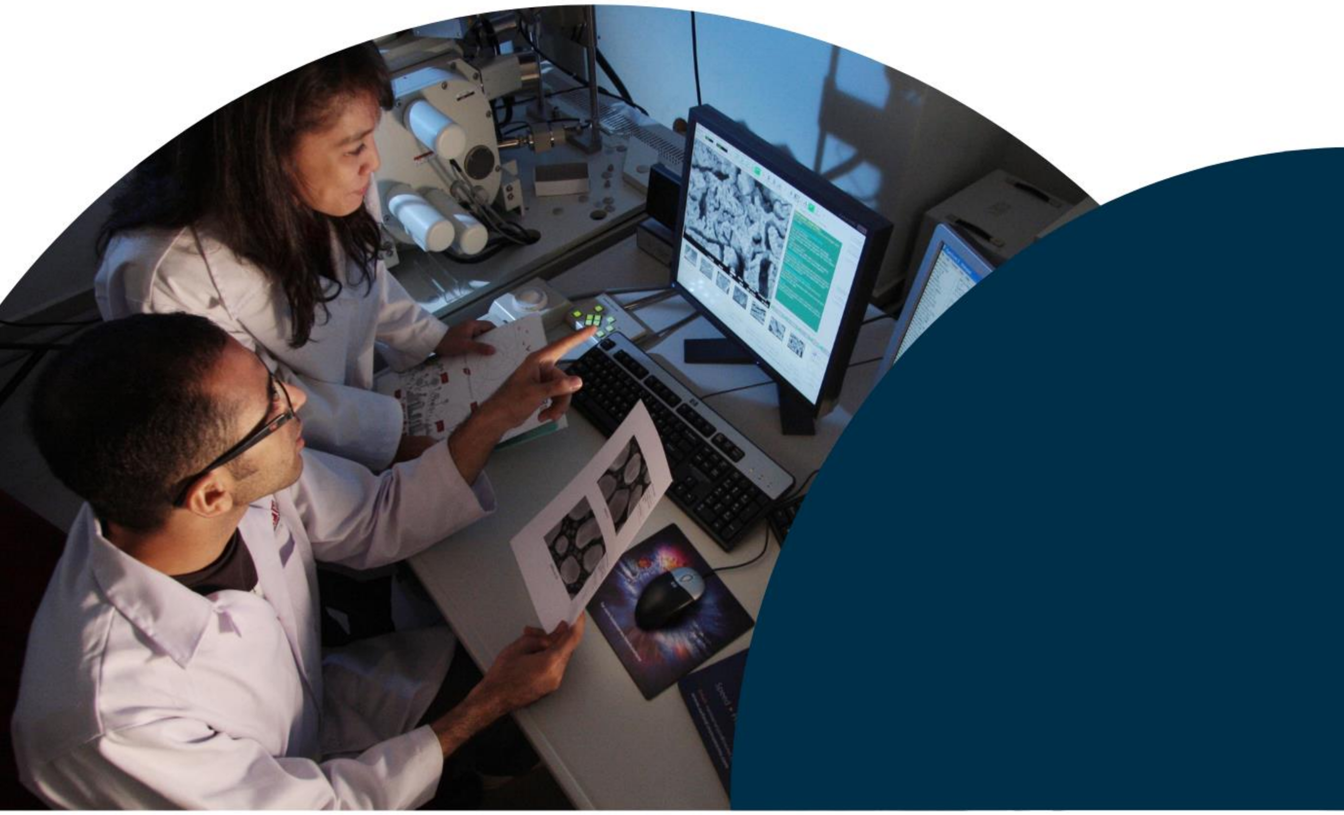
OCTOBER 2023

COMPILATION OF RESEARCH PAPERS ON STEM

SCIENCE, TECHNOLOGY, ENGINEERING & MATHEMATICS



EDITOR,

YUS AZNITA USTADI

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CHAPTER IN BOOK

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FOREWORD

It is with great pleasure that I introduce this compelling compilation of research papers on STEM (Science, Technology, Engineering, and Mathematics) in October 2023.

The field of STEM encompasses a vast range of disciplines, each playing a crucial role in shaping our present and future. This collection brings together a selection of research papers that explore few aspects of STEM, showcasing the remarkable breadth and depth of knowledge being generated across these domains.

This book serves as evidence to the collaborative spirit that defines the scientific community. It is a celebration of the collective achievements of researchers, scholars, and scientists who dedicate their lives to unravelling the mysteries of the natural world and advancing our understanding of the universe.

I extend my gratitude to the authors whose work graces these pages, as well as the publisher's team who have made this compilation possible. Their dedication and passion for advancing knowledge in STEM are truly commendable.

I hope that this compilation inspires readers to appreciate the wonders of STEM and encourages them to embark on their own journeys of discovery and inquiry. May this book serve as a source of inspiration and a catalyst for further exploration and innovation.

Editor

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Production of Prebiotics Fermented Guava (*Psidium guajava*) Drinks

Nordalila Ahmad, Rinani Shima Abd Rashid*, Masniza Mohamed, Farah Salina Hussin & Noriza Ahmad.

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ABSTRACT

This study aimed to explore the potential of various prebiotics, including fructo-oligosaccharide (FOS), inulin, isomalto-oligosaccharide (IMO), and gum arabic (GA), in promoting lactic acid bacteria (LAB) development in fermented guava drinks. *Lactobacillus* spp. was used as the LAB strain. The guava drinks were fermented for 72 hours at 30 °C under anaerobic circumstances, then kept at 4 °C for 14 days. The drinks also contained 1% (v/v) probiotics and 6% (w/v) of various prebiotics. The pH and sensory assessment were calculated, along with the total viable count. Among the tested prebiotics, gum arabic exhibited the most favorable microbial growth, while FOS showed the lowest microbial population. The pH reduction of all fermented drinks was observed, with IMO demonstrating the highest pH reduction. Regarding overall acceptance, the guava drink, added with IMO was most preferred by the panellist. These findings suggest that probiotics can thrive in sterilized guava drinks supplemented with prebiotics, indicating their potential use as prebiotic health food.

Keywords: Guava, Fermentation, prebiotic, *Lactobacillus* spp.

1. INTRODUCTION

A prebiotic is a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health (Gibson et al., 2010). Prebiotics have also been shown to have effects on health, such as increased certain minerals, colonization resistance, constipation relief, and reduction in serum lipids and cholesterol (Du et al., 2020; Zábranský et al., 2021).

Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2001). Nowadays, probiotics are popular as food supplements based on numerous scientific studies on maintaining a healthy gut microbiota and may protect against a host of gastrointestinal disorders such as irritable syndromes of the bowel and colon cancer (Amara & Shibl, 2015)

Studies have shown that by using both the benefits of prebiotics and probiotics into synergy, the number of good bacteria in the digestive system increases to better health (Sekhon & Jairath, 2010; Upasana, 2022). Most industrial probiotic food products mainly belong to dairy products. People with lactose intolerance and issues of cholesterol level in milk will be the disadvantages in obtaining health benefits from probiotic products. To overcome this problem, it has been proposed that fruit juice might serve as a beneficial environment for growing probiotic bacteria. (Frediansyah, 2021).

Guava (*Psidium guajava* Linn.), is one of a tropical fruit which had been cultivated mainly in Asian countries. It is a super fruit with unique flavor, taste, and health-promoting qualities. These fruits are a good source of carbohydrates and vitamin C, as well as a fairly good source of vitamin A and B complex, calcium, phosphorus, and fiber (Bongha, 2020). In this study, guava is used to produce juice, which is later added with probiotics and prebiotics.

2. METHODOLOGY

2.1. Materials

Fresh guava fruits were obtained from the local market. The fruit was then kept at 4 °C until prior use. The medium-sized guava fruits, semi-ripe and slightly firm, with a slight greenish-yellow color, were chosen as the sample. Inulin, fructo-oligosaccharides (FOS), isomalto-oligosaccharides (IMO) and gum arabic were obtained from CK Chemical Sdn. Bhd., Malaysia. *L. acidophilus* FTCC 0291, *L. casei* FTCC 0442, and *L. bulgaricus* FTCC 0411, all in slant agar, were bought from the Food Technology Research Center, MARDI, Serdang. All cultures were maintained on MRS slant agar at 4 °C and subcultured every month. All microbiological culture media were obtained from Oxoid Ltd. (Hampshire, England). Malaysia. All other chemicals were analytical grade and obtained from Merck, Darmstadt Co., or Sigma Chemical Co.

2.2. Inoculum and seed medium preparation

A single colony of individual *Lactobacillus* spp. from MRS plate agar was transferred into 15 ml MRS broth in test tubes. The broths were then placed into an anaerobic jar (Oxoid Ltd., Hampshire, England) which contained Anerogen envelopes (Oxoid Ltd., Hampshire, England) to create anaerobic environment and incubated at 37 °C for 24 hours. Total viable count *of Lactobacillus* spp*.* was counted, and populations in the range of 10^6 – 10^8 CFU/mL were obtained.

2.3. Guava drinks preparation and fermentation of prebiotic guava drink

Guava fruits were blended with the following formulation: 55% (w/v) of guava, 5% (w/v) of sugar, and 40% (v/v) of water. The guava drinks were then filtered by using cheese cloth. The pH was measured using pH meter. Fermentation experiments were conducted in an anaerobic jar filled with 120 ml of guava drinks in bottles. All the bottles containing guava drinks were pasteurized at 70 °c for 20 minutes; then, the guava drinks were cooled to 45 °c before all the probiotic cultures were inoculated into the drink. 6% (w/v) of fos, imo, inulin, and gum arabic, respectively, were added to the samples. the guava drinks without the addition of prebiotics were served as control. a mixture of 1% (v/v) 10^6 – 10^8 cfu/ml of each lactobacillus spp. being inoculated into each bottle. all samples were then incubated for 72 hours at a temperature 37 °c before further storage in the refrigerator at 4 °c for up to 14 days. samples were taken at various time intervals up to 14 days for chemical and microbiological analysis.

2.4. Microbiological analysis

Viable plate counts were determined by the standard plate method with MRS agar at 0, 36, and 72 hours during fermentation and at day 0, 4, 8, 10 and 14 day of cold storage. Pour plate count was conducted by using the MRS agar. The plates are incubated at 37°C for 3 days. The number of bacteria growth was counted and expressed as colony forming unit or CFU/mL.

2.5. Measurement of pH

The pH of guava fruit drink was measured with a pH meter (Toledo 320, Mettler).

2.6. Sensory evaluation test

30 panelists were selected from among the students in UniKL. The panelists were asked to rate their acceptance of each attribute in the guava drink. A range of hedonic scales from 1 to 5 was used. 1 indicates extremely dislike, while 5 indicates extremely like. Each sample was numbered randomly according to permutation to avoid bias. The guava drinks were maintained and served at 4 °C. The result from the hedonic test was then calculated by using SPSS. The graphs were plotted based on the results obtained.

3. RESULTS AND DISCUSSION

3.1. Lactic acid bacteria count in prebiotic guava drinks

Figure 1 shows lactic acid bacteria count (LABC) of LAB in guava drink contain 6% (w/v) of different types of prebiotics. The results of LABC of all guava drinks samples ranges from 6.40 ± 1.16 to 7.20 ± 1.14 log_{10} CFU/mL. From the results, the total plate count started to increase exponentially from 10^6 to around 10^8 through the 240 hour. As shown in the Figure 1, guava drink containing gum arabic had a better growth stimulatory effect on the LAB compared with the other prebiotics. Gum arabic been recognized as important ingredient in the industrial production of beverages as it stabilize emulsions and suspensions besides harmonizing the texture of the drink (Benech, 2008, Varastegani, 2018).

From Figure 1, all products were in the acceptable range for minimum beneficial health effects at 10^6 – 10^8 CFU/mL. Generally, the viability of bacteria was sufficient for 14 days, and their numbers decreased but usually were not below 10^6 CFU/mL (Gustaw et al., 2011).

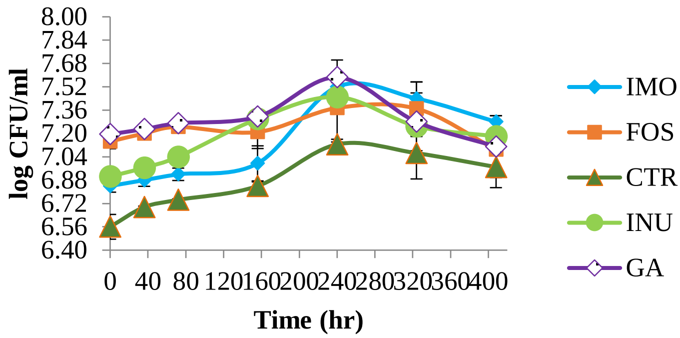

Figure 1 Total viable count on *Lactobacillus* spp. in different types of prebiotic guava drink. Values are mean ± SD of duplicates. Isomalto-oligosaccharides (IMO); Fructo-oligosaccharides (FOS); Inulin (INU), Gum Arabic (GA); Control (CTR).

The decreasing number of viable cell counts occurred after 320 hours of storage. All the prebiotic products decrease bacteria count on reaching the storage at 400 hours and above. Probiotic organism viability is influenced by various variables, including fermentation duration, oxygen permeability of the package, and storage temperature (Gurram et al., 2021). Lactic acid created during manufacture and cold storage is also a factor that affects it (Joan & Paula, 2017).

3.2 pH changes in prebiotic guava drinks

Based on Figure 2, the guava drinks fermented at 37 °C showed a slower reduction in pH value. The initial pH values range from 4.72 ± 0.04 to 4.30 ± 0.12, while the final pH value ranges from 3.95 ± 0.03 to 3.17 ± 0.05. The pH value for all prebiotic samples was significantly lower than the control sample.

