727558	1.1962852

	34	-2.25000000*	.41642246	.001	-3.7845205	-.7154795
	35	-.55882353	.41642246	.987	-2.0933441	.9756970
33	11	-12.18627451*	.41642246	.000	-13.7207950	-10.6517540
	12	-14.59313726*	.41642246	.000	-16.1276578	-13.0586167
	13	-14.31862745*	.41642246	.000	-15.8531480	-12.7841069
	14	-16.04901961*	.41642246	.000	-17.5835401	-14.5144991
	15	-14.25000000*	.41642246	.000	-15.7845205	-12.7154795
	21	1.53921569*	.41642246	.049	.0046952	3.0737362
	22	.98039216	.41642246	.562	-.5541284	2.5149127
	23	1.29411765	.41642246	.172	-.2404029	2.8286382
	24	1.06862745	.41642246	.428	-.4658931	2.6031480
	25	1.27450980	.41642246	.188	-.2600107	2.8090303
	31	-.76470588	.41642246	.865	-2.2992264	.7698146
	32	.33823529	.41642246	1.000	-1.1962852	1.8727558
	34	-1.91176471*	.41642246	.005	-3.4462852	-.3772442
	35	-.22058824	.41642246	1.000	-1.7551088	1.3139323
34	11	-10.27450980*	.41642246	.000	-11.8090303	-8.7399893
	12	-12.68137255*	.41642246	.000	-14.2158931	-11.1468520
	13	-12.40686274*	.41642246	.000	-13.9413833	-10.8723422
	14	-14.13725490*	.41642246	.000	-15.6717754	-12.6027344
	15	-12.33823529*	.41642246	.000	-13.8727558	-10.8037148
	21	3.45098039*	.41642246	.000	1.9164599	4.9855009
	22	2.89215686*	.41642246	.000	1.3576363	4.4266774
	23	3.20588235*	.41642246	.000	1.6713618	4.7404029
	24	2.98039216*	.41642246	.000	1.4458716	4.5149127
	25	3.18627451*	.41642246	.000	1.6517540	4.7207950
	31	1.14705882	.41642246	.322	-.3874617	2.6815793
	32	2.25000000*	.41642246	.001	.7154795	3.7845205
	33	1.91176471*	.41642246	.005	.3772442	3.4462852
	35	1.69117647*	.41642246	.020	.1566559	3.2256970
35	11	-11.96568627*	.41642246	.000	-13.5002068	-10.4311658
	12	-14.37254902*	.41642246	.000	-15.9070695	-12.8380285
	13	-14.09803921*	.41642246	.000	-15.6325597	-12.5635187
	14	-15.82843137*	.41642246	.000	-17.3629519	-14.2939108

	15	-14.02941176*	.41642246	.000	-15.5639323	-12.4948912
	21	1.75980392*	.41642246	.013	.2252834	3.2943244
	22	1.20098039	.41642246	.259	-.3335401	2.7355009
	23	1.51470588	.41642246	.056	-.0198146	3.0492264
	24	1.28921569	.41642246	.176	-.2453048	2.8237362
	25	1.49509804	.41642246	.062	-.0394225	3.0296186
	31	-.54411765	.41642246	.990	-2.0786382	.9904029
	32	.55882353	.41642246	.987	-.9756970	2.0933441
	33	.22058824	.41642246	1.000	-1.3139323	1.7551088
	34	-1.69117647*	.41642246	.020	-3.2256970	-.1566559

*. The mean difference is significant at the 0.05 level.

Table. A.2.4. Statistical analysis of total flavonoids contents in Jujube samples (n=2), comparison between leaves, fruit, and seed contents of 5 cultivars.

ANOVA

Total_flavonoids

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	132.918	14	9.494	248.770	.000
Within Groups	.572	15	.038		
Total	133.491	29			

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Total_flavonoids	.339	30	.000	.752	30	.000

a. Lilliefors Significance Correction

Multiple Comparisons

Dependent Variable: Total_flavonoids

Tukey HSD

(I) Part_of_tree_cultivar	(J) Part_of_tree_cultivar	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
11	12	.59191176	.19535703	.244	-.1884723	1.3722958
	13	-.08823529	.19535703	1.000	-.8686193	.6921487
	14	-1.13970588*	.19535703	.002	-1.9200899	-.3593218
	15	.84558824*	.19535703	.028	.0652042	1.6259723
	21	4.30091912*	.19535703	.000	3.5205351	5.0813032
	22	4.40937500*	.19535703	.000	3.6289910	5.1897590
	23	4.36755515*	.19535703	.000	3.5871711	5.1479392
	24	4.66167279*	.19535703	.000	3.8812888	5.4420568
	25	4.69797794*	.19535703	.000	3.9175939	5.4783620
	31	4.84181985*	.19535703	.000	4.0614358	5.6222039
	32	3.96268382*	.19535703	.000	3.1822998	4.7430679
	33	4.16718750*	.19535703	.000	3.3868035	4.9475715
	34	4.42270221*	.19535703	.000	3.6423182	5.2030862
	35	4.21750919*	.19535703	.000	3.4371252	4.9978932
12	11	-.59191176	.19535703	.244	-1.3722958	.1884723
	13	-.68014706	.19535703	.120	-1.4605311	.1002370
	14	-1.73161765*	.19535703	.000	-2.5120017	-.9512336
	15	.25367647	.19535703	.986	-.5267076	1.0340605
	21	3.70900735*	.19535703	.000	2.9286233	4.4893914
	22	3.81746324*	.19535703	.000	3.0370792	4.5978473
	23	3.77564338*	.19535703	.000	2.9952593	4.5560274
	24	4.06976103*	.19535703	.000	3.2893770	4.8501451
	25	4.10606618*	.19535703	.000	3.3256821	4.8864502
	31	4.24990809*	.19535703	.000	3.4695241	5.0302921
	32	3.37077206*	.19535703	.000	2.5903880	4.1511561
	33	3.57527574*	.19535703	.000	2.7948917	4.3556598
	34	3.83079044*	.19535703	.000	3.0504064	4.6111745
	35	3.62559743*	.19535703	.000	2.8452134	4.4059815
13	11	.08823529	.19535703	1.000	-.6921487	.8686193
	12	.68014706	.19535703	.120	-.1002370	1.4605311
	14	-1.05147059*	.19535703	.004	-1.8318546	-.2710866
	15	.93382353*	.19535703	.012	.1534395	1.7142076