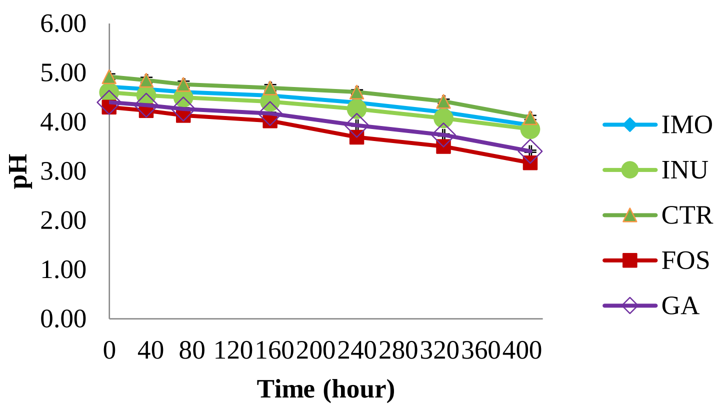

Figure 2 pH changes on Lactobacillus spp. in different types of prebiotic guava drink. Values are mean ± SD of duplicates. Isomalto-oligosaccharides (IMO); Fructo-oligosaccharides (FOS); Inulin (INU), Gum Arabic (GA); Control (CTR).

Based on Figure 2, all prebiotic samples exhibited a declining trend in pH. The guava drink with added FOS decreased from 4.30 ± 0.08 to 3.17 ± 0.02. This can be attributed to

FOS having a shorter chain length than inulin, resulting in faster consumption by probiotic bacteria, increased lactic acid production, and lower pH values. Aryana and Mc Grew (2007) reported a similar observation regarding the effect of inulin chain length on fat-free plain yogurts. *Lactobacillus* spp. demonstrated relative acid tolerance, as the total count remained stable throughout the fermentation, suggesting their suitability for fruit drink fermentation. The slight decrease in pH during cold storage at 4°C may be attributed to the probiotic strains' inability to survive in the stressful, highly acidic guava drink environment and the relatively low temperature. Yoon et al. (2004) obtained similar results for fermented tomato juice. The pH values of the guava drinks supplemented with different prebiotics were close to the pH of fermented milk, as suggested by Saw et al. (2011), indicating that the drinks provided the necessary nutrients for the growth and acid production of LAB strains. In the pH range of 3.17 to 3.95, further growth and acid production of most LAB cultures would be retarded.

3.3 Sensory Acceptance Analysis

Five attributes of the fermented guava drinks were selected: sourness, sweetness, smooth texture, fruity smell, viscosity, and overall acceptability.

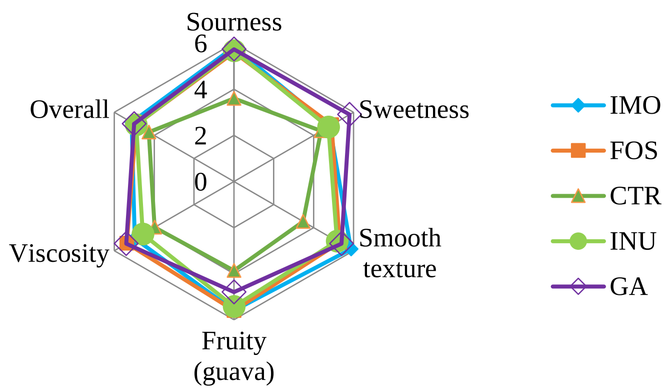

Figure 3 The spider web chart of the attributes for each guava drink of different prebiotics.

From Figure 3, for the overall acceptance, the highest mean value was obtained from guava drink supplemented with 6% (w/v) IMO followed by guava drink with 6% (w/v) gum arabic which has the mean value of 5.13 ± 0.66 and 5.0 ± 0.51, respectively. The overall acceptability shows only small differences for panelists in choosing the best prebiotic guava drink. Sensory investigation is important to indicate that either prebiotic guava drink affects the content of individual taste-aroma compounds. It can, therefore, be assumed that key parameters like total soluble solid titratable acidity will determine the final sensory attributes including the overall acceptability of the beverage.

4. CONCLUSION

In this study, the probiotic consisting of *Lactobacillus* spp. was capable of growing well in different types of prebiotics, which are 6% (w/v) of isomalto-oligosaccharides (IMO), fructo-oligosaccharides (FOS), inulin and gum arabic (GA). Guava drinks with the addition of 6% (w/v) of gum arabic have beneficial effects on the amount of lactic acid and the viability of lactic acid bacteria. while IMO showing a better effect in terms of pH reduction.

The suitable type of prebiotics is determined in this study so that the probiotic guava drink will have better growth and the shelf life might be increased compared to the probiotic guava drink without any addition of prebiotics. This may become an important factor as *Lactobacillus* spp., are suitable for use as probiotic cultures in health beverages from guava drinks.

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Potential Pharmacotherapy of Tualang Honey Against Oxidative Stress and Angiogenesis in Diabetic Retinopathy

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ABSTRACT
Honey has been used since ancient times for its nutritional as well as curative properties. Tualang honey (TH) is collected from wild honeybees' hives on Tualang trees found in the Malaysian rain forest. It has been used traditionally for the treatment of various diseases, where its therapeutic value has extremely been related to its antioxidant and anti-angiogenesis properties. This study therefore assessed the potential pharmacotherapeutic effects of TH against diabetic retinopathy (DR) disease by ameliorating oxidative stress (OS) and angiogenesis mechanisms. The online PubMed and Medline search engines were used to collect publications published between 2010 and 2022. This was accomplished by studying the abstracts and full papers of all included sources using a single or combination of keywords such as Tualang Honey, DR, oxidative stress and angiogenesis. All publications cited were published in English-language.

Keywords: Tualang Honey, diabetic retinopathy, oxidative stress, angiogenesis.

1. INTRODUCTION

Diabetes mellitus (DM) depicts a group of metabolic disorders indicated by elevated blood glucose level and have a higher risk of morbidity as well as mortality than the normal population (Alam et al., 2021). Diabetic retinopathy (DR) is one of the common microvascular complications of DM which leads to vision impairment and blindness. DR is classified into non-proliferative (NPDR), which is characterized by increased vascular permeability, and proliferative (PDR), in which neovascularization and vitreous haemorrhage are prominent features. If remain untreated, retinal detachment and blindness may occur. As a result of visual impairment, the social life and employment can be affected which further deteriorate a person's quality of life. There are multiple

biochemical and metabolic abnormalities in the retina that contribute to DR pathogenesis (Tangvarasittichai & Tangvarasittichai, 2018).
Currently, there is no specific treatment exist and outcomes are relatively inconsistent despite maximal treatment. The development of alternative therapies is of utmost critical. While there are available approaches for DR, including laser therapy and medication, there is no substantial scientific evidence to suggest that Tualang honey (TH) can directly treat or cure this condition. For this review, we are trying to discuss on the possibility of TH against oxidative stress (OS) and angiogenesis mechanisms in DR.

2. DIABETIC RETINOPATHY

DR is a common complication of diabetes and is a leading cause of vision loss and blindness worldwide. The epidemiology of DR involves various factors such as the prevalence of diabetes, duration of diabetes, glycaemic control, and access to healthcare. According to the International Diabetes Federation (IDF), as of 2021, approximately 463 million adults (age 20-79) were living with diabetes globally. DR affects around one-third of people with diabetes, making it one of the most common microvascular complications of the disease. There are two main types of DR, firstly, NPDR: In the early stages, the blood vessels in the retina weaken and develop small bulges called microaneurysms. As the condition progresses, the blood vessels may leak blood or fluid into the retina, causing swelling or oedema. This can result in blurred or distorted vision. Secondly, proliferative PDR: In advanced stages, the lack of oxygen and nutrients due to damaged blood vessels triggers the growth of new, abnormal blood vessels in the retina. These new vessels are fragile and prone to bleeding, leading to the formation of scar tissue. The scar tissue can pull on the retina, causing it to detach and potentially leading to severe vision loss.

Hyperglycaemia-induced OS is known to be one of the most important mechanisms involved in microvascular complication of DM. Overproduction of superoxide, an oxygen free radical, through dysregulation in mitochondrial electron-transport chain was shown to be the main cause of OS in hyperglycaemic environment. The key biochemical signalling involved are polyol pathway, advanced glycation end products (AGEs) as well as protein kinase C (PKC) activation which leads to occurrence of mainly OS and angiogenesis that further damage the retinas in DR (Sadikan, 2022). Overall, OS plays a significant role in the development and progression of DR by damaging retinal cells, promoting inflammation, and contributing to vascular dysfunction. Strategies aimed at reducing OS and restoring antioxidant balance have shown potential in mitigating the progression of DR in preclinical and clinical studies.

Angiogenesis, the formation of new blood vessels, is another critical process in the development and progression of DR. In DR, it occurs in response to retinal ischemia caused by damage to the retinal microvasculature. Vascular endothelial growth factor (VEGF) is a key molecule involved in angiogenesis and plays a crucial role in promoting the growth of new blood vessels. The hypoxic conditions in the retina stimulate the overproduction of VEGF which can bind to specific receptors on endothelial cells, initiating a cascade of events that lead to neovascularization. Understanding the mechanisms underlying abnormal angiogenesis in DR has led to the development of targeted therapies aimed at inhibiting VEGF and other pro-angiogenic factors.

3. TUALANG HONEY

TH is a type of honey derived from the hives of the Tualang tree (Koompassia excelsa), which grows in Southeast Asian rainforests, primarily in Malaysia, Thailand, and Indonesia. TH is well-known for its unique flavour and medicinal benefits. The Tualang tree may reach heights of 250 feet, and its honey is collected from colonies high up in the branches. The honey is generated by Apis dorsata bees, which construct their combs on the outside of the hive, as opposed to most other honeybee species, which build their combs inside. TH is an uncommon and very valuable honey that is frequently sold at a premium price. It has a dark, reddish-brown colour and a thick, viscous consistency. Its distinct flavour and medicinal characteristics make it a popular culinary and therapeutic ingredient.

In recent years, TH has gained popularity beyond its traditional use in Southeast Asia. TH has been used in traditional medicine to cure several diseases, including sore throat, cough, wounds, and digestive problems. It is also thought to have anti-inflammatory and immune-boosting properties (Mohamed et al., 2010). Over time, the demand for TH has expanded beyond the local communities. Its reputation for unique flavour and potential health benefits has attracted attention from researchers and honey enthusiasts worldwide. As a result, there has been a growing interest in commercial production and marketing of TH.

3.1. Antioxidant

TH has been shown to have potent antioxidant properties, which may help protect against OS. OS occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them with antioxidants. This can result in damage to cells and tissues, leading to various health problems.

Studies have found that TH contains a range of antioxidants, including phenolic acids, flavonoids, and enzymes. These compounds have been shown to scavenge free radicals, reduce oxidative damage, and protect against inflammation. One study published by Mohd Sairazi et al. (2017) investigated the effects of TH on OS in rats with brain neurodegeneration. The results showed that rats fed with TH had lower superoxide dismutase (SOD), glutathione peroxidase (GPx), malondialdehyde (MDA) and fasting plasma glucose (FPG) and significantly increased body weight, catalase activity, total antoxidant status, total glutathione (TGSH), reduced glutathione (GSH) and reduced glutathione:oxidized glutathione (GSH: GSSG) ratio compared to the control group (Erejuwa et al., 2009). Other studies also found similar amelioration against OS markers in diabetic nephropathy (Omotayo et al., 2010), anxiety-like behaviour (Al-Rahbi et al., 2014), reproductive abnormalities (Ruslee et al., 2020), and bone metabolism (Mohd Effendy et al., 2012). It has been previously proven to have phenolic compound that able to neutralize free radicals thus can reduce the retinal OS by restoring endogenous antioxidant capacity and reducing pro-oxidant level. This natural product has also been found effective against range of diseases such as neurodegenerative brain disorders, cardiovascular problems, DM, diabetic liver disease, chronic obstructive pulmonary disease (COPD), and obesity (Kishore et al., 2011). Overall, these studies suggest that TH may be a useful dietary supplement for reducing OS and promoting overall health. However, more research is needed to determine the optimal dosage and duration of TH supplementation and to understand its mechanisms of action.

3.2. Anti-angiogenesis

Angiogenesis is the process by which new blood vessels are formed in the body. While angiogenesis is a normal and important process in the body, it can also contribute to the abnormal growth and spread of cancer cells. Anti-angiogenic compounds are substances that can inhibit the formation of the new blood vessels, and therefore may be useful in the treatment of certain diseases such as DR. Other than playing role against OS, TH also has anti-angiogenesis properties which may be useful in preventing the DR progression by inhibition of abnormal blood vessels growth or neovascularization.

TH has been identified in study by Vaskular et al. (2022) and Roy et al. (2020) to suppress the angiogenic events in endothelial cells induced by VEGF. They found that the anti-angiogenic effects of MTH mainly targets endothelial cell through inhibition cell proliferation, migration and tube formation capacity, via suppression of matrix metalloproteinase-2 (MMP-2) secretion by the endothelial cells. Other study has found that TH was able to inhibit the formation of new blood vessels in the chorioallantoic membrane (CAM) of chicken embryos. The CAM is a thin membrane that surrounds the developing embryo and is often used as a model to study angiogenesis. Although there are many studies mentioned the reduction of VEGF as a determinant of angiogenesis, TH inhibits VEGF without showing significant difference compared to control group of breast cancer cells (Kadir et al., 2013). This effect has been thoroughly investigated against cancers such as breast, lung, colon and cervical cancer cell lines and conditions such as endothelial hyperpermeability and dysfunction (Firdaus et al., 2018). Hence, there is needs to conduct more experiments to reveal the pharmacological effects of TH against angiogenesis parameters.

4. TUALANG HONEY AND DIABETIC RETINOPATHY

TH, like other types of honey, contains natural sugars and various bioactive compounds. Some types of honey have been studied for potential health benefits due to their antioxidant and anti-angiogenesis properties (Figure 1). However, there is limited research specifically linking TH to the treatment or prevention of DR.

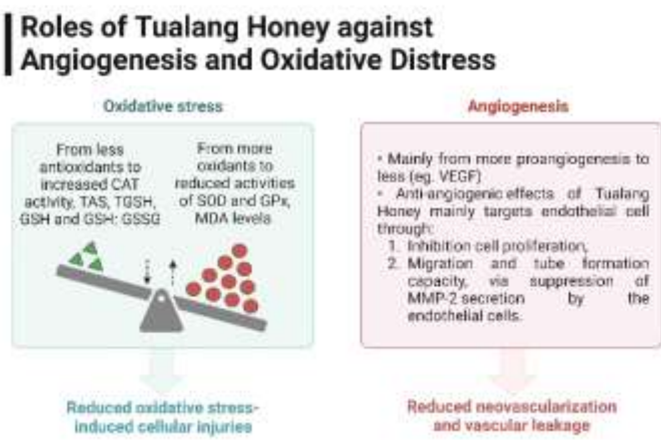

Figure 1: Roles of TH against OS and angiogenesis.

Findings of interrelated biomarkers are promising, more research is needed to determine if TH has antioxidant and anti-angiogenic properties in humans especially whether it could be used as a treatment for DR. As far as author concerns no research has been done to investigate potential mechanisms underlying the possible protective effects of TH against DR. Better understanding about the efficacy of TH towards improving retinal parameters in DR through its antioxidant and anti-angiogenesis properties can be generated through experimental *in vivo, in vitro* or *ex vivo* studies and lastly through human trials. Most importantly, the findings may prove TH as a therapeutic agent for DR that can benefit many people all over the world in future.

Although exploring complementary approaches like diet and supplements can be interesting, it is important to prioritize treatments that have been well-studied and proven effective for managing DR. Patients must always consult with a medical professional before making any changes to treatment plan or incorporating new supplements or foods into daily routine.

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Green Tea and Retinal Vascular Diseases

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ABSTRACT
Retinal vascular diseases, such as diabetic retinopathy (DR) and age-related macular degeneration (AMD) involve damage or dysfunction of the blood vessels in the retina. The issues regarding the treatment of these disorders are suboptimal outcome, invasive and focus is only given at later stage of the diseases. So, search for alternative therapy is crucial. Green tea (GT) which is renowned for its potential health benefits, due to rich content of antioxidants and various bioactive compounds have been studied for cardiovascular health, weight management, cancer prevention, brain function, diabetes control and oral and skin healths. However, studies regarding GT effects on retinal vascular diseases particularly DR and AMD are limited. This review provides details discussion on how GT can provide protective effects against retinal vascular diseases according to the articles published between 2006 and 2023 with English-language version. Overall, there is cross-linking mechanisms of action of GT especially its key component, epigallocatechin gallate (EGCG) against DR and AMD primarily due to their antioxidant, anti-inflammatory and anti-angiogenic properties in multiple signalling and molecular biomarkers. However, it is important to note that while there is promising research in this area, more studies are needed to fully understand the extent of GT's benefits for retinal vascular diseases. Research in human trials is crucial to establish a direct link and determine optimal dosages for its therapeutic benefits.

Keywords: Green tea, retinal vascular diseases, diabetic retinopathy, age-related macular degeneration, therapeutic effects

1. INTRODUCTION

Retinal vascular diseases are a group of eye conditions that affect the blood vessels in the retina, which is the light-sensitive tissue at the back of the eye. These conditions can lead to various visual problems and, if left untreated, can result in vision loss. The common retinal vascular diseases include diabetic retinopathy (DR) and age-related macular degeneration (AMD) (Pemp & Schmetterer, 2008).

DR is a complication of diabetes and is a leading cause of blindness in adults. It occurs when high blood sugar levels damage the blood vessels in the retina, leading to swelling, leakage, and the growth of abnormal blood vessels. Whereas AMD, affects the macula, a small but critical part of the retina at the back of the eye, which is responsible for central vision and allows people to see fine details clearly. It is a common eye condition and a leading cause of vision loss among people over the age of 50. Treatment for retinal vascular diseases depends on the specific condition and its severity. It may include laser therapy, intravitreal injections, anti-VEGF medications, vitrectomy (surgical removal of the vitreous gel), and management of underlying medical conditions like diabetes or hypertension.

However, the treatment approach for both retinal vascular diseases is limited and generally invasive. Another most crucial issue with the treatment is suboptimal and focus is given at later stage of the disease. Hence, search of other alternative approaches is extremely important. Green tea (GT), a well-known antioxidant may provide potential therapy against the disease mechanisms involved in retinal vascular pathology. This review highlighted the cross-linking between the therapeutic properties of GT against DR and AMD molecular pathogenesis.

2. GREEN TEA

GT is a popular and widely consumed type of tea that is known for its numerous health benefits and distinct flavour. GT originated in China, where it has been consumed for thousands of years. It is also widely produced in countries like Japan, Korea, and various other parts of Asia. GT is made from the leaves of the *Camellia sinensis* plant. The leaves are minimally processed, which helps retain their natural green colour. Unlike black tea, which is oxidized, GT is not oxidized or is only minimally oxidized (Shrivastava et al., 2018). There are various varieties of GT, and the flavour and aroma can vary depending on factors such as the region grown in, the specific tea cultivar, and how it is processed. Some popular varieties include Sencha, Matcha, Dragonwell, and Gunpowder, among others. To make GT, typically loose tea leaves or tea bags are steeped in hot water, usually around 175°F (80°C) for a few minutes. It is essential not to use boiling water, as it can make the tea taste bitter. GT has a fresh, vegetal, and somewhat grassy flavour. Some people find it slightly astringent, especially if over-brewed. GT contains caffeine, but in lower amounts than coffee. The caffeine content can vary depending on the type of GT and how it is prepared. Some people enjoy GT as a milder alternative to coffee.

GT is often lauded for its potential health benefits. It is rich in antioxidants, particularly catechins and various other bioactive compounds, which are believed to have a range of health-promoting properties (Cabrera et al., 2006). These may include improved heart health, reduced risk of certain types of cancer, weight management, and enhanced cognitive function. Recently, GT, has been studied for its potential benefits in promoting eye health and reducing the risk of certain ocular diseases. While research is ongoing, there is some evidence to suggest that GT may have a positive impact on eye health and the prevention of eye-related conditions.

GT contains a variety of bioactive compounds that contribute to its flavour, aroma, and potential health benefits. The key compounds found in GT included in Table 1. These compounds work together to give GT its unique taste and aroma, and they are also thought to contribute to its potential health benefits. The high concentration of catechins, particularly EGCG, is often cited as one of the primary reasons for GT's positive effects on health. However, the specific composition of these compounds can vary depending on factors such as the type of GT, its processing, and the growing conditions.

Table 1 Key compounds found in GT

Compounds	Description
Catechins	• A type of natural polyphenol and are the primary antioxidants in GT. • They are responsible for many of the potential health benefits associated with GT. • The major catechins in GT include epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG), which is the most abundant and widely studied catechin.
Caffeine	• GT contains caffeine, although in lower amounts compared to coffee. • Caffeine is a natural stimulant that can provide a mild energy boost. • It can also contribute to the taste and aroma of the tea.
L-theanine	• It is an amino acid found in GT. • It has a calming effect and can counteract the stimulating effects of caffeine, resulting in a balanced and focused state of alertness. • L-theanine is known for promoting relaxation and mental clarity.
Polyphenols	• GT is rich in polyphenolic compounds, of which catechins are a major subgroup. • Polyphenols have antioxidant properties and play a significant role in the potential health benefits of GT.
Flavonoids	• Flavonoids are a class of plant compounds with antioxidant properties. • GT is a good source of various flavonoids, which can contribute to its colour, flavour, and health-promoting effects
Theaflavins	• While theaflavins are more commonly associated with black tea, small amounts of these compounds can also be found in GT. • They are formed during the oxidation of tea leaves and contribute to the flavour and colour of the tea.
Vitamins and Minerals	• GT contains small amounts of vitamins and minerals, including vitamin C, vitamin B2 (riboflavin), folic acid, and manganese.
Chlorophyll	• Chlorophyll is the green pigment in plants, and it is responsible for the green colour of GT leaves. • It has antioxidant properties and contributes to the visual appeal of the tea.
Amino Acids	• Besides L-theanine, GT contains other amino acids that can affect the tea's flavour and aroma.

3. RETINAL VASCULAR DISEASES AND GREEN TEA

3.1. Diabetic Retinopathy

DR is a potentially serious ocular condition that can affect people with diabetes. It occurs when high blood sugar levels damage the blood vessels in the retina, the light-sensitive tissue at the back of the eye. Over time, this damage can lead to vision problems and, in severe cases, can cause blindness. There are two main types of DR, firstly, non-proliferative DR (NPDR): In the early stages, small blood vessels in the retina weaken and may leak fluid or tiny amounts of blood. This can cause the retina to swell and lead to the development of fatty deposits. Many people with diabetes have mild NPDR, which may not cause significant vision problems and secondly, proliferative DR (PDR): As the disease progresses, new, abnormal blood vessels can grow on the surface of the retina. These blood vessels are fragile and can bleed, causing significant vision loss (Stitt et al., 2016).
The pathogenesis of DR is multifactorial and involves several key mechanisms. Hyperglycaemia leads to metabolic abnormalities, including increased formation of advanced glycation end products (AGEs), activation of protein kinase C (PKC), and increased polyol pathway activity. These processes contribute to cellular damage and alter signalling within the retinal cells. Hyperglycaemia-induced oxidative stress (OS) also plays a crucial role in DR. Reactive oxygen species (ROS) and free radicals are generated in excess, causing damage to cellular components like lipids, proteins, and DNA. OS further

exacerbates inflammation and cellular injury which is another hallmark of DR. OS can trigger release of inflammatory mediators such as cytokines, chemokines, and adhesion molecules. These molecules promote leukocyte adhesion and migration into the retinal tissue, contributing to vascular and tissue damages. Another important event in DR is vascular dysfunction and microangiopathy in which hyperglycaemia damages the endothelial cells lining the retinal blood vessels. This leads to increased vascular permeability and altered blood flow. Microangiopathy, characterized by capillary basement membrane thickening and pericyte loss, is a distinctive feature of DR which impairs retinal blood vessel function. Next, progressive damage to retinal blood vessels results in areas of ischemia and reduced oxygen levels hypoxia. Hypoxia can further trigger the release of angiogenic factors, such as vascular endothelial growth factor (VEGF), which promote the growth of abnormal and fragile blood vessels known as neovascularization. While GT contains compounds with potential health benefits, including antioxidants and anti-inflammatory properties, there is limited direct evidence to suggest that GT can prevent or treat DR. The relationship between GT consumption and DR is an area of ongoing research, and more studies are needed to establish a clear cause-and-effect relationship.

3.2. Age-related Macular Degeneration

AMD is another complex ocular condition that primarily affects the macula, the central part of the retina responsible for sharp and central vision. The pathogenesis of AMD involves a combination of genetic, environmental, and aging-related factors. The exact mechanisms are not fully understood, but several key factors play a role in the development and progression of AMD (Lim et al., 2012).

The primary risk factor for AMD is age. As individuals grow older, the risk of developing AMD increases. This suggests that cumulative cellular damage and aging-related changes contribute to the development of the condition. Next, OS can occur when there is an imbalance between the production of harmful free radicals and the body's ability to neutralize them with antioxidants. OS can damage the retinal cells and lead to the formation of drusen, which are small yellow deposits under the retina and a hallmark of early AMD. Chronic inflammation in the retina is also believed to play a role in the development and progression of AMD. Inflammatory processes can disrupt the balance of immune cells and molecules, potentially contributing to tissue damage and the formation of abnormal blood vessels (neovascular AMD). Other than that, RPE is a layer of cells that supports the photoreceptor cells in the retina. Dysfunction of the RPE can lead to the accumulation of waste products and the death of photoreceptor cells, contributing to vision loss in AMD. Changes in the blood supply to the retina and choroid, including altered perfusion and the growth of abnormal blood vessels, have also been observed in AMD.

3.3. Roles of Green Tea in Retinal Vascular Diseases

GT, with its potential health benefits related to antioxidants, and anti-inflammatory properties, has been studied in the context of retinal vascular diseases, including conditions such as DR and AMD. GT contains a group of antioxidants called catechins, with EGCG being one of the most potent. These antioxidants can help to combat OS in the body, including the retina. EGCG can increase the activity of endogenous antioxidant enzymes in the body, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase in DR (Fanaro et al., 2023; Silva et al., 2013) and AMD (Pawlowska et al., 2019). These enzymes work to counteract OS by breaking down harmful ROS and free radicals. GT also contains compounds that have anti-inflammatory properties, which may help reduce inflammation in the retinal blood vessels. It reduces the expression of interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrotic factor-alpha (TNF-α) through inhibition of nuclear factor-kappa B (NF-κB) pathway, which plays a role in promoting both inflammation and OS (Lee et al., 2011).