	21	4.38915441*	.19535703	.000	3.6087704	5.1695384
	22	4.49761029*	.19535703	.000	3.7172263	5.2779943
	23	4.45579044*	.19535703	.000	3.6754064	5.2361745
	24	4.74990809*	.19535703	.000	3.9695241	5.5302921
	25	4.78621324*	.19535703	.000	4.0058292	5.5665973
	31	4.93005515*	.19535703	.000	4.1496711	5.7104392
	32	4.05091912*	.19535703	.000	3.2705351	4.8313032
	33	4.25542279*	.19535703	.000	3.4750388	5.0358068
	34	4.51093750*	.19535703	.000	3.7305535	5.2913215
	35	4.30574449*	.19535703	.000	3.5253605	5.0861285
14	11	1.13970588*	.19535703	.002	.3593218	1.9200899
	12	1.73161765*	.19535703	.000	.9512336	2.5120017
	13	1.05147059*	.19535703	.004	.2710866	1.8318546
	15	1.98529412*	.19535703	.000	1.2049101	2.7656782
	21	5.44062500*	.19535703	.000	4.6602410	6.2210090
	22	5.54908088*	.19535703	.000	4.7686968	6.3294649
	23	5.50726103*	.19535703	.000	4.7268770	6.2876451
	24	5.80137868*	.19535703	.000	5.0209946	6.5817627
	25	5.83768382*	.19535703	.000	5.0572998	6.6180679
	31	5.98152574*	.19535703	.000	5.2011417	6.7619098
	32	5.10238971*	.19535703	.000	4.3220057	5.8827737
	33	5.30689338*	.19535703	.000	4.5265094	6.0872774
	34	5.56240809*	.19535703	.000	4.7820241	6.3427921
	35	5.35721507*	.19535703	.000	4.5768310	6.1375991
15	11	-.84558824*	.19535703	.028	-1.6259723	-.0652042
	12	-.25367647	.19535703	.986	-1.0340605	.5267076
	13	-.93382353*	.19535703	.012	-1.7142076	-.1534395
	14	-1.98529412*	.19535703	.000	-2.7656782	-1.2049101
	21	3.45533088*	.19535703	.000	2.6749468	4.2357149
	22	3.56378676*	.19535703	.000	2.7834027	4.3441708
	23	3.52196691*	.19535703	.000	2.7415829	4.3023509
	24	3.81608456*	.19535703	.000	3.0357005	4.5964686
	25	3.85238971*	.19535703	.000	3.0720057	4.6327737
	31	3.99623162*	.19535703	.000	3.2158476	4.7766157

	32	3.11709559*	.19535703	.000	2.3367116	3.8974796
	33	3.32159926*	.19535703	.000	2.5412152	4.1019833
	34	3.57711397*	.19535703	.000	2.7967299	4.3574980
	35	3.37192096*	.19535703	.000	2.5915369	4.1523050
21	11	-4.30091912*	.19535703	.000	-5.0813032	-3.5205351
	12	-3.70900735*	.19535703	.000	-4.4893914	-2.9286233
	13	-4.38915441*	.19535703	.000	-5.1695384	-3.6087704
	14	-5.44062500*	.19535703	.000	-6.2210090	-4.6602410
	15	-3.45533088*	.19535703	.000	-4.2357149	-2.6749468
	22	.10845588	.19535703	1.000	-.6719282	.8888399
	23	.06663603	.19535703	1.000	-.7137480	.8470201
	24	.36075368	.19535703	.846	-.4196304	1.1411377
	25	.39705882	.19535703	.756	-.3833252	1.1774429
	31	.54090073	.19535703	.351	-.2394833	1.3212848
	32	-.33823529	.19535703	.893	-1.1186193	.4421487
	33	-.13373162	.19535703	1.000	-.9141157	.6466524
	34	.12178309	.19535703	1.000	-.6586009	.9021671
	35	-.08340993	.19535703	1.000	-.8637940	.6969741
22	11	-4.40937500*	.19535703	.000	-5.1897590	-3.6289910
	12	-3.81746324*	.19535703	.000	-4.5978473	-3.0370792
	13	-4.49761029*	.19535703	.000	-5.2779943	-3.7172263
	14	-5.54908088*	.19535703	.000	-6.3294649	-4.7686968
	15	-3.56378676*	.19535703	.000	-4.3441708	-2.7834027
	21	-.10845588	.19535703	1.000	-.8888399	.6719282
	23	-.04181985	.19535703	1.000	-.8222039	.7385642
	24	.25229779	.19535703	.987	-.5280862	1.0326818
	25	.28860294	.19535703	.962	-.4917811	1.0689870
	31	.43244485	.19535703	.654	-.3479392	1.2128289
	32	-.44669118	.19535703	.612	-1.2270752	.3336929
	33	-.24218750	.19535703	.991	-1.0225715	.5381965
	34	.01332721	.19535703	1.000	-.7670568	.7937112
	35	-.19186581	.19535703	.999	-.9722498	.5885182
23	11	-4.36755515*	.19535703	.000	-5.1479392	-3.5871711
	12	-3.77564338*	.19535703	.000	-4.5560274	-2.9952593

	13	-4.45579044*	.19535703	.000	-5.2361745	-3.6754064
	14	-5.50726103*	.19535703	.000	-6.2876451	-4.7268770
	15	-3.52196691*	.19535703	.000	-4.3023509	-2.7415829
	21	-.06663603	.19535703	1.000	-.8470201	.7137480
	22	.04181985	.19535703	1.000	-.7385642	.8222039
	24	.29411765	.19535703	.956	-.4862664	1.0745017
	25	.33042279	.19535703	.907	-.4499612	1.1108068
	31	.47426471	.19535703	.530	-.3061193	1.2546487
	32	-.40487132	.19535703	.734	-1.1852554	.3755127
	33	-.20036765	.19535703	.998	-.9807517	.5800164
	34	.05514706	.19535703	1.000	-.7252370	.8355311
	35	-.15004596	.19535703	1.000	-.9304300	.6303381
24	11	-4.66167279*	.19535703	.000	-5.4420568	-3.8812888
	12	-4.06976103*	.19535703	.000	-4.8501451	-3.2893770
	13	-4.74990809*	.19535703	.000	-5.5302921	-3.9695241
	14	-5.80137868*	.19535703	.000	-6.5817627	-5.0209946
	15	-3.81608456*	.19535703	.000	-4.5964686	-3.0357005
	21	-.36075368	.19535703	.846	-1.1411377	.4196304
	22	-.25229779	.19535703	.987	-1.0326818	.5280862
	23	-.29411765	.19535703	.956	-1.0745017	.4862664
	25	.03630515	.19535703	1.000	-.7440789	.8166892
	31	.18014706	.19535703	.999	-.6002370	.9605311
	32	-.69898897	.19535703	.102	-1.4793730	.0813951
	33	-.49448529	.19535703	.472	-1.2748693	.2858987
	34	-.23897059	.19535703	.992	-1.0193546	.5414134
	35	-.44416360	.19535703	.619	-1.2245476	.3362204
25	11	-4.69797794*	.19535703	.000	-5.4783620	-3.9175939
	12	-4.10606618*	.19535703	.000	-4.8864502	-3.3256821
	13	-4.78621324*	.19535703	.000	-5.5665973	-4.0058292
	14	-5.83768382*	.19535703	.000	-6.6180679	-5.0572998
	15	-3.85238971*	.19535703	.000	-4.6327737	-3.0720057
	21	-.39705882	.19535703	.756	-1.1774429	.3833252
	22	-.28860294	.19535703	.962	-1.0689870	.4917811
	23	-.33042279	.19535703	.907	-1.1108068	.4499612