Other than that, EGCG and other components of GT have been investigated for their potential anti-angiogenic effects. Studies have suggested that EGCG can inhibit the activity of various molecules and signalling pathways involved in promoting angiogenesis (Blasiak et al., 2023), such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and matrix metalloproteinases (MMPs) (Sartippour et al., 2002). GT has also been associated with a mild reduction in blood pressure. Maintaining healthy blood pressure is crucial for the health of blood vessels, including those in the retina. GT compounds have also demonstrated potential neuroprotective effects, which could be beneficial for preserving the normal physiology of retinal cells and blood vessels (Onakpoya et al., 2014).

4. CONCLUSION

Management of retinal vascular diseases typically involves regular monitoring, lifestyle modifications, and, in some cases, medical or surgical interventions. GT consumption can be a part of a healthy diet, but it should not be considered a sole or primary treatment for these conditions. This is because the effectiveness of GT as an antioxidant can vary from person to person, and individual results may differ depend on various factors, including genetics, diet, and overall lifestyle. It is important to note that while there is some evidence to suggest that GT may have a positive impact on retinal vascular health in laboratory and animal studies, the research is not yet conclusive, and more robust clinical trials are needed to establish a clear cause-and-effect relationship against retinal vascular disorders. Nevertheless, incorporating GT into a balanced diet, along with other antioxidant-rich foods, may contribute to a reduction in OS and offer potential health benefits.

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Pressurized Hot Water Extraction of Hydrolysable Tannins from *Pereskia sacharosa*

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ABSTRACT
Hydrolysable tannins (HTs) consist a group of complex polyphenols with versatile applications in the food, pharmaceutical, and cosmetic industries due to their anti-cancer, anti-angiogenic, antioxidant, anti-inflammatory, and anti-ulcerative properties. *Pereskia sacharosa*, an obscure tropical plant is a potential source of hydrolysable tannins. This study investigates the extraction of hydrolysable tannins from *P. sacharosa* using pressurized hot water extraction (PHWE). In this research, the optimal conditions for the extraction process including; temperature and extraction time, to maximize HTs yield at constant pressure 1 atm, were investigated by response surface methodology (RSM) using Central Composite Design (CCD). Additionally, the chemical profile of the extracted tannins is analysed using ferric chloride method, allowing for the identification of hydrolysable tannin compounds. The results demonstrate that *P. sacharosa* is a promising source of hydrolysable tannins with the highest HTs obtained is 85.6 wt %. The optimum conditions for the extraction of hydrolysable tannins involve employing PHWE at a temperature of 120°C for a duration of 20 minutes to obtain the highest yield of HTs from this plant. This study provides valuable insights into the utilisation of underexplored plant or herbs resources for the sustainable production of bioactive compounds with significant health-related implications.

Keywords: Pressurized hot water extraction, *Pereskia sacharosa*, hydrolysable tannins

1. INTRODUCTION

Pereskia sacharosa or better known as '*Jarum Tujuh Bilah'* in Malay terms, is a type of tree found in Malaysia and other ASEAN countries. This plant, belonging to the *cactaceae* family, is one of the most interesting, unusual and exotic members of cactus genus. In fact, it is a relatively hardy plant that can adapt to almost any climate except the tundra. The optimal conditions for its growth are bright sunlight and a tropical climate

(Jacqueline, 2017). Apart from its use as flowering hedges, it has been traditionally used for traditional medicine and food preparation, as well as in the treatment of various diseases. It is also known as 'Tree Cancer' (Jacqueline, 2017). This plant contains high tannin contents, which offer various health benefits to humans. Moreover, the existence of tannins has been used in traditional medicine for their potential anti-inflammatory, antioxidant, and antimicrobial properties.

Pressurized hot water extraction (PHWE) is selected as the method of choice for tannin extraction from *P. sacharosa* for several reasons. PHWE is reported to also exhibit shorter extraction time, lower costs of the extracting agent, and an environmentally compatible technique (Mohd Jusoh et al., 2019). It offers several advantages, including enhanced selectivity, reduced solvent consumption, and the ability to extract a wide range of polar compounds, such as tannins. Additionally, PHWE operates under elevated temperature and pressure conditions, which can aid in breaking down complex plant matrices and promoting efficient tannin extraction. By utilizing PHWE, this research aims to maximize tannin yield from *P. sacharosa* while minimizing the environmental impact of the extraction process.

2. LITERATURE REVIEW

Several previous studies have explored various methods for the extraction of tannins from plant materials. Common methods include solvent extraction (using organic solvents like methanol or ethanol), solid-liquid extraction, microwave-assisted extraction, and pressurized hot water extraction (PHWE). The choice of extraction method can significantly impact tannin yield, composition, and quality. Studies have shown that PHWE offers distinct advantages, such as reduced solvent use, enhanced selectivity, and environmental friendliness when compared to traditional organic solvent-based extraction methods (Plaza & Marina, 2023). Optimizing the parameters of PHWE, including temperature, pressure, and extraction time, can be challenging due to the complex nature of plant matrices and the interactions between different compounds. Hydrolysable tannins are a class of polyphenolic compounds and important groups of secondary metabolites that occur in wood, bark, leaves, and galls (Ky et al., 2016). These compounds include simple gallic acid derivatives, gallotannins (GTs), and ellagitannins (ETs) and characterized by their ability to undergo hydrolysis. HTs will be decomposed into less complex phenolic compounds when subjected to heat or exposure to acidic conditions. HTs exhibit anti-cancer, anti-angiogenic, antioxidant, anti-inflammatory, and anti-ulcerative properties. Their antioxidant activity has been confirmed using several systems (Amarowicz and Janiak, 2019). As a result, the extraction and analysis of hydrolysable tannins from natural sources have become a topic of interest for both the food and pharmaceutical industries.

3. METHODOLGY

3.1. Collection and Preparation of Plant Material

P. Sacharosa fresh leaves were procured from Taman Desa Serdang, Malaysia, where the plant grows naturally on the nearby hillside. The plants that had just been picked were washed and dried using the air-drying process. Next, the air-dried leaves were ground into small pieces. In order to prevent contamination from dirt and insects, the ground leaves were then kept inside an airtight container before the experiment was conducted.

3.2. Experimental Design

Response surface methodology (RSM) was used in the design of the experiment and to determine an adequate model for hydrolysable tannins extraction using the PHWE technique. The experiment had been designed with two variables in mind, namely time and temperature. For the experiment, 13 runs in total were planned. The temperature range considered was from 100°C to 145°C, and the second factor was the duration of the extraction procedure, ranging from 5 to 40 minutes with constant pressure at 106 Kpa (1 atm).

3.3. Water Extraction at Elevated Temperatures and Pressure

Approximately 5 g of air-dried *P. Sacharosa* leaves and deionized water (100 mL) were carefully mixed. The mixture was subsequently homogenized for 20 minutes at a speed of 1100–1500 rpm. Upon completion of the homogenization procedure, the homogenized solution was transferred from the beaker into a blue-capped bottle (as amber bottles should be avoided due to the applied pressure). The solution was then autoclaved using temperature and time parameters generated by Design Expert software, employing Response Surface Method, Central Composite Design (CCD). Once the PHWE process was completed, the crude yields of the plants were dried in the oven at 60-70°C for 24 hours. Subsequently, the extracted crude yields were analyzed to determine their hydrolysable tannin content.

3.4. Preparation of Ferric Chloride

A 5% ferric chloride solution using 97% pure ferric chloride was prepared. Approximately 12.89 g of anhydrous $FeCl_3$ was weighed, and 50 mL of distilled water was added to a beaker and stirred. Another 200 mL of distilled water was then poured into the beaker and homogenized using a magnetic stirrer for 30 minutes. The 5% $FeCl_3$ solution was then transferred into a 250 mL volumetric flask and adjusted as necessary with distilled water to reach the desired volume. It was shaken before use.

3.5. Determination of Hydrolysable Tannins Process

After the extracted crude yields had been dried, they were placed in a beaker with a sufficient quantity of water was added. The beaker was then heated over a heating mantle for a few minutes. After boiling, the contents of the beaker were filtered through filter paper to remove the residue, and the liquid extract was collected in the same beaker. Then, 2 mL of the extract solution was transferred into a test tube. Finally, a few drops of ferric chloride solution were added to the test tube, and the reaction was observed. If the colour changed to blue-black, it indicated the presence of hydrolysable tannins. If the solution turned brownish green or dark green, it contained condensed tannins. The collected weight of extracted sample is calculated as Eq. (1) below;

$$\text{Yield (Y)} = \frac{\text{Weight of Dried Extract}}{\text{Weight of Dried Plant Sample}} \times 100 \qquad \text{Eq. (1)}$$

4. RESULTS & DISCUSSION

4.1. Effect of the variables on hydrolysable tannins (HTs) yield using pressurized hot water extraction (PHWE)

In this study, the central composite design (CCD) was applied to investigate the effect of independent variables (temperature and flow rate) on extraction yield of *P. sacharosa* samples. Thirteen experimental runs were conducted, including three replicates at the central point.

Table 2 ANOVA analysis for the regression model of the PEGP sample yields

Source	SS	DF	MS	F-value	p-value	
Model	1.2	2	0.5987	14.56	0.0011	**significant**
X_1	0.8114	1	0.8114	19.73	0.0013	
X_2	0.3861	1	0.3861	9.39	0.012	
Residual	0.4112	10	0.0411			
Lack of Fit	0.4112	6	0.0685	0.4714	0.8667	**not significant**
Pure Error	3.49	4	0.1453			
Cor Total	1.61	12				

X_1: temperature; X_2: time; SS: sum of squares; DF: degrees of freedom; MS: mean square; F-value: Fisher value; p-value: probability value

The yield of HTs ranged from 62.15 wt% to 85.6 wt% under the applied PHWE conditions. The optimum conditions for achieving the highest yield were a temperature of 120 °C and a time of 20 minutes, which allowed for a HTs yield of up to 86.5 wt% in the *P.sacharosa* sample. The experimental yield was close to the predicted yield (85.88 wt%), which demonstrates the validation of the optimized condition. Additionally, to confirm the experimental design's reproducibility, the central point was analyzed in triplicate. The results obtained in these analyses showed consistent yields across samples, with mean value 84.80 wt% ± 0.15 wt%. As shown in Table 1, the p-values of linear coefficients (X_1: pressure and X_2: temperature) were all lower than 0.05. This indicates the significant effects of these parameters on HTs yield. The lack of fit tests were not significant ($p = 0.8667$), indicating that the models had adequate accuracy for predicting HTs yield using any combination of independent factors within the range of this study. The relationship between the HTs response (yield) and independent variables (temperature and time) is shown below in Eq. (2):

$$\text{HTs Yield (Y)} = 0.310295 + 0.032008\, X_1 - 0.022078 X_2 \qquad \text{Eq. (2)}$$

where X_1 (temperature) and X_2 (time) are the coded variables. Eq. (2) and the data from the variance analysis (ANOVA) demonstrated that the yield of the HTs was affected by the two variables (temperature and time) through a linear effect. The individual and interactive effects from both process variables yield is also shown in Figure 1 by three-dimensional (3D) surface plots: when the slope of the response surface is relatively steep, it means that the response value has greater effect with the change of extraction conditions. The HTs yield increased with the increase in temperature with longer time. In previous studies, a high temperature associated with pressure, keeping water liquid, affects the mass transfer rate to favor extraction, enhancing the solubility of the solute and the diffusion coefficient

(Adetunji et al., 2017; Plaza & Marina, 2019; Smiderle et al., 2017). Numerous studies have explored time as an important variable in PHWE pectin extraction (Hosseini et al.,2016; Chaharbaghi et al., 2017; Colodel et al., 2018) because extended contact times between extracting solvent and plant material provide greater mass transfer of solid particles in the solution (Pasandide et al., 2017). The presence of hydrolysable tannins is confirmed by the $FeCl_3$ test. Hydrolysable tannins turn dark blue when ferric chloride solution is added to their aqueous solution, while non-hydrolysable tannins turn dark green (Figure 2).

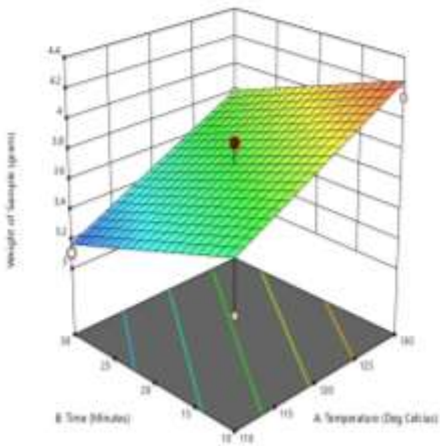

Figure 1: Response surface plot showing the interaction between temperature and time on HTs yield

Figure 2: Ferric chloride test for HTs

5. CONCLUSION

The extraction of hydrolysable tannins from *P. sacharosa* through pressurized hot water (PHWE) was influenced by temperature and time, while maintaining a constant pressure at 106 kPa (1 atm). Based on the experimental design, the combination of the constant pressure, 120°C temperature, and 20 minutes of extraction time resulted in a significantly higher HTs yield of 86.5 wt%. Furthermore, a validation test successfully confirmed the presence of HTs in the *P. sacharosa* samples, as indicated by the solution turning blue. Considering the quality of the extracted HTs and its environmentally friendly approach, it is evident that PHWE presents a promising technique for the extraction of these HTs.

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Probiotics: The Challenges of Survival in the Gut

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ABSTRACT
Within the intricate landscape of the gut, probiotics face various challenges, especially hostile acidic conditions. Probiotics exhibit the potential to exert significant influence over the human intestinal microbiota's composition, thwart the proliferation of pathogenic bacteria dwelling in the intestines, and fortify the intestinal barrier, ultimately augmenting immune function. Critical to their efficacy, probiotics must demonstrate robust survival capabilities in the gut milieu. Consequently, *in vitro* and *in vivo* methods have been developed to meticulously evaluate the potential effectiveness of probiotics across the entirety of the gastrointestinal digestive system, providing valuable insights into their potential as agents for promoting health.

Keywords: Probiotic, survival, gastrointestinal digestive

1. INTRODUCTION

1.1 Probiotics and their importance in gut health.

Probiotics are living bacteria that, when taken in adequate quantities, bring benefits to the health of the host by colonising the body as defined by the World Health Organisation (WHO) and Food and Agriculture Organisation (FAO) of the United Nations both defined probiotics as living microorganisms that, upon consumption in adequate quantities, confer health advantages to the host by establishing colonisation within the body (Markowiak et al., 2018). They are frequently encountered in various fermented food products, including yoghurt, kefir, and sauerkraut, alongside their presence in dietary supplements. Probiotics also have the potential to modulate the composition of the human intestinal microbiota, suppress the colonisation of pathogenic bacteria within the intestines, and boost the resilience of the intestinal barrier, thereby bolstering immune function (Wang et al., 2021). According to Milani et al. (2017), probiotics comprise a wide range of species and can be broadly categorised into three primary groups: *Bifidobacteria*, *Lactobacillus* and others. *Lactobacillus* is a significant probiotic in the field of research on human gut microbes, exhibiting a strong correlation with human health. In addition to its ability to synthesise high beneficial vitamins and amino acids and enhance mineral absorption, it plays a significant role in modulating intestinal microbiota by effectively suppressing the proliferation of harmful microbes.

The human stomach is also generally colonised to *bifidobacteria*, which are known to improve host health. Other probiotic microorganisms that are frequently included in fermented foods and supplements are *Bacillus coagulans*, *Saccharomyces boulardii*, and *Streptococcus thermophilus*. Every probiotic strain has distinct qualities and possible health advantages of its own; also, the efficacy of a given strain may vary depending on the health status of the individual and other circumstances and more research is needed to fully understand their mechanisms of action (Wolfe et al., 2023).

2. PROBING THE GUT: UNVEILING THE METHODS ON ASSESSING THE SURVIVAL AND VIABILITY OF PROBIOTICS

Despite the considerable potential of probiotics as therapeutic agents for gastrointestinal illnesses, their uses continue to encounter challenges. Furthermore, it is necessary to have a minimum viable count of approximately ($\sim 10^6$ CFU/g) in order to have positive effects. *Lactobacillus* and *Bifidobacterium* are specific bacteria that exhibit great susceptibility to aerobic and high-temperature surroundings. In addition, it is essential for probiotics to withstand the acidic conditions of the stomach and the presence of bile throughout the process of gastrointestinal transit (Han et al., 2023). Therefore, several *in vitro* and *in vivo* methods have been developed to assess the potential efficacy of probiotics throughout the gastrointestinal digestive system.

2.1 *In vitro* method.

In vitro assays are commonly used to assess the technological resilience of probiotics, including their capacity for proliferation, concentration, stabilisation, and integration into a final product exhibiting favourable sensory attributes. The objective of the gastrointestinal simulation is to assess the viability and abundance of probiotic cells in the stomach, duodenum, and ileum in the context of enzymatic activity and pH fluctuations (Da Silva et al., 2021). According to Hussin et al. (2021), the simulation to assess the survival of probiotics against gastrointestinal conditions was performed at pH 1.2 (added with pepsin) for the first 2 hours to mimic stomach digestion, followed by increasing the pH to 6.8 (added with pancreatin) for the next 4 hours to mimic intestinal digestion in duodenum and ileum. Survival was assessed by collecting an aliquot of sample from different sections of the gastrointestinal tract for counting of viable cells as shown in Figure 1. However, the efficacy of this assay in predicting *in vivo* functionality has not been thoroughly established.

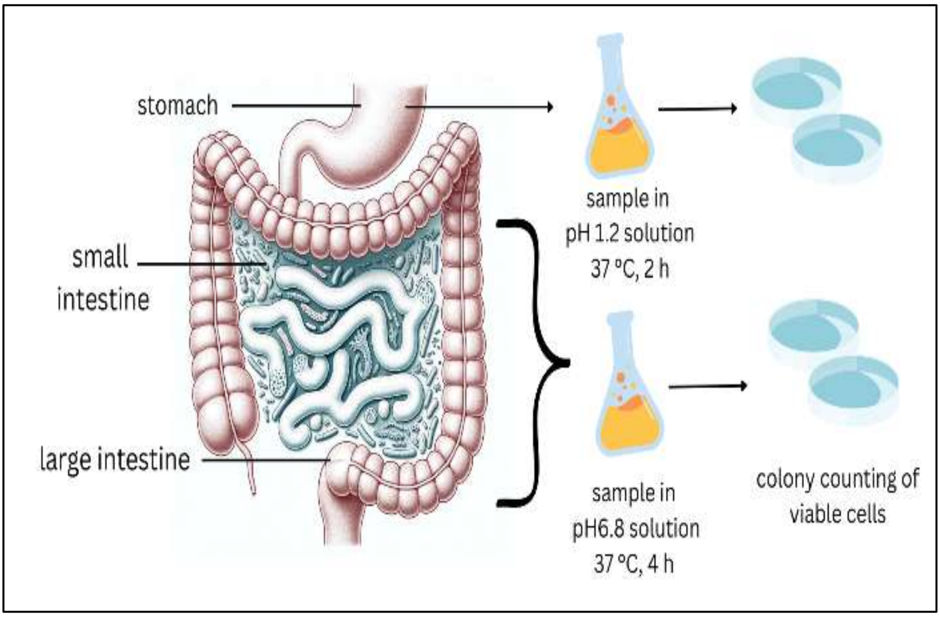

Figure 1: *In vi*tro assay to determine probiotic survival in gastrointestinal digestive system.

2.2 *In vivo* method

In vivo models are far more expensive and time consuming, but they offer a more precise evaluation of the delivery systems' potential efficacy in practical scenarios. In order to assess the endurance and effectiveness of probiotics within a living organism, it is imperative to conduct well-regulated investigations employing animal models and human subjects (Sanders, 2008).

The viable bacteria present in faecal samples following ingestion of the probiotic supplement or food is one popular approach for measuring the survival and viability of probiotics in the gastrointestinal system. This approach can assess how many of the probiotics consumed survived the acidic environment of the stomach and colonised the gut. The utilisation of animal models, such as mice, rats, or pigs, in an *in vivo* approach offers significant insights into the advantageous impacts of probiotic consumption, as well as the interactions between the sample and the microbiota of the host. Furthermore, animal models have the potential to obtain samples from different parts of the gastrointestinal tract, which is impossible to do in clinical trials. It is necessary to take into account that the efficacy of these approaches may differ based on numerous aspects, such as the particular probiotic strain employed, the dosage and administration technique, and the unique attributes of the host's gastrointestinal system. Animal models serve as a means to substantiate the outcomes observed in in vitro experiments; nonetheless, it is imperative to corroborate these findings through clinical trials conducted on human subjects. (Mukherjee et al, 2022; Khan et al., 2019).

3. CHALLENGES ENCOUNTERED BY PROBIOTICS IN THE GUT

The presence of living bacteria in different sections of the gastrointestinal tract is constrained by the prevailing environmental conditions. The bacteria's passage through the acidic environment of the stomach, which is extremely negative for living bacteria, is crucial for the proper colonization of the intestine by probiotics. After being taken orally, a probiotic product travels down the oesophagus and into the stomach, where it is digested by the hydrochloric acid that is present in the gastric fluid. When it comes to probiotics, the presence of hydrochloric acid has an has an adverse impact on the microorganisms present, resulting in a reduction in their viability (Bernatek et al., 2022).

One crucial characteristic of the intestinal microbiota, particularly in the context of administered probiotic microorganisms, is their ability to withstand additional gastrointestinal conditions, particularly their capacity to tolerate and thrive in the presence of bile salts. Particularly for probiotic microorganisms that are administered, the intestinal microbiota's resistance to additional gastrointestinal conditions, particularly its tolerance and proliferation in the presence of bile salts, is an essential characteristic. Bacterial defence mechanisms against bile may include the production of exopolysaccharides, the synthesis of various surface proteins and fatty acids, and special transport mechanisms. Numerous bacterial species possess the enzymatic capability to hydrolyze bile salts. For example, cholylglycine hydrolase is an intracellular enzyme that is constitutively expressed and plays an essential role in the hydrolysis of bile salts. Specifically, it catalyses the hydrolysis of the amide bond between glycine or taurine and the steroid nucleus of bile acids (*Lactobacillus* spp., *Bifidobacterium* spp., *Clostridium* spp., and *Bacteroides* spp.) (de Vos et al., 2022).

The action of bile salt hydrolase has the potential to aid in the detoxification of bile, create possibilities for bacteria to use the amino acids that are produced as a source of carbon and nitrogen, or enhance the incorporation of cholesterol into the cell wall. The process of deconjugating bile salts may have a direct correlation with the decrease in serum cholesterol levels, as conjugated bile salts are synthesised de novo from serum cholesterol. There are also evidences of the capacity of microbes to either assimilate or bind ingested cholesterol to their cell wall, or to remove it through co-precipitation with cholic acid that is produced. Certain gut bacteria have the ability to synthesise cholesterol reductase, an enzyme that facilitates the transformation of cholesterol into insoluble coprostanol. This resultant coprostanol is then eliminated from the body through faecal excretion, thereby contributing to the reduction of exogenous cholesterol levels. (Hernández-Gómez et al., 2021).

4. BENEFITS OF PROBIOTICS

Nowadays, probiotics have emerged as a significant category of advantageous bacteria that are ingested or taken as supplements and can reside within both food products and the intestinal tract. The ingestion of probiotics has been shown to have a beneficial impact on the composition of the intestinal microflora and its interaction with various immune cells, leading to enhancements in immunological activities as shown in Table 1. It is consequently commonly accepted that probiotics have health-promoting and immunomodulatory capabilities. Undoubtedly, these microorganisms exhibit a great degree of reliability in mitigating the occurrence of diverse illnesses. Therefore, the consumption of probiotics may offer cost-effective alternative approaches for the therapy of diseases (Mazziotta et al., 2023).

Table 3 Health benefits of probiotics

Health benefits	*Probiotic*
Improved resistance to foodborne illness	*Bifidobacterium lactis* *S. boulardii*
Improved immune system	*Lacticaseibacillus casei, Lacticaseibacillus rhamnosus* *Lactobacillus acidophilus* *Bifidobacterium longum* *Bifidobacterium lactis*
Increased resistance malignancy	*Lacticaseibacillus casei* *Bifidobacterium longum*
Prevented allergies	*Bifidobacterium lactis* *Lacticaseibacillus rhamnosus* *Lactobacillus, Lactococcus, Pediococcus and Leuconostoc*
Reduced cholesterol	*Lactobacillus acidophilus*
Reduced lactose intolerance	*Lactobacillus bulgaricus, Bifidobacterium bifidum* *Lactobacillus acidophilus*

Source: Ibrahim et al. (2023).

5. CONCLUSION

In summary, while probiotics hold significant potential for treating gastrointestinal illnesses, their practical application faces challenges, including the need for a minimum viable count, susceptibility to environmental conditions, and the need to withstand stomach acidity and bile. Various methods have been developed to assess their efficacy in the gastrointestinal tract. Nonetheless, probiotics have clearly demonstrated their health-promoting and immunomodulatory capabilities, making them a reliable and cost-effective approach for disease therapy.

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Relevance and Use of Honey and Lemon Water for Cough

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ABSTRACT
Coughing is a symptom rather than a disease. Coughing is a natural reflex that helps clear the airways of irritants, mucus, or foreign particles to maintain respiratory health. However, a persistent or chronic cough can be a symptom of an underlying disease or condition. Some common diseases and conditions associated with coughing include respiratory infections, asthma, and chronic obstructive pulmonary disease (COPD). The treatment approach will depend on the specific disease or condition causing the cough. The combination of honey and lemon water is thought to provide beneficial effects against cough, however, there is a limited works of literature that specifically provide a fundamental understanding with combination these two natural substances against cough symptoms. This paper provides a discussion on the relevance of using this mixture by emphasizing its underlying actions against cough. Overall, this review supports the concept of using honey and lemon water in cough due to their multiple nutritional content and health benefits. When consuming, it is essential to do so in moderation and consider individual dietary and medical needs.

Keywords: Cough, honey, lemon water, health benefits, underlying effects.

1. INTRODUCTION

Coughing is a common reflex action that helps to clear your throat and airways of irritants, such as mucus, dust, or foreign particles (Mansour et al., 2021). It can be caused by various factors, including respiratory infections, allergies, irritants (such as smoke or pollution), and sometimes as a symptom of underlying health conditions such as asthma or chronic obstructive pulmonary disease (COPD). The prevalence of cough may vary with age and gender. Children and the elderly may be more susceptible to cough due to different factors, and there may be gender differences in the occurrence of chronic cough. The prevalence of cough may differ between geographic regions due to environmental factors, climate, and exposure to various allergens and pollutants. Other than that, certain occupations or workplaces with exposure to dust, fumes, or irritants may have a higher prevalence of work-related cough. Cough can impact public health due to its association with the transmission of respiratory infections, decreased quality of life, and healthcare costs related to the diagnosis and treatment of cough-related conditions (Turner & Birring, 2023).