	24	-.03630515	.19535703	1.000	-.8166892	.7440789
	31	.14384191	.19535703	1.000	-.6365421	.9242259
	32	-.73529412	.19535703	.075	-1.5156782	.0450899
	33	-.53079044	.19535703	.376	-1.3111745	.2495936
	34	-.27527574	.19535703	.973	-1.0556598	.5051083
	35	-.48046875	.19535703	.512	-1.2608528	.2999153
31	11	-4.84181985*	.19535703	.000	-5.6222039	-4.0614358
	12	-4.24990809*	.19535703	.000	-5.0302921	-3.4695241
	13	-4.93005515*	.19535703	.000	-5.7104392	-4.1496711
	14	-5.98152574*	.19535703	.000	-6.7619098	-5.2011417
	15	-3.99623162*	.19535703	.000	-4.7766157	-3.2158476
	21	-.54090073	.19535703	.351	-1.3212848	.2394833
	22	-.43244485	.19535703	.654	-1.2128289	.3479392
	23	-.47426471	.19535703	.530	-1.2546487	.3061193
	24	-.18014706	.19535703	.999	-.9605311	.6002370
	25	-.14384191	.19535703	1.000	-.9242259	.6365421
	32	-.87913603*	.19535703	.020	-1.6595201	-.0987520
	33	-.67463235	.19535703	.126	-1.4550164	.1057517
	34	-.41911765	.19535703	.693	-1.1995017	.3612664
	35	-.62431066	.19535703	.190	-1.4046947	.1560734
32	11	-3.96268382*	.19535703	.000	-4.7430679	-3.1822998
	12	-3.37077206*	.19535703	.000	-4.1511561	-2.5903880
	13	-4.05091912*	.19535703	.000	-4.8313032	-3.2705351
	14	-5.10238971*	.19535703	.000	-5.8827737	-4.3220057
	15	-3.11709559*	.19535703	.000	-3.8974796	-2.3367116
	21	.33823529	.19535703	.893	-.4421487	1.1186193
	22	.44669118	.19535703	.612	-.3336929	1.2270752
	23	.40487132	.19535703	.734	-.3755127	1.1852554
	24	.69898897	.19535703	.102	-.0813951	1.4793730
	25	.73529412	.19535703	.075	-.0450899	1.5156782
	31	.87913603*	.19535703	.020	.0987520	1.6595201
	33	.20450368	.19535703	.998	-.5758804	.9848877
	34	.46001838	.19535703	.572	-.3203657	1.2404024
	35	.25482537	.19535703	.985	-.5255587	1.0352094

33	11	-4.16718750*	.19535703	.000	-4.9475715	-3.3868035
	12	-3.57527574*	.19535703	.000	-4.3556598	-2.7948917
	13	-4.25542279*	.19535703	.000	-5.0358068	-3.4750388
	14	-5.30689338*	.19535703	.000	-6.0872774	-4.5265094
	15	-3.32159926*	.19535703	.000	-4.1019833	-2.5412152
	21	.13373162	.19535703	1.000	-.6466524	.9141157
	22	.24218750	.19535703	.991	-.5381965	1.0225715
	23	.20036765	.19535703	.998	-.5800164	.9807517
	24	.49448529	.19535703	.472	-.2858987	1.2748693
	25	.53079044	.19535703	.376	-.2495936	1.3111745
	31	.67463235	.19535703	.126	-.1057517	1.4550164
	32	-.20450368	.19535703	.998	-.9848877	.5758804
	34	.25551471	.19535703	.985	-.5248693	1.0358987
	35	.05032169	.19535703	1.000	-.7300623	.8307057
34	11	-4.42270221*	.19535703	.000	-5.2030862	-3.6423182
	12	-3.83079044*	.19535703	.000	-4.6111745	-3.0504064
	13	-4.51093750*	.19535703	.000	-5.2913215	-3.7305535
	14	-5.56240809*	.19535703	.000	-6.3427921	-4.7820241
	15	-3.57711397*	.19535703	.000	-4.3574980	-2.7967299
	21	-.12178309	.19535703	1.000	-.9021671	.6586009
	22	-.01332721	.19535703	1.000	-.7937112	.7670568
	23	-.05514706	.19535703	1.000	-.8355311	.7252370
	24	.23897059	.19535703	.992	-.5414134	1.0193546
	25	.27527574	.19535703	.973	-.5051083	1.0556598
	31	.41911765	.19535703	.693	-.3612664	1.1995017
	32	-.46001838	.19535703	.572	-1.2404024	.3203657
	33	-.25551471	.19535703	.985	-1.0358987	.5248693
	35	-.20519301	.19535703	.998	-.9855770	.5751910
35	11	-4.21750919*	.19535703	.000	-4.9978932	-3.4371252
	12	-3.62559743*	.19535703	.000	-4.4059815	-2.8452134
	13	-4.30574449*	.19535703	.000	-5.0861285	-3.5253605
	14	-5.35721507*	.19535703	.000	-6.1375991	-4.5768310
	15	-3.37192096*	.19535703	.000	-4.1523050	-2.5915369
	21	.08340993	.19535703	1.000	-.6969741	.8637940

	22	.19186581	.19535703	.999	-.5885182	.9722498
	23	.15004596	.19535703	1.000	-.6303381	.9304300
	24	.44416360	.19535703	.619	-.3362204	1.2245476
	25	.48046875	.19535703	.512	-.2999153	1.2608528
	31	.62431066	.19535703	.190	-.1560734	1.4046947
	32	-.25482537	.19535703	.985	-1.0352094	.5255587
	33	-.05032169	.19535703	1.000	-.8307057	.7300623
	34	.20519301	.19535703	.998	-.5751910	.9855770

*. The mean difference is significant at the 0.05 level.

Table. A.2.5. Statistical analysis of total FRAP in Jujube samples (n=3), comparison between leaves, fruit, and seed contents of 5 cultivars.

Tests of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
FRAP	Based on Mean	554779958728439 200000000000000 0.000	14	15	.000
	Based on Median	554779958728439 200000000000000 0.000	14	15	.000
	Based on Median and with adjusted df	554779958728439 200000000000000 0.000	14	2.107	.000
	Based on trimmed mean	508360252710487 300000000000000 0.000	14	15	.000

Tests of Normality

		Kolmogorov-Smirnov[a]		
	Part_of_tree_cultivar	Statistic	df	Sig.
FRAP	11	.260	2	.
	12	.260	2	.
	13	.260	2	.
	14	.260	2	.
	15	.260	2	.
	21	.260	2	.
	22	.260	2	.
	23	.260	2	.
	24	.260	2	.
	25	.260	2	.

	31	.260	2	.
	32	.260	2	.
	33	.260	2	.
	34	.260	2	.
	35	.260	2	.

a. Lilliefors Significance Correction

ANOVA

FRAP

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	154430.565	14	11030.755	36.790	.000
Within Groups	4497.498	15	299.833		
Total	158928.064	29			

Multiple Comparisons

Dependent Variable: FRAP

Tukey HSD

(I) Part_of_tree_cultivar	(J) Part_of_tree_cultivar	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
11	12	-53.73241975	17.31569323	.218	-122.9026499	15.4378104
	13	-138.65849265*	17.31569323	.000	-207.8287228	-69.4882625
	14	-82.58204110*	17.31569323	.013	-151.7522712	-13.4118110
	15	-71.52902995*	17.31569323	.039	-140.6992601	-2.3587998
	21	77.71366751*	17.31569323	.021	8.5434374	146.8838977
	22	73.20591418*	17.31569323	.033	4.0356840	142.3761443
	23	74.46808511*	17.31569323	.029	5.2978550	143.6383152
	24	80.77893978*	17.31569323	.015	11.6087096	149.9491699
	25	82.40173098*	17.31569323	.013	13.2315008	151.5719611
	31	62.02668590	17.31569323	.102	-7.1435442	131.1969160
	32	59.14172376	17.31569323	.134	-10.0285064	128.3119539
	33	57.87955284	17.31569323	.150	-11.2906773	127.0497830
	34	73.02560404*	17.31569323	.034	3.8553739	142.1958342
	35	78.25459791*	17.31569323	.020	9.0843678	147.4248281
12	11	53.73241975	17.31569323	.218	-15.4378104	122.9026499
	13	-84.92607290*	17.31569323	.010	-154.0963030	-15.7558428

	14	-28.84962135	17.31569323	.915	-98.0198515	40.3206088
	15	-17.79661020	17.31569323	.998	-86.9668403	51.3736199
	21	131.44608726*	17.31569323	.000	62.2758571	200.6163174
	22	126.93833393*	17.31569323	.000	57.7681038	196.1085641
	23	128.20050486*	17.31569323	.000	59.0302747	197.3707350
	24	134.51135953*	17.31569323	.000	65.3411294	203.6815897
	25	136.13415073*	17.31569323	.000	66.9639206	205.3043809
	31	115.75910565*	17.31569323	.000	46.5888755	184.9293358
	32	112.87414351*	17.31569323	.001	43.7039134	182.0443737
	33	111.61197258*	17.31569323	.001	42.4417424	180.7822027
	34	126.75802379*	17.31569323	.000	57.5877937	195.9282539
	35	131.98701766*	17.31569323	.000	62.8167875	201.1572478
13	11	138.65849265*	17.31569323	.000	69.4882625	207.8287228
	12	84.92607290*	17.31569323	.010	15.7558428	154.0963030
	14	56.07645155	17.31569323	.177	-13.0937786	125.2466817
	15	67.12946270	17.31569323	.061	-2.0407674	136.2996928
	21	216.37216016*	17.31569323	.000	147.2019300	285.5423903
	22	211.86440683*	17.31569323	.000	142.6941767	281.0346370
	23	213.12657776*	17.31569323	.000	143.9563476	282.2968079
	24	219.43743243*	17.31569323	.000	150.2672023	288.6076626
	25	221.06022363*	17.31569323	.000	151.8899935	290.2304538
	31	200.68517855*	17.31569323	.000	131.5149484	269.8554087
	32	197.80021642*	17.31569323	.000	128.6299863	266.9704466
	33	196.53804549*	17.31569323	.000	127.3678153	265.7082756
	34	211.68409670*	17.31569323	.000	142.5138666	280.8543268
	35	216.91309056*	17.31569323	.000	147.7428604	286.0833207
14	11	82.58204110*	17.31569323	.013	13.4118110	151.7522712
	12	28.84962135	17.31569323	.915	-40.3206088	98.0198515
	13	-56.07645155	17.31569323	.177	-125.2466817	13.0937786
	15	11.05301115	17.31569323	1.000	-58.1172190	80.2232413
	21	160.29570861*	17.31569323	.000	91.1254785	229.4659388
	22	155.78795528*	17.31569323	.000	86.6177251	224.9581854
	23	157.05012621*	17.31569323	.000	87.8798961	226.2203563
	24	163.36098088*	17.31569323	.000	94.1907507	232.5312110

	25	164.98377208*	17.31569323	.000	95.8135419	234.1540022
	31	144.60872700*	17.31569323	.000	75.4384969	213.7789571
	32	141.72376487*	17.31569323	.000	72.5535347	210.8939950
	33	140.46159394*	17.31569323	.000	71.2913638	209.6318241
	34	155.60764515*	17.31569323	.000	86.4374150	224.7778753
	35	160.83663901*	17.31569323	.000	91.6664089	230.0068692
15	11	71.52902995*	17.31569323	.039	2.3587998	140.6992601
	12	17.79661020	17.31569323	.998	-51.3736199	86.9668403
	13	-67.12946270	17.31569323	.061	-136.2996928	2.0407674
	14	-11.05301115	17.31569323	1.000	-80.2232413	58.1172190
	21	149.24269746*	17.31569323	.000	80.0724673	218.4129276
	22	144.73494413*	17.31569323	.000	75.5647140	213.9051743
	23	145.99711506*	17.31569323	.000	76.8268849	215.1673452
	24	152.30796973*	17.31569323	.000	83.1377396	221.4781999
	25	153.93076093*	17.31569323	.000	84.7605308	223.1009911
	31	133.55571585*	17.31569323	.000	64.3854857	202.7259460
	32	130.67075371*	17.31569323	.000	61.5005236	199.8409839
	33	129.40858279*	17.31569323	.000	60.2383526	198.5788129
	34	144.55463399*	17.31569323	.000	75.3844039	213.7248641
	35	149.78362786*	17.31569323	.000	80.6133977	218.9538580
21	11	-77.71366751*	17.31569323	.021	-146.8838977	-8.5434374
	12	-131.44608726*	17.31569323	.000	-200.6163174	-62.2758571
	13	-216.37216016*	17.31569323	.000	-285.5423903	-147.2019300
	14	-160.29570861*	17.31569323	.000	-229.4659388	-91.1254785
	15	-149.24269746*	17.31569323	.000	-218.4129276	-80.0724673
	22	-4.50775334	17.31569323	1.000	-73.6779835	64.6624768
	23	-3.24558241	17.31569323	1.000	-72.4158125	65.9246477
	24	3.06527227	17.31569323	1.000	-66.1049579	72.2355024
	25	4.68806347	17.31569323	1.000	-64.4821667	73.8582936
	31	-15.68698161	17.31569323	1.000	-84.8572118	53.4832485
	32	-18.57194375	17.31569323	.997	-87.7421739	50.5982864
	33	-19.83411467	17.31569323	.995	-89.0043448	49.3361155
	34	-4.68806347	17.31569323	1.000	-73.8582936	64.4821667
	35	.54093040	17.31569323	1.000	-68.6292997	69.7111605