Honey obtained from natural sources has antibacterial properties. This can be beneficial when the cough is caused by a bacterial infection or to prevent further complications from a viral infection. Whereas, lemon is a good source of vitamin C, which can help support the body's immune system and may provide some relief from cold symptoms. There has been a discussion on honey and lemon that is used as home remedies to alleviate cough symptoms, especially when dealing with a common cold or sore throat. However, there are limited studies to elaborate on the underlying effects of honey and lemon against symptoms of cough. The current review provides extensive evidence, especially in terms of mechanisms of action of honey and lemon and possible cross-linking of pathways against cough.

2. COUGH

2.1 Pathogenesis

Coughing is a common reflex action that helps to clear the throat and airways of irritants, mucus, or foreign particles. It is a natural defence mechanism of the body to protect the respiratory system. The epidemiology of cough, or the study of its occurrence and distribution in populations, can provide valuable insights into various aspects of public health. Cough can be associated with various underlying pathological conditions. The causes of cough can be broadly categorized into acute and chronic conditions, with different pathologies contributing to each (Lucanska et al., 2020).

Table 1: Various factors affecting cough.

Factors	Conditions	Explanation
Infections	Viral Respiratory Infections	Infections like the common cold, influenza, and COVID-19 can lead to cough as the body's response to the infection and the production of mucus.
	Bacterial Infections	Conditions like bronchitis or pneumonia can cause coughing as a result of lung inflammation and the presence of infection.
	Tuberculosis	A bacterial infection caused by *Mycobacterium tuberculosis* can result in a chronic cough as it primarily affects the lungs.
Allergies	Allergic Rhinitis	Inflammation and irritation of the nasal passages due to allergens can lead to postnasal drip and cough.
	Asthma	An allergic reaction in the airways can cause bronchoconstriction and increased mucus production, leading to coughing.
Chronic Respiratory Conditions	COPD	Conditions like chronic bronchitis and emphysema can cause chronic cough due to airway obstruction and inflammation.
	Bronchiectasis	A chronic condition characterized by damage to the bronchial tubes, leading to excess mucus production and persistent cough.

	Interstitial Lung Disease (ILD)	ILD encompasses a group of lung disorders, including idiopathic pulmonary fibrosis, which can result in a dry, persistent cough.
	Cystic Fibrosis	A genetic condition that causes thick, sticky mucus in the airways, leading to cough and respiratory issues.
Gastroesophageal Conditions	Gastroesophageal Reflux Disease (GERD)	Acid reflux from the stomach can irritate the throat and trigger a chronic cough.
	Laryngopharyngeal Reflux (LPR)	Similar to GERD, LPR involves stomach acid affecting the throat and can cause coughing.
Environmental and Occupational Factors	Exposure to Irritants	Inhaling smoke, air pollutants, dust, or fumes from certain chemicals can lead to cough.
	Occupational Hazards	Certain occupations with exposure to respiratory irritants may result in work-related cough.
Neurological Conditions	Psychogenic Cough	Coughing can sometimes be caused or exacerbated by psychological factors, including stress or anxiety.
Medications	Angiotensin-Converting Enzyme (ACE) Inhibitors	Some medications in this class can cause a persistent dry cough as a side effect.

2.2 Physiological Processes

The cough reflex is triggered when receptors in the airways, such as those in the throat, trachea, or bronchi, are irritated or stimulated. This irritation can result from a wide range of factors, including mucus buildup, foreign objects, smoke, dust, or infection. Once the sensory nerves detect irritation, they send signals to the brain through the afferent (incoming) pathway of the nervous system. These signals travel along sensory nerves to the brainstem (Taylor-Clark, 2016).

The brainstem, specifically the medulla oblongata and pons, plays a central role in coordinating the cough reflex (Pantaleo et al., 2002). It receives sensory input from the afferent pathway and processes this information. The brainstem then sends signals via motor nerves through the efferent pathway to initiate the cough response. These motor nerves control the muscles involved in coughing. Coughing involves the coordinated contraction of several muscle groups, including the diaphragm, intercostal muscles, and abdominal muscles. These muscles contract to increase pressure in the chest cavity. The glottis, the opening between the vocal cords in the larynx, closes to prevent the entry of air into the lungs. The contraction of respiratory muscles increases the pressure in the chest cavity. This pressure forces air out of the lungs. The glottis suddenly opens, and a strong, rapid exhalation of air occurs. This high-velocity expiratory airflow helps dislodge and propel irritants, mucus, or foreign particles out of the airways. The sound produced during a cough result from the turbulent flow of air through the narrowed glottis. The expelled air, along with any irritants, is expelled from the body, and the airway is cleared.

3. HONEY AND LEMON

3.1 Honey

Honey is a naturally sweet material made by honeybees obtained from the nectar of flowers (Anklam, 1998). Honey can be obtained from honeybees (such as *Apidae*) and stingless bees (such as *Apidae*) which are significantly depending on their geographic origin. Honey's flavour and colour can vary depending on the types of flowers from which the nectar was collected by bees. Different floral sources give rise to various varieties of honey, such as clover honey, acacia honey, and lavender. It is important to choose high-quality, pure honey. Some commercial honey products may be processed or diluted with additives like sugar syrup. Pure, raw, and unprocessed honey is often preferred for its potential health benefits. Honey is primarily composed of sugars, with the two main sugars being fructose and glucose. It also contains smaller amounts of water, minerals, vitamins, amino acids, and antioxidants.

Honey has been used for therapeutic purposes in the majority of ancient societies (Kaškonienė & Venskutonis, 2010). The first report about honey is obtained from Sumerian tablet writing from between 2100 and 2000 BC mentioning details of its use as an ointment and a medicine. Currently, the major use of honey for antibacterial, antioxidant, anti-inflammatory, antidiabetic, and cardioprotective have been widely reported to have medical effects (Kadir et al., 2013). Honey helps to protect cells from damage caused by free radicals. It is a natural energy source due to its carbohydrate content, making it a suitable sweetener. It is also known for its potential wound-healing benefits when applied topically.

3.2 Lemon

Citrus limon (Lemon) is a member of the *Rutaceae* family of evergreen tree species, which is commonly found in South Asia. Lemon is a citrus fruit known for its tangy and refreshing flavour. It is a versatile fruit that is widely used in culinary, medicinal, and household applications. Different varieties of lemons exist, including Eureka and Lisbon, which are commonly found in grocery stores, as well as Meyer lemons, which are smaller and sweeter.

Lemon is sometimes used as a home remedy for a variety of health concerns. Before the discovery of vitamin C, it had been used for the treatment of treatment for scurvy. In medicine, it has been used to treat high blood pressure, the cold, and cough treatment (Mabberley, 2004). Also, lemon juice with warm water is thought to have detoxifying and digestive benefits.

4. HONEY AND LEMON IN COUGH

4.1 Preparation

To make a honey and lemon for cough remedy, the ingredients include 1 to 2 tablespoons of honey in combination of juice of half a lemon (approximately 1 to 2 tablespoons) (Figure 1).

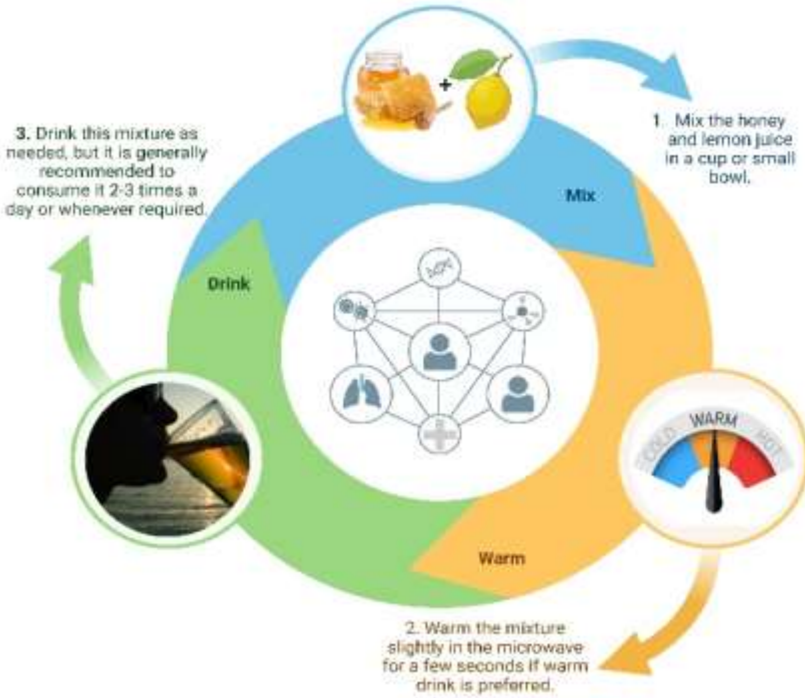

Figure 1: Preparation of honey and lemon water for cough treatment

4.2 Relevance of Using Combination of Honey and Lemon in Cough

Honey and lemon is a natural remedy that is often used to soothe and relieve cough symptoms, particularly when dealing with a common cold or a mild respiratory infection (Rajai et al., 2022). Some of the beneficial characteristics of honey and lemon in cough are described in Table 2.

Table 2: Effects of honey and lemon on cough

Source	Effects	Description
Honey	Anti-inflammatory	Honey has natural soothing and anti-inflammatory properties. It can help ease the irritation and inflammation in throat and airways, which can lead to coughing.
	Cough Suppressant	Honey can act as a natural cough suppressant. It can reduce the frequency and severity of coughing episodes, allowing sleep and rest more comfortably.
	Antibacterial properties	Honey has mild antibacterial properties. This can be beneficial when the cough is due to a bacterial infection, or it can help prevent secondary bacterial infections during a viral illness.
Lemon	Immune booster	Lemons are a good source of vitamin C, which is known to support the immune system. Maintaining a healthy immune system can help to fight off infections that may be causing cough.
	Reduce irritation	Lemon juice can have a soothing effect on a sore throat, which often accompanies coughing. The acidity of lemon juice can help break up mucus and reduce throat irritation.
	Hydration	Staying well-hydrated is important when having a cough. Lemon juice mixed with warm water can provide extra hydration, which can help keep the throat moist and reduce irritation.

5. CONCLUSION

Combination of honey and lemon water can be a helpful therapy due to its various health benefits, but it should not replace medical treatment when needed. If cough is persistent, severe, or accompanied by other concerning symptoms, such as high fever, difficulty breathing, chest pain, or coughing up blood, it is important to proceed with a proper evaluation and treatment. While research is ongoing, honey and lemon can be complementary agents for mild coughs and is not a substitute for addressing the underlying cause of more serious coughs. Extensive evaluation on *in vitro, in vivo, ex vivo*, human trials, or even formulation study is crucial to provide further knowledge regarding molecular pathways of lemon and honey against symptoms of cough. Extensive evaluation on this is necessary to benefit many people suffered from multifactorial conditions that lead to cough.

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Potential Effects of Zamzam Water in Type 2 Diabetes Mellitus (T2DM)

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ABSTRACT
Muslims have utilised Zamzam water for thousands of years to treat illnesses and quench their thirst. Zamzam water is mineral-rich alkaline water. The important metal ions sodium, potassium, magnesium, and calcium are abundant in Zamzam water. Therefore, this review explored at the possible therapeutic effects of Zamzam water on type 2 diabetic mellitus (T2DM) disease by reducing oxidative stress (OS) and inflammation. The online search engines PubMed and Medline were utilised to collect publications published between 2000 and 2022. This was performed by examining abstracts and complete studies using keywords such as Zamzam water, diabetes, type 2 diabetes, oxidative stress, and inflammation. All of the listed publications were written in English.

Keywords: Zamzam water, type 2 diabetes mellitus, antioxidant, inflammation

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic, complicated metabolic disease that affects a sizable portion of the world's population. DM has an epidemic-like prevalence throughout the world, and its alarming rising trend continues. According to the data from International Federal Diabetes (IDF), there were an estimated 463 million persons worldwide who were diagnosed with diabetes in 2019. It is estimated that the number will rise to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045 if suitable preventive measures are not implemented (Federation, 2017). Hyperglycaemia, the term for elevated blood glucose levels, is a hallmark of the disease. This happens when the body either doesn't create enough insulin or doesn't use the insulin it does produce well. The hormone insulin, which the pancreas produces, is essential for controlling blood sugar levels and facilitating the uptake of glucose into cells, which allows for the production of energy. As a result, prolonged disruption of normal bodily functions can result in various problems that could impact the body's several organ systems (Son & Accili, 2023).
At present, managing this illness involves using insulin or anti-diabetic medications, increasing physical exercise, and maintaining a nutritious diet (Ansari et al., 2023). Despite this, the anti-diabetic medications on the market today are far from ideal. Use of herbal

medicines, dietary elements or supplements, and other natural products like honey or Zamzam water are all included in this alternative approach to diabetes treatment. In this review, we aim to explore the potential benefits of Zamzam water for type 2 diabetes mellitus, specifically in relation to its anti-inflammatory and antioxidant properties.

2. TYPE 2 DIABETIC MELLITUS (T2DM)

One of the main characteristics of type 2 diabetes mellitus (T2DM) is dysregulation of the metabolism of proteins, lipids, and carbohydrates. T2DM can be brought on by insufficient insulin production, insulin resistance, or a combination of the two. Of the three main types of diabetes, T2DM accounts for over 90% of cases and is far more common than type 1 diabetes (T1DM) or gestational diabetes (Kale, 2023). Chronic exposure to high glucose initiates a number of pathways that lead to abnormal hepatic glucose production, insulin resistance in peripheral tissues, impaired insulin secretion from the β-cells of the pancreatic islets, and decreased glucose utilisation in peripheral tissues. In individuals with diabetes mellitus, chronic hyperglycaemia can exacerbate other metabolic abnormalities and harm multiple organ systems. This can result in life-threatening and incapacitating health complications, the most common being microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular (a 2- to 4-fold increased risk of cardiovascular diseases). The development of insulin resistance and T2DM also associated with such genetic predisposition, environmental factors, nutrition, physical inactivity, and obesity (Lu et al., 2023).