22	11	-73.20591417*	17.31569323	.033	-142.3761443	-4.0356840
	12	-126.93833392*	17.31569323	.000	-196.1085641	-57.7681038
	13	-211.86440682*	17.31569323	.000	-281.0346370	-142.6941767
	14	-155.78795527*	17.31569323	.000	-224.9581854	-86.6177251
	15	-144.73494412*	17.31569323	.000	-213.9051743	-75.5647140
	21	4.50775334	17.31569323	1.000	-64.6624768	73.6779835
	23	1.26217093	17.31569323	1.000	-67.9080592	70.4324011
	24	7.57302560	17.31569323	1.000	-61.5972045	76.7432557
	25	9.19581680	17.31569323	1.000	-59.9744133	78.3660469
	31	-11.17922827	17.31569323	1.000	-80.3494584	57.9910019
	32	-14.06419041	17.31569323	1.000	-83.2344206	55.1060397
	33	-15.32636134	17.31569323	1.000	-84.4965915	53.8438688
	34	-.18031013	17.31569323	1.000	-69.3505403	68.9899200
	35	5.04868374	17.31569323	1.000	-64.1215464	74.2189139
23	11	-74.46808510*	17.31569323	.029	-143.6383152	-5.2978550
	12	-128.20050485*	17.31569323	.000	-197.3707350	-59.0302747
	13	-213.12657775*	17.31569323	.000	-282.2968079	-143.9563476
	14	-157.05012620*	17.31569323	.000	-226.2203563	-87.8798961
	15	-145.99711505*	17.31569323	.000	-215.1673452	-76.8268849
	21	3.24558241	17.31569323	1.000	-65.9246477	72.4158125
	22	-1.26217093	17.31569323	1.000	-70.4324011	67.9080592
	24	6.31085467	17.31569323	1.000	-62.8593755	75.4810848
	25	7.93364587	17.31569323	1.000	-61.2365843	77.1038760
	31	-12.44139920	17.31569323	1.000	-81.6116293	56.7288309
	32	-15.32636134	17.31569323	1.000	-84.4965915	53.8438688
	33	-16.58853227	17.31569323	.999	-85.7587624	52.5816979
	34	-1.44248106	17.31569323	1.000	-70.6127112	67.7277491
	35	3.78651281	17.31569323	1.000	-65.3837173	72.9567429
24	11	-80.77893978*	17.31569323	.015	-149.9491699	-11.6087096
	12	-134.51135953*	17.31569323	.000	-203.6815897	-65.3411294
	13	-219.43743243*	17.31569323	.000	-288.6076626	-150.2672023
	14	-163.36098088*	17.31569323	.000	-232.5312110	-94.1907507
	15	-152.30796973*	17.31569323	.000	-221.4781999	-83.1377396
	21	-3.06527227	17.31569323	1.000	-72.2355024	66.1049579

	22	-7.57302560	17.31569323	1.000	-76.7432557	61.5972045
	23	-6.31085467	17.31569323	1.000	-75.4810848	62.8593755
	25	1.62279120	17.31569323	1.000	-67.5474389	70.7930213
	31	-18.75225388	17.31569323	.997	-87.9224840	50.4179763
	32	-21.63721601	17.31569323	.990	-90.8074462	47.5330141
	33	-22.89938694	17.31569323	.984	-92.0696171	46.2708432
	34	-7.75333573	17.31569323	1.000	-76.9235659	61.4168944
	35	-2.52434187	17.31569323	1.000	-71.6945720	66.6458883
25	11	-82.40173098*	17.31569323	.013	-151.5719611	-13.2315008
	12	-136.13415073*	17.31569323	.000	-205.3043809	-66.9639206
	13	-221.06022363*	17.31569323	.000	-290.2304538	-151.8899935
	14	-164.98377208*	17.31569323	.000	-234.1540022	-95.8135419
	15	-153.93076093*	17.31569323	.000	-223.1009911	-84.7605308
	21	-4.68806347	17.31569323	1.000	-73.8582936	64.4821667
	22	-9.19581680	17.31569323	1.000	-78.3660469	59.9744133
	23	-7.93364587	17.31569323	1.000	-77.1038760	61.2365843
	24	-1.62279120	17.31569323	1.000	-70.7930213	67.5474389
	31	-20.37504508	17.31569323	.994	-89.5452752	48.7951851
	32	-23.26000721	17.31569323	.982	-92.4302374	45.9102229
	33	-24.52217814	17.31569323	.972	-93.6924083	44.6480520
	34	-9.37612693	17.31569323	1.000	-78.5463571	59.7941032
	35	-4.14713307	17.31569323	1.000	-73.3173632	65.0230971
31	11	-62.02668590	17.31569323	.102	-131.1969160	7.1435442
	12	-115.75910565*	17.31569323	.000	-184.9293358	-46.5888755
	13	-200.68517855*	17.31569323	.000	-269.8554087	-131.5149484
	14	-144.60872700*	17.31569323	.000	-213.7789571	-75.4384969
	15	-133.55571585*	17.31569323	.000	-202.7259460	-64.3854857
	21	15.68698161	17.31569323	1.000	-53.4832485	84.8572118
	22	11.17922828	17.31569323	1.000	-57.9910019	80.3494584
	23	12.44139921	17.31569323	1.000	-56.7288309	81.6116293
	24	18.75225388	17.31569323	.997	-50.4179763	87.9224840
	25	20.37504508	17.31569323	.994	-48.7951851	89.5452752
	32	-2.88496214	17.31569323	1.000	-72.0551923	66.2852680
	33	-4.14713306	17.31569323	1.000	-73.3173632	65.0230971

	34	10.99891814	17.31569323	1.000	-58.1713120	80.1691483
	35	16.22791201	17.31569323	.999	-52.9423181	85.3981422
32	11	-59.14172376	17.31569323	.134	-128.3119539	10.0285064
	12	-112.87414351*	17.31569323	.001	-182.0443737	-43.7039134
	13	-197.80021641*	17.31569323	.000	-266.9704466	-128.6299863
	14	-141.72376486*	17.31569323	.000	-210.8939950	-72.5535347
	15	-130.67075371*	17.31569323	.000	-199.8409839	-61.5005236
	21	18.57194375	17.31569323	.997	-50.5982864	87.7421739
	22	14.06419041	17.31569323	1.000	-55.1060397	83.2344206
	23	15.32636134	17.31569323	1.000	-53.8438688	84.4965915
	24	21.63721601	17.31569323	.990	-47.5330141	90.8074462
	25	23.26000721	17.31569323	.982	-45.9102229	92.4302374
	31	2.88496214	17.31569323	1.000	-66.2852680	72.0551923
	33	-1.26217093	17.31569323	1.000	-70.4324011	67.9080592
	34	13.88388028	17.31569323	1.000	-55.2863499	83.0541104
	35	19.11287415	17.31569323	.997	-50.0573560	88.2831043
33	11	-57.87955283	17.31569323	.150	-127.0497830	11.2906773
	12	-111.61197258*	17.31569323	.001	-180.7822027	-42.4417424
	13	-196.53804548*	17.31569323	.000	-265.7082756	-127.3678153
	14	-140.46159393*	17.31569323	.000	-209.6318241	-71.2913638
	15	-129.40858278*	17.31569323	.000	-198.5788129	-60.2383526
	21	19.83411467	17.31569323	.995	-49.3361155	89.0043448
	22	15.32636134	17.31569323	1.000	-53.8438688	84.4965915
	23	16.58853227	17.31569323	.999	-52.5816979	85.7587624
	24	22.89938694	17.31569323	.984	-46.2708432	92.0696171
	25	24.52217814	17.31569323	.972	-44.6480520	93.6924083
	31	4.14713306	17.31569323	1.000	-65.0230971	73.3173632
	32	1.26217093	17.31569323	1.000	-67.9080592	70.4324011
	34	15.14605121	17.31569323	1.000	-54.0241789	84.3162814
	35	20.37504508	17.31569323	.994	-48.7951851	89.5452752
34	11	-73.02560404*	17.31569323	.034	-142.1958342	-3.8553739
	12	-126.75802379*	17.31569323	.000	-195.9282539	-57.5877937
	13	-211.68409669*	17.31569323	.000	-280.8543268	-142.5138666
	14	-155.60764514*	17.31569323	.000	-224.7778753	-86.4374150

	15	-144.55463399*	17.31569323	.000	-213.7248641	-75.3844039
	21	4.68806347	17.31569323	1.000	-64.4821667	73.8582936
	22	.18031013	17.31569323	1.000	-68.9899200	69.3505403
	23	1.44248106	17.31569323	1.000	-67.7277491	70.6127112
	24	7.75333573	17.31569323	1.000	-61.4168944	76.9235659
	25	9.37612693	17.31569323	1.000	-59.7941032	78.5463571
	31	-10.99891814	17.31569323	1.000	-80.1691483	58.1713120
	32	-13.88388028	17.31569323	1.000	-83.0541104	55.2863499
	33	-15.14605121	17.31569323	1.000	-84.3162814	54.0241789
	35	5.22899387	17.31569323	1.000	-63.9412363	74.3992240
35	11	-78.25459791*	17.31569323	.020	-147.4248281	-9.0843678
	12	-131.98701766*	17.31569323	.000	-201.1572478	-62.8167875
	13	-216.91309056*	17.31569323	.000	-286.0833207	-147.7428604
	14	-160.83663901*	17.31569323	.000	-230.0068692	-91.6664089
	15	-149.78362786*	17.31569323	.000	-218.9538580	-80.6133977
	21	-.54093040	17.31569323	1.000	-69.7111605	68.6292997
	22	-5.04868374	17.31569323	1.000	-74.2189139	64.1215464
	23	-3.78651281	17.31569323	1.000	-72.9567429	65.3837173
	24	2.52434187	17.31569323	1.000	-66.6458883	71.6945720
	25	4.14713307	17.31569323	1.000	-65.0230971	73.3173632
	31	-16.22791201	17.31569323	.999	-85.3981422	52.9423181
	32	-19.11287415	17.31569323	.997	-88.2831043	50.0573560
	33	-20.37504507	17.31569323	.994	-89.5452752	48.7951851
	34	-5.22899387	17.31569323	1.000	-74.3992240	63.9412363

*. The mean difference is significant at the 0.05 level.

Table. A.2.6. Statistical analysis of total FRAP in Jujube samples (n=1), comparison between leaves, fruit, and seed contents of 5 cultivars.

ANOVA

Total_triterpene_content

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	455769.406	14	32554.958	286.163	.000
Within Groups	3412.905	30	113.763		
Total	459182.311	44			

Multiple Comparisons

Dependent Variable: Total_triterpene_content

Tukey HSD

(I) Part_of_tree_cultivar	(J) Part_of_tree_cultivar	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
11	12	18.61861860	8.70875013	.702	-13.4732044	50.7104416
	13	-156.39639643*	8.70875013	.000	-188.4882194	-124.3045734
	14	-9.12912913	8.70875013	.999	-41.2209521	22.9626939
	15	-130.21021020*	8.70875013	.000	-162.3020332	-98.1183872
	21	172.72672670*	8.70875013	.000	140.6349037	204.8185497
	22	160.65465463*	8.70875013	.000	128.5628316	192.7464776
	23	145.15915914*	8.70875013	.000	113.0673361	177.2509821
	24	139.60360358*	8.70875013	.000	107.5117806	171.6954266
	25	166.36036033*	8.70875013	.000	134.2685373	198.4521833
	31	148.04204202*	8.70875013	.000	115.9502190	180.1338650
	32	106.27027027*	8.70875013	.000	74.1784473	138.3620933
	33	87.23123123*	8.70875013	.000	55.1394082	119.3230542
	34	67.56156153*	8.70875013	.000	35.4697385	99.6533845
	35	94.94894893*	8.70875013	.000	62.8571259	127.0407719
12	11	-18.61861860	8.70875013	.702	-50.7104416	13.4732044
	13	-175.01501503*	8.70875013	.000	-207.1068380	-142.9231920
	14	-27.74774773	8.70875013	.147	-59.8395707	4.3440753
	15	-148.82882880*	8.70875013	.000	-180.9206518	-116.7370058
	21	154.10810810*	8.70875013	.000	122.0162851	186.1999311
	22	142.03603603*	8.70875013	.000	109.9442130	174.1278590
	23	126.54054054*	8.70875013	.000	94.4487175	158.6323635
	24	120.98498498*	8.70875013	.000	88.8931620	153.0768080
	25	147.74174173*	8.70875013	.000	115.6499187	179.8335647
	31	129.42342342*	8.70875013	.000	97.3316004	161.5152464
	32	87.65165167*	8.70875013	.000	55.5598287	119.7434747
	33	68.61261263*	8.70875013	.000	36.5207896	100.7044356
	34	48.94294293*	8.70875013	.000	16.8511199	81.0347659
	35	76.33033033*	8.70875013	.000	44.2385073	108.4221533
13	11	156.39639643*	8.70875013	.000	124.3045734	188.4882194
	12	175.01501503*	8.70875013	.000	142.9231920	207.1068380
	14	147.26726730*	8.70875013	.000	115.1754443	179.3590903
	15	26.18618623	8.70875013	.208	-5.9056368	58.2780092
	21	329.12312314*	8.70875013	.000	297.0313001	361.2149461
	22	317.05105106*	8.70875013	.000	284.9592281	349.1428741
	23	301.55555557*	8.70875013	.000	269.4637326	333.6473786
	24	296.00000001*	8.70875013	.000	263.9081770	328.0918230