Numerous ample evidence discovered that reactive oxygen species (ROS) and oxidative stress are major causative factors that lead to the pathogenesis of insulin resistance, impaired insulin secretion and glucose utilisation, abnormal hepatic glucose production, and ultimately overt T2DM through the activation of various proinflammatory mediators, transcriptional mediated molecular pathways, and metabolic pathways. It has been demonstrated that the primary cause of oxidative stress in a hyperglycaemic environment is an excess of superoxide, an oxygen free radical, caused by dysregulation in the mitochondrial electron-transport chain. As a result, cascades of ROS production begin, increasing the oxidation of lipids, proteins, and carbohydrates. Following metabolism, the oxidation of polyunsaturated fatty acids yields a variety of lipid peroxidation end products, most notably malondialdehyde (MDA), 4-hydroxy-nonenal (HNE), and 4-oxy-2-nonenal (ONE) (Singh et al., 2022). In preclinical and clinical trials, strategies targeted at lowering oxidative stress and reestablishing antioxidant balance have demonstrated promise in slowing the progression of T2DM.

Prolonged exposure to high glucose, triglyceride (TG), and free fatty acids (FFA) levels has been shown to activate NADPH oxidase, which in turn increases oxidative stress. Once oxidative stress is created, it increases the production of many proinflammatory mediators, including TNF-α, IL-1β-dependent cytokines and chemokines, IL-β, and IL-6. The role of inflammatory mediators and responses in the production of oxidative stress during the pathophysiology and development of T2DM has been briefly emphasised in a few studies. According to these studies, inflammatory responses is associated with oxidative stress and have a major role in the pathophysiology of T2DM (Ménégaut et al., 2023). The development of tailored therapy aiming at decreasing inflammation has resulted from an understanding of the processes behind the inflammatory response in the pathogenesis of diabetes mellitus.

3. ZAMZAM WATER

Zamzam, or natural water, is consumed by millions of Muslims worldwide according to their religious beliefs. According to a Youssef et al., Zamzam water is perfect for drinking (class I water quality index) and has no microbiological growth. It has been discovered that this natural water is alkaline and mineral-rich, suggesting that it may have antioxidant properties. Calcium (Ca), magnesium (Mg), sodium (Na), and chloride (Cl) were discovered to be more concentrated in Zamzam water, but harmful elements including arsenic (As), cadmium (Cd), lead (Pb), and selenium (Se) were found to be below the maximum toxic limits established by various regulatory bodies (Youssef, 2016). Zamzam water is extremely healthy for us to drink since it can stimulate aquaporins, a type of water channel that helps to regulate the flow of water between cells and facilitates its transportation (A. F. M. Ali et al., 2009).
Zamzam water has potential medicinal therapy as antioxidant, anti-inflammatory, anticancer, improved reproductive system, anti-diabetes and dental caries. In reproduction system, Zamzam water was used in the treatment of implantation failure, for stimulation of endometrial prolactin, α and β defensin, luteinizing hormone (LH), endometrial vascular endothelial growth factor (VEGF), and angiopoietin receptors. Recently, Ali et al. demonstrated that Zamzam water stimulates stem cells' differentiation in the endometrium. This phenomenon is triggered due to the high calcium and magnesium content of Zamzam. Also, Zamzam water also plays a vital role as coenzyme during the formation of immunoglobulin (A. Ali et al., 2009). Because of its distinct mineral makeup, Zamzam water offers a number of functional and nutraceutical benefits that combine with other ingredients to either promote positive effects or prevent negative ones.

3.1. Potential Effects of Zamzam Water as Antioxidant

Evidence of the potent antioxidant property of Zamzam water suggests that it may offer oxidative stress protection. Reactive oxygen species (ROS) generation and the body's capacity to counteract them with antioxidants are out of balance, which leads to oxidative stress. This may cause harm to tissues and cells, which could result in a number of health complications.

Zamzam water has been reported to increase human cell lymphocytes resistance to DNA strand breaks brought on in vitro by H_2O_2. This may be due to the alkaline pH of Zamzam water, which also has a high concentration of several minerals that are necessary for the functioning of antioxidant enzymes. A number of elements found in naturally alkaline water, including zinc, magnesium, and selenium, help to produce the antioxidant enzymes catalase, superoxide dismutase, and glutathione peroxidase. For instance, combining dapagliflozin and Zamzam water treatment dramatically increased the activity of SOD, CAT, and GPX antioxidant enzymes, with a large decrease in MDA, thereby abrogating diabetic wound oxidative damage (Taha et al., 2023). This is also consistent with another finding that study at the effects of consuming Zamzam water on people with non-insulin-dependent diabetic mellitus. According to his findings, the patients' levels of glutathione, superoxide dismutase, and antioxidant capacity are much higher in the Zamzam water-drinking group (Bamosa et al., 2013).

Another investigation by Yazdi et al., into the anti-radiation effect of Zamzam water on mice's bone marrow following gamma radiation revealed that the alkaline water's ability to reduce cytotoxic effects (Yazdi et al., 2017). The antioxidant mechanisms of Zamzam alkaline water were studied by Abdullah et al. The experiment involved two groups: the first group was given a high dose of gentamicin as a control, and the second group received Zamzam water for 21 days. The results showed that Zamzam water does not exhibit any toxicity in normal rats and total antioxidant in rats stressed with gentamicin overdose (Abdullah et al., 2012). Furthermore, according to the study on Zamzam water's potential as an antioxidant agent, it may be able to protect rats from the toxicity of carbon tetrachloride on their livers (Saif et al., 2014).

3.2. Potential Effects of Zamzam Water as Anti-inflammation

Of all the multifactorial pathophysiology involved in the dissemination of type 2 diabetes, the most promising one involves the role of pro-inflammatory cytokines, such as IL-1β, TNF-α, and IL-6, which are released from adipose tissues and cause inflammation in both the corresponding tissue and the β-cells of the pancreatic islets, ultimately leading to insulin resistance.
Zamzam water has been identified in study Taha et al., possesses anti-inflammatory effects. According to this study, dapagliflozin and Zamzam water treatment together shorten the inflammatory stage by upregulating antioxidant enzyme levels and inhibiting the NF-κB transcription factor. As a result, there are fewer CD45 cells and pro-inflammatory cytokines such TNFα, IL-1β, and IL-6. This combination treatment has synergistic effect sped up the proliferative phase and reduced the inflammatory process, which had anti-inflammatory effects on diabetic wounds (Taha et al., 2023). However, further research is necessary to determine the pharmacological effects of Zamzam water, particularly its anti-inflammatory properties in pancreatic β-cells.

4. ZAMZAM WATER AND T2DM

Zamzam water has therapeutic potential on curing diabetes mellitus. On its own, Zamzam water consumption for eight weeks reduced hyperglycaemia and was linked to a considerable increase in blood insulin levels, which were further supported by a notable release of insulin secretion from the isolated islets of Langerhans in vitro. When gliclazide was added, Zamzam water's ability to release insulin was more noticeable. These results add to the body of evidence supporting earlier research suggesting that Zamzam water's antioxidant properties reduce insulin resistance and blood sugar levels.

Another intriguing study by AlJuwaie et al., on the in vivo study, the diabetic rats were fed Zamzam water as their sole hydration source while in vivo. Following a 10-week period of Zamzam water consumption, the following factors were measured: lipid oxidation, superoxide dismutase antioxidant enzyme (SOD), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, insulin resistance, and fasting blood glucose. According to these results, drinking Zamzam water for ten weeks lowers blood insulin, fasting blood sugar, and insulin resistance (AlJuwaie et al., 2020). Zamzam water has no effect on body composition, lipids, or redox homeostasis. The other noteworthy finding is that type 2 diabetic patients who drank Zamzam water for two months saw a significant decrease in their HbA1c; surprisingly, there was no corresponding decrease in the patients' fasting blood glucose levels (Bamosa et al., 2013).

Although the results of linked biomarkers are encouraging, more investigation is required to ascertain whether Zamzam water possesses anti-inflammatory and antioxidant properties, especially in relation to insulin resistance and T2DM. A deeper comprehension of Zamzam water's effectiveness in enhancing insulin sensitivity in T2DM in human trials.

Above all, the results might support the use of Zamzam water as a treatment agent for T2DM, which could have global benefits in the future.

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Effect of Different Environmental Exposure on TSPC Tensile Strength Under Long Term Curing Time

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ABSTRACT

TSPC compressive strength has achieved 59.2 MPa after 3 days of curing in room temperature. Study on the effect of long term environmental exposure on TSPC compressive strength results in increased strength for indoor exposure (62.2 MPa) and aggressive environmental exposure (71.7 MPa) but decreased in strength for tropical climate exposure (56.6 MPa). For tensile strength, TSPC has a splitting tensile strength of 6.08 MPa under short term indoor curing condition. This article presents the effect of long term environmental exposure on TSPC tensile strength through splitting tensile test experiment. Findings show that after 12 month of exposure, TSPC splitting tensile strength increased but with different rates. The observation on average splitting tensile strength for every sample indicates that IND-12 (Aggressive environmental exposure) has the highest value with 10.36 MPa. Meanwhile, the other two samples LAB-12 (Indoor control exposure) and OD-12 (Tropical climate exposure) have shown 7.92 MPa and 8.37 MPa. Failure modes of TSPC for long term curing have shown splitting of the sample along diametrical line with some parts peeling out from middle section. Then, through stress versus strain curve plotted, the study revealed that during test load application, all TSPC specimen has shown linear trend up to maximum load and after that the specimen continue to fracture without further strain gain indicating a perfect brittle damage behavior.

Keywords: TSPC; Durability; Tropical Climate; Aggressive Environment; Tensile Strength

1. INTRODUCTION

TSPC is introduced in 2018 after Faidzal et. al (2018) report that with uniform graded fine TS particles (<1mm) and Unsaturated Polyester Resin (UPR) mixture with 70:30 ratio, cast into cylindrical sample for compression test, the compressive strength achievement (58.21 MPa) is comparable with cement concrete material which make it as suitable replacement for the cement concrete material. TS is an industrial waste from Tin extraction process and the application of TS particles as aggregates may promotes sustainability by recycling waste materials. In case of the matrix selection, the consumption of UPR is suitable due to lower cost and adequate strength. According to Jo et al. (2006), polymer concrete made with UPR can achieve a very high tensile strength (22.4 MPa in flexural and 7.85 MPa in splitting test). Review on TSPC studies in literatures has indicated that optimum particle size, aggregate grading, resin aggregate ratio and strength enhancement potential by fiber reinforced polymer confinement have been carried out (Manda et. al, 2022). In addition to that, Amirnuddin et. al (2021) has observed the effect of TSPC exposure on tropical climate and aggressive environment towards compressive strength. Rambabu and Mahindrakar (2017) report that long term environmental exposure on concrete structures has affected the physical and mechanical properties of the material. The material degradation depends on the exposure condition such as sunshine, temperature, rain, wind, chemical and the effect varies based on duration, intensity and sequence. The study conclude that concrete material preparation must undergo essential research based on weathering exposure on where it is to be applied. Review on previous literatures indicated that in the case of polymer concrete, there was a limited study found regarding the strength measurement in long term after the specimens has been prepared (Sokolowska, 2020). In the study, Sokolowska found that there was significant enhancement on polymer concrete compressive strength after several years of curing. The most recent articles by Cakir (2022) has summarized the studies related to long term exposure on polymer concrete strength which concluded that UPR based polymer concrete achieved 80% strength after 7 days. Besides curing time, the environmental exposure on long term cured UPR based polymer concrete has also counter balance the strength enhancement to affect the durability of UPR based polymer concrete depending on exposure condition. According to Gao et. al (2019), humidity and temperature exposure is two major factors that will reduced the mechanical properties of UPR based polymer concrete. Therefore, for TSPC as one of the UPR based polymer concrete, study by Amirnuddin et.al (2021) regarding tropical climate and aggressive environmental exposure on the materials has provided fundamental description in TSPC material design based on environmental interaction.

According to a study by Amirnuddin et. al (2021), the 18 months of long-term environmental exposure on TSPC has been defined as indoor for control specimen, outdoor for general tropical climate weathering exposure and industrial area for aggressive environmental exposure. Indoor area selected was inside composite material laboratory and outdoor area was outside the similar laboratory, located inside Universiti Teknologi Malaysia, Skudai campus. Indoor curing condition was at 280C normal room temperature and humidity range of 70%-90% neglecting the effect of ultraviolet radiations (UV) exposure. For outdoor curing condition, the tropical weathering exposure is expected with high temperature due to UV exposure and high humidity due to rainfall. This condition is caused by equatorial geographical location of Malaysia which generally has evenly distributed hot and humid weather throughout the year. Finally, for aggressive environmental exposure, the TSPC specimens are exposed to open area in Pasir Gudang

Industrial area. The area has high heavy metal content, high in acidity based on PH data measurement, high in dissolved oxygen concentration (DO) which caused thicker atmosphere resulting in more sunlight dispersion, high in salinity (PPT) due to located near sea area and high in turbidity (NTU). All of the reported environmental exposure data are based on Amirnuddin et. al (2021) which referred from Malaysian Meteorological Department. Compressive strength of TSPC after cured for three days at room temperature is 59.2MPa (Hassan et. al, 2020). Long term environmental exposure under various conditions on TSPC curing was then examined after 18 months of curing period. For indoor (control) and industrial area (aggressive) curing environment, the compressive strength of TSPC specimens increased to 62.2 MPa and 71.7 MPa, while for outdoor (tropical climate) curing environment the strength decreased to 56.6 MPa (Amirnuddin et. al, 2021). Various factors may become the caused for the changes in compressive strength measurement such as prolong curing time, temperature and humidity exposure. Besides, other than compressive strength, the tensile strength of TSPC is also an important parameter to be investigated. A study by Manda et. al (2022) has report that the average splitting tensile for TSPC samples cured for three days at room temperature is 6.08 MPa which is about 10.44% from TSPC compressive strength (59.2 MPa). According to extensive literature reviews, there was no information found regarding the effect of different environmental exposure on TSPC tensile strength under long term curing time. Therefore, the focus of this study is to examine the similar long term exposure on TSPC tensile strength.

2. MATERIALS & MECHANICAL TESTINGS

According to Faidzal et. al (2018), Shakil and Hassan (2020) and Amirnuddin et. al (2021), TSPC is prepared by applying uniform graded of fine Tin Slag particles (<1mm) as aggregates and Unsaturated Polyester resin (UPR) as matrix binder. The ratio of resin to aggregate is 30:70 according to previous study while the weight percentage of Methyl Ethyl Ketone Peroxide (MEKP) as hardener for UPR is 2% as recommend by the resin manufacturer. The specimen preparation is based on ASTM C192/470 standard specification for molds for forming concrete test cylinders vertically. After the specimens have been casted, it was then cured under long term and specific environmental exposure based on literatures. Similar with Amirnuddin et. al (2021) study, all of the specimens were divided into three group each for indoor condition (control), outdoor condition (tropical climate) and industrial condition (aggressive).The long term exposure period is 12 months and the locations was inside and outside composite material laboratory, Universiti Teknologi Malaysia, Skudai for indoor and outdoor exposure. Meanwhile, for aggressive environmental exposure, the specimens are placed at Pasir Gudang industrial area. The samples are coded based on exposure condition-exposure period-specimen number as IND-12-1 (Industrial exposure for 12 months of specimen 1), LAB-12-1 (Inside laboratory exposure for 12 months of specimen 1) and OD-12-1 (Outside laboratory exposure for 12 months of specimen 1). Table 1 presents the description of each sample and specimen code. The comparison between three days curing and 18 months long term curing under various environmental condition exposures will be made with data as report by Manda et. al (2022). The comparison between long term curing under different exposure for TSPC compressive strength and splitting tensile strength will be made based on Amirnuddin et. al (2021).

Table 4 Description on test samples and specimen code

Sample Code	Specimen Number	Exposure Condition	Environmental Description	Exposure Period
IND-12-1	*1*	*Pasir Gudang Industrial Area*	*Aggressive Environment*	
IND-12-2	*2*			
IND-12-3	*3*			
LAB-12-1	*1*	*Inside Composite Material Laboratory*	*Control Environment*	
LAB-12-2	*2*			*12 Months*
LAB-12-3	*3*			
OD-12-1	*1*	*Outside Composite Material Laboratory*	*Tropical Climate Environment*	
OD-12-2	*2*			
OD-12-3	*3*			

After the period of 12 months, all of the specimens were gathered to undergo a splitting tensile test. The specimens were marked on its diametrical section as suggested by ASTM C496 for splitting tensile test specimen. Instron 100kN universal testing machine was employed for the mechanical testing process. The compressive load was applied along a line on diametrical section and the opposite line was located on the bottom plate of the testing machine. Constant loading rate application is within the range of 0.7 to 1.4 MPa/min until failure of the specimens. Figure 1 (a) shows the specimens preparation process and (b) the testing machine setup. Based on specimens' size and loading parameters, the splitting tensile strength was calculated based on equation (1) below, where T is the splitting tensile strength (MPa), P is the maximum applied load indicated by the testing machine (N), l is length of the specimen (mm) and d is diameter of the specimen (mm). The calculation and methods of average splitting tensile strength measurement are made based on Manda et. al (2022) and ASTM C496.

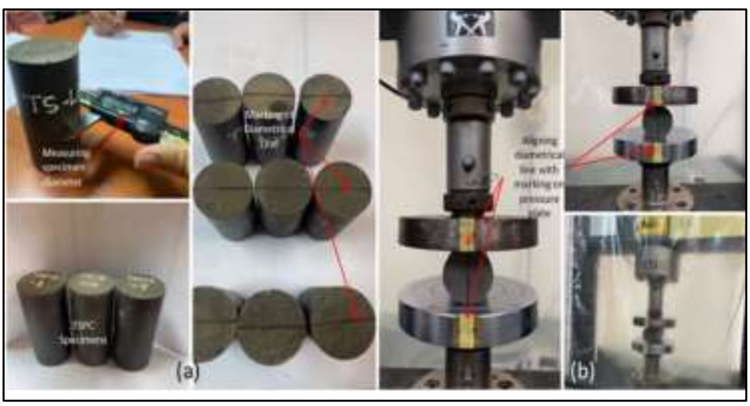

Figure 1: Samples and mechanical testing (a) Specimen preparation process and diametrical line marking (b) Testing machine setup for splitting tensile test.

$$T = \frac{2P}{\pi l d} \quad (1)$$

3. RESULTS & DISCUSSIONS

Splitting Tensile Strength

The test results are as present in Table 2. All of the specimen sizes measured are acceptable where the length are within the range of 100 mm to 102 mm (± 2 mm), while the diameter are within the range of 47.5 mm to 48.5 mm (± 1 mm) except for IND-12-3 specimen with has an excess of 0.06 mm (48.56 mm) in diameter compared to others. However, because of very small difference, the measurement has been accepted considering the consistency in maximum load applied to break the specimen. For sample which exposed to aggressive environment at Pasir Gudang Industrial Area, the maximum load required to break specimen IND-12-1, IND-12-2 and IND-12-3 is 79.04 kN, 70.84 kN and 87.64 kN. Observed that specimen IND-12-3 requires the highest load to be broken as its diameter was also a little bit larger than IND-12-1 and IND-12-2. The splitting tensile strength for IND-12 samples is obtained using equation (1). The calculation indicates that IND-12-1, IND-12-2 and IND-12-3 have splitting tensile strength of 10.38 MPa, 9.3 MPa and 11.39 MPa. The average splitting tensile strength for IND-12 sample is 10.36 MPa with tensile modulus 0.657 GPa.

Then for control sample which cured at room temperature inside composite material laboratory, the maximum load required to break specimen LAB-12-1, LAB-12-2 and LAB-12-3 is 64.2 kN, 55 kN and 60.48 kN. Observed that specimen LAB-12-2 requires the smallest load to be broken with just 55 kN. The splitting tensile strength for LAB-12 samples is also obtained using equation (1). The calculation indicates that LAB-12-1, LAB-12-2 and LAB-12-3 have splitting tensile strength of 8.54 MPa, 7.27 MPa and 7.94 MPa. The average splitting tensile strength for LAB-12 sample is 7.92 MPa with tensile modulus 0.591 GPa.Finally, for sample which exposed to tropical climate at outdoor area of composite material laboratory, the maximum load required to break specimen OD-12-1, OD-12-2 and OD-12-3 is 63.09 kN, 62.32 kN and 64.88 kN. Observed that all specimens for OD-12 sample has shown the highest consistency in maximum load measurement with all in the range of 62 kN to 65 kN only. Similarly, the splitting tensile strength for OD-12 samples is obtained using equation (1). The calculation indicates that OD-12-1, OD-12-2 and OD-12-3 have splitting tensile strength of 8.3 MPa, 8.21 MPa and 8.59 MPa. The average splitting tensile strength for OD-12 sample is 8.37 MPa with tensile modulus 0.672 GPa.

The observation on average splitting tensile strength for every sample indicates that IND-12 has the highest value with 10.36 MPa. Meanwhile, the other two samples LAB-12 and OD-12 have shown 7.92 MPa and 8.37 MPa. In comparison with normal short-term curing as report by Manda et. al (2022), the average splitting tensile strength is just 6.08 MPa. These findings have revealed that long term curing has increased the splitting tensile strength of TSPC in general. Then, the second parameter is environmental exposure which shows that aggressive environment has provided the highest rate of splitting tensile strength enhancement compared with control and tropical climate environment. Similarly, the compressive strength for long term exposure of TSPC under tropical climate and aggressive environmental exposure according to Amirnuddin et. al (2021) has also shown highest rate of strength enhancement in aggressive environment from 59.2 MPa to 71.7 MPa followed by indoor exposure with strength increased to 62.2 MPa. However, in the study, tropical climate exposure has reduced the compressive strength from 59.2 MPa to 56.6 MPa. The percentage of splitting tensile strength of TSPC for long term exposure is about 14.45% for aggressive environment, 12.73% for indoor control environment and 14.79% for tropical climate environment. Another finding is on tensile modulus value for each sample. Tensile modulus represents the rate of loading towards deformation of specimen. Higher tensile modulus indicating higher stiffness compared to lower value of modulus. Higher stiffness means that a specimen is hardly deformed under loading application. In this experimental study, OD-12 has shown highest tensile modulus (0.672 GPa) followed by IND-12 (0.657 GPa) and LAB-12 (0.591 GPa). The results indicate that even though splitting tensile strength of OD-12 has the lowest enhancement rate, it has however increased the stiffness of the sample. The summary of all data that has been discussed is summarized in Table 2.

Table 5 Summary of experimental test results

Sample	Length, l	Diameter, d	Max. Load, P	Sp.Tens. Strength,T	Tensile Modulus
	(mm)	*(mm)*	*(kN)*	*(MPa)*	*(GPa)*
IND-12-1	*101.57*	*47.74*	*79.04*	*10.38*	*0.642*
IND-12-2	*101.43*	*47.78*	*70.84*	*9.3*	*0.62*
IND-12-3	*100.87*	*48.56*	*87.64*	*11.39*	*0.708*
AVERAGE	**101.29**	**48.03**	**79.17**	**10.36**	**0.657**
LAB-12-1	*100.67*	*47.52*	*64.2*	*8.54*	*0.655*
LAB-12-2	*101.07*	*47.63*	*55*	*7.27*	*0.66*
LAB-12-3	*101.01*	*47.98*	*60.48*	*7.94*	*0.458*
AVERAGE	**100.92**	**47.71**	**59.89**	**7.92**	**0.591**
OD-12-1	*101.04*	*47.86*	*63.09*	*8.3*	*0.611*
OD-12-2	*101.18*	*47.76*	*62.32*	*8.21*	*0.612*
OD-12-3	*100.89*	*47.66*	*64.88*	*8.59*	*0.793*
AVERAGE	**101.04**	**47.76**	**63.43**	**8.37**	**0.672**

Failure Mode

The fracture appearance of every samples (IND-12, LAB-12 and OD-12) have shared similar pattern. The splitting of each sample occurs along the diametrical line which causes the specimen to separate into two sections namely right and left section. In addition to that, there was also a small part peeling off from the middle section of the specimen during breakage. This condition occurs as during the mechanical testing, it is observed that there was a formation of two crack lines where first crack is right on the diametrical line and then another small branch crack propagate from the main diametrical line crack. The first crack line has result in splitting of the specimen into two large sections and the smaller crack has caused the smaller section peeling out from middle section of the specimen. The fracture process also occurs in quick sudden together with a little explosion. These patterns of failure modes are similar with previous study by Manda et. al (2022) which has named those cracks as primary and secondary crack. Figure 2 has showed general failure modes on test specimens with Figure 2 (a) represent the failure modes footage on one of the OD-12 specimen and Figure 2 (b) represents similar failure patterns on one of the IND-12 specimen.

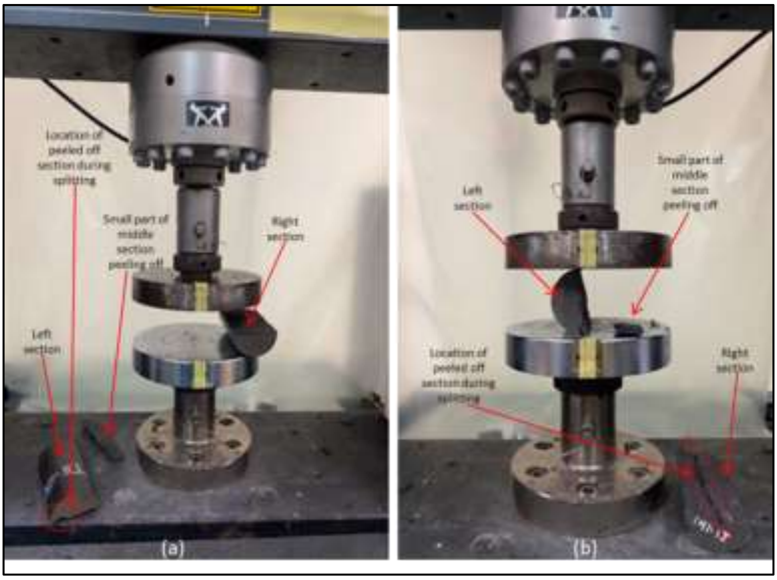

Figure 2: Failure modes of TSPC specimens (a) Failure modes of OD-12 sample.
(b) Failure modes of IND-12 sample

Stress versus Strain Curve

Stress versus strain curve shows the behavior of a material under gradually applied loads. Generally, from the curve, material response towards applied load may be observed starting from a linear response, yielding, non-linear response and fracture behavior. In the mechanical testing that has been performed, the stress versus strain curve has been plotted for each of the specimen from sample IND-12, LAB-12 and OD-12 groups. Besides stress and strain relationship, this curve also represents the load versus deformation relationship where both curves are similar in shape. Other than maximum loads, average strength and failure modes, the analysis on stress versus