	25	322.75675677*	8.70875013	.000	290.6649338	354.8485798
	31	304.43843845*	8.70875013	.000	272.3466154	336.5302615
	32	262.66666670*	8.70875013	.000	230.5748437	294.7584897
	33	243.62762767*	8.70875013	.000	211.5358047	275.7194507
	34	223.95795797*	8.70875013	.000	191.8661350	256.0497810
	35	251.34534537*	8.70875013	.000	219.2535224	283.4371684
14	11	9.12912913	8.70875013	.999	-22.9626939	41.2209521
	12	27.74774773	8.70875013	.147	-4.3440753	59.8395707
	13	-147.26726730*	8.70875013	.000	-179.3590903	-115.1754443
	15	-121.08108107*	8.70875013	.000	-153.1729041	-88.9892581
	21	181.85585584*	8.70875013	.000	149.7640328	213.9476788
	22	169.78378376*	8.70875013	.000	137.6919608	201.8756068
	23	154.28828827*	8.70875013	.000	122.1964653	186.3801113
	24	148.73273271*	8.70875013	.000	116.6409097	180.8245557
	25	175.48948947*	8.70875013	.000	143.3976665	207.5813125
	31	157.17117115*	8.70875013	.000	125.0793481	189.2629942
	32	115.39939940*	8.70875013	.000	83.3075764	147.4912224
	33	96.36036037*	8.70875013	.000	64.2685374	128.4521834
	34	76.69069067*	8.70875013	.000	44.5988677	108.7825137
	35	104.07807807*	8.70875013	.000	71.9862551	136.1699011
15	11	130.21021020*	8.70875013	.000	98.1183872	162.3020332
	12	148.82882880*	8.70875013	.000	116.7370058	180.9206518
	13	-26.18618623	8.70875013	.208	-58.2780092	5.9056368
	14	121.08108107*	8.70875013	.000	88.9892581	153.1729041
	21	302.93693690*	8.70875013	.000	270.8451139	335.0287599
	22	290.86486483*	8.70875013	.000	258.7730418	322.9566878
	23	275.36936934*	8.70875013	.000	243.2775463	307.4611923
	24	269.81381378*	8.70875013	.000	237.7219908	301.9056368
	25	296.57057053*	8.70875013	.000	264.4787475	328.6623935
	31	278.25225222*	8.70875013	.000	246.1604292	310.3440752
	32	236.48048047*	8.70875013	.000	204.3886575	268.5723035
	33	217.44144143*	8.70875013	.000	185.3496184	249.5332644
	34	197.77177173*	8.70875013	.000	165.6799487	229.8635947
	35	225.15915913*	8.70875013	.000	193.0673361	257.2509821
21	11	-172.72672670*	8.70875013	.000	-204.8185497	-140.6349037
	12	-154.10810810*	8.70875013	.000	-186.1999311	-122.0162851
	13	-329.12312314*	8.70875013	.000	-361.2149461	-297.0313001
	14	-181.85585584*	8.70875013	.000	-213.9476788	-149.7640328
	15	-302.93693690*	8.70875013	.000	-335.0287599	-270.8451139
	22	-12.07207207	8.70875013	.982	-44.1638951	20.0197509
	23	-27.56756757	8.70875013	.153	-59.6593906	4.5242554
	24	-33.12312312*	8.70875013	.038	-65.2149461	-1.0313001

	25	-6.36636637	8.70875013	1.000	-38.4581894	25.7254566
	31	-24.68468468	8.70875013	.282	-56.7765077	7.4071383
	32	-66.45645644*	8.70875013	.000	-98.5482794	-34.3646334
	33	-85.49549547*	8.70875013	.000	-117.5873185	-53.4036725
	34	-105.16516517*	8.70875013	.000	-137.2569882	-73.0733422
	35	-77.77777777*	8.70875013	.000	-109.8696008	-45.6859548
22	11	-160.65465463*	8.70875013	.000	-192.7464776	-128.5628316
	12	-142.03603603*	8.70875013	.000	-174.1278590	-109.9442130
	13	-317.05105106*	8.70875013	.000	-349.1428741	-284.9592281
	14	-169.78378376*	8.70875013	.000	-201.8756068	-137.6919608
	15	-290.86486483*	8.70875013	.000	-322.9566878	-258.7730418
	21	12.07207207	8.70875013	.982	-20.0197509	44.1638951
	23	-15.49549549	8.70875013	.889	-47.5873185	16.5963275
	24	-21.05105105	8.70875013	.522	-53.1428741	11.0407720
	25	5.70570570	8.70875013	1.000	-26.3861173	37.7975287
	31	-12.61261261	8.70875013	.975	-44.7044356	19.4792104
	32	-54.38438436*	8.70875013	.000	-86.4762074	-22.2925614
	33	-73.42342340*	8.70875013	.000	-105.5152464	-41.3316004
	34	-93.09309310*	8.70875013	.000	-125.1849161	-61.0012701
	35	-65.70570570*	8.70875013	.000	-97.7975287	-33.6138827
23	11	-145.15915914*	8.70875013	.000	-177.2509821	-113.0673361
	12	-126.54054054*	8.70875013	.000	-158.6323635	-94.4487175
	13	-301.55555557*	8.70875013	.000	-333.6473786	-269.4637326
	14	-154.28828827*	8.70875013	.000	-186.3801113	-122.1964653
	15	-275.36936934*	8.70875013	.000	-307.4611923	-243.2775463
	21	27.56756757	8.70875013	.153	-4.5242554	59.6593906
	22	15.49549549	8.70875013	.889	-16.5963275	47.5873185
	24	-5.55555556	8.70875013	1.000	-37.6473786	26.5362674
	25	21.20120120	8.70875013	.511	-10.8906218	53.2930242
	31	2.88288288	8.70875013	1.000	-29.2089401	34.9747059
	32	-38.88888887*	8.70875013	.007	-70.9807119	-6.7970659
	33	-57.92792790*	8.70875013	.000	-90.0197509	-25.8361049
	34	-77.59759760*	8.70875013	.000	-109.6894206	-45.5057746
	35	-50.21021020*	8.70875013	.000	-82.3020332	-18.1183872
24	11	-139.60360358*	8.70875013	.000	-171.6954266	-107.5117806
	12	-120.98498498*	8.70875013	.000	-153.0768080	-88.8931620
	13	-296.00000001*	8.70875013	.000	-328.0918230	-263.9081770
	14	-148.73273271*	8.70875013	.000	-180.8245557	-116.6409097
	15	-269.81381378*	8.70875013	.000	-301.9056368	-237.7219908

	21	33.12312312*	8.70875013	.038	1.0313001	65.2149461
	22	21.05105105	8.70875013	.522	-11.0407720	53.1428741
	23	5.55555556	8.70875013	1.000	-26.5362674	37.6473786
	25	26.75675675	8.70875013	.184	-5.3350663	58.8485798
	31	8.43843844	8.70875013	.999	-23.6533846	40.5302614
	32	-33.33333331*	8.70875013	.036	-65.4251563	-1.2415103
	33	-52.37237235*	8.70875013	.000	-84.4641954	-20.2805493
	34	-72.04204205*	8.70875013	.000	-104.1338651	-39.9502190
	35	-44.65465465*	8.70875013	.001	-76.7464777	-12.5628316
25	11	-166.36036033*	8.70875013	.000	-198.4521833	-134.2685373
	12	-147.74174173*	8.70875013	.000	-179.8335647	-115.6499187
	13	-322.75675677*	8.70875013	.000	-354.8485798	-290.6649338
	14	-175.48948947*	8.70875013	.000	-207.5813125	-143.3976665
	15	-296.57057053*	8.70875013	.000	-328.6623935	-264.4787475
	21	6.36636637	8.70875013	1.000	-25.7254566	38.4581894
	22	-5.70570570	8.70875013	1.000	-37.7975287	26.3861173
	23	-21.20120120	8.70875013	.511	-53.2930242	10.8906218
	24	-26.75675675	8.70875013	.184	-58.8485798	5.3350663
	31	-18.31831831	8.70875013	.723	-50.4101413	13.7735047
	32	-60.09009007*	8.70875013	.000	-92.1819131	-27.9982671
	33	-79.12912910*	8.70875013	.000	-111.2209521	-47.0373061
	34	-98.79879880*	8.70875013	.000	-130.8906218	-66.7069758
	35	-71.41141140*	8.70875013	.000	-103.5032344	-39.3195884
31	11	-148.04204202*	8.70875013	.000	-180.1338650	-115.9502190
	12	-129.42342342*	8.70875013	.000	-161.5152464	-97.3316004
	13	-304.43843845*	8.70875013	.000	-336.5302615	-272.3466154
	14	-157.17117115*	8.70875013	.000	-189.2629942	-125.0793481
	15	-278.25225222*	8.70875013	.000	-310.3440752	-246.1604292
	21	24.68468468	8.70875013	.282	-7.4071383	56.7765077
	22	12.61261261	8.70875013	.975	-19.4792104	44.7044356
	23	-2.88288288	8.70875013	1.000	-34.9747059	29.2089401
	24	-8.43843844	8.70875013	.999	-40.5302614	23.6533846
	25	18.31831831	8.70875013	.723	-13.7735047	50.4101413
	32	-41.77177175*	8.70875013	.003	-73.8635948	-9.6799487
	33	-60.81081079*	8.70875013	.000	-92.9026338	-28.7189878
	34	-80.48048049*	8.70875013	.000	-112.5723035	-48.3886575
	35	-53.09309309*	8.70875013	.000	-85.1849161	-21.0012701
32	11	-106.27027027*	8.70875013	.000	-138.3620933	-74.1784473
	12	-87.65165167*	8.70875013	.000	-119.7434747	-55.5598287

	13	-262.66666670*	8.70875013	.000	-294.7584897	-230.5748437
	14	-115.39939940*	8.70875013	.000	-147.4912224	-83.3075764
	15	-236.48048047*	8.70875013	.000	-268.5723035	-204.3886575
	21	66.45645644*	8.70875013	.000	34.3646334	98.5482794
	22	54.38438436*	8.70875013	.000	22.2925614	86.4762074
	23	38.88888887*	8.70875013	.007	6.7970659	70.9807119
	24	33.33333331*	8.70875013	.036	1.2415103	65.4251563
	25	60.09009007*	8.70875013	.000	27.9982671	92.1819131
	31	41.77177175*	8.70875013	.003	9.6799487	73.8635948
	33	-19.03903903	8.70875013	.671	-51.1308620	13.0527840
	34	-38.70870873*	8.70875013	.008	-70.8005317	-6.6168857
	35	-11.32132133	8.70875013	.990	-43.4131443	20.7705017
33	11	-87.23123123*	8.70875013	.000	-119.3230542	-55.1394082
	12	-68.61261263*	8.70875013	.000	-100.7044356	-36.5207896
	13	-243.62762767*	8.70875013	.000	-275.7194507	-211.5358047
	14	-96.36036037*	8.70875013	.000	-128.4521834	-64.2685374
	15	-217.44144143*	8.70875013	.000	-249.5332644	-185.3496184
	21	85.49549547*	8.70875013	.000	53.4036725	117.5873185
	22	73.42342340*	8.70875013	.000	41.3316004	105.5152464
	23	57.92792790*	8.70875013	.000	25.8361049	90.0197509
	24	52.37237235*	8.70875013	.000	20.2805493	84.4641954
	25	79.12912910*	8.70875013	.000	47.0373061	111.2209521
	31	60.81081079*	8.70875013	.000	28.7189878	92.9026338
	32	19.03903903	8.70875013	.671	-13.0527840	51.1308620
	34	-19.66966970	8.70875013	.625	-51.7614927	12.4221533
	35	7.71771770	8.70875013	1.000	-24.3741053	39.8095407
34	11	-67.56156153*	8.70875013	.000	-99.6533845	-35.4697385
	12	-48.94294293*	8.70875013	.000	-81.0347659	-16.8511199
	13	-223.95795797*	8.70875013	.000	-256.0497810	-191.8661350
	14	-76.69069067*	8.70875013	.000	-108.7825137	-44.5988677
	15	-197.77177173*	8.70875013	.000	-229.8635947	-165.6799487
	21	105.16516517*	8.70875013	.000	73.0733422	137.2569882
	22	93.09309310*	8.70875013	.000	61.0012701	125.1849161
	23	77.59759760*	8.70875013	.000	45.5057746	109.6894206
	24	72.04204205*	8.70875013	.000	39.9502190	104.1338651
	25	98.79879880*	8.70875013	.000	66.7069758	130.8906218
	31	80.48048049*	8.70875013	.000	48.3886575	112.5723035
	32	38.70870873*	8.70875013	.008	6.6168857	70.8005317
	33	19.66966970	8.70875013	.625	-12.4221533	51.7614927
	35	27.38738740	8.70875013	.160	-4.7044356	59.4792104
35	11	-94.94894893*	8.70875013	.000	-127.0407719	-62.8571259

	12	-76.33033033*	8.70875013	.000	-108.4221533	-44.2385073
	13	-251.34534537*	8.70875013	.000	-283.4371684	-219.2535224
	14	-104.07807807*	8.70875013	.000	-136.1699011	-71.9862551
	15	-225.15915913*	8.70875013	.000	-257.2509821	-193.0673361
	21	77.77777777*	8.70875013	.000	45.6859548	109.8696008
	22	65.70570570*	8.70875013	.000	33.6138827	97.7975287
	23	50.21021020*	8.70875013	.000	18.1183872	82.3020332
	24	44.65465465*	8.70875013	.001	12.5628316	76.7464777
	25	71.41141140*	8.70875013	.000	39.3195884	103.5032344
	31	53.09309309*	8.70875013	.000	21.0012701	85.1849161
	32	11.32132133	8.70875013	.990	-20.7705017	43.4131443
	33	-7.71771770	8.70875013	1.000	-39.8095407	24.3741053
	34	-27.38738740	8.70875013	.160	-59.4792104	4.7044356

*. The mean difference is significant at the 0.05 level.

Table. A.3.1. Statistical analysis of cell MTT assay results (cell death %) (n=2), comparison between Li 2, Chico, Sihong leaf extract treatment on HCT116 cells at 5 mg/mL concentration.

ANOVA

CELL_DEATH_PERCENTAGE

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	19.647	2	9.823	12.707	.034
Within Groups	2.319	3	.773		
Total	21.966	5			

Multiple Comparisons

Dependent Variable: CELL_DEATH_PERCENTAGE

Tukey HSD

(I) TREATMENT	(J) TREATMENT	Std. Error	Sig.	95% Confidence Interval

		Mean Difference (I-J)			Lower Bound	Upper Bound
1	2	1.12849	.87923	.493	-2.5456	4.8026
	3	4.27640*	.87923	.033	.6023	7.9505
2	1	-1.12849	.87923	.493	-4.8026	2.5456
	3	3.14790	.87923	.074	-.5262	6.8220
3	1	-4.27640*	.87923	.033	-7.9505	-.6023
	2	-3.14790	.87923	.074	-6.8220	.5262

*. The mean difference is significant at the 0.05 level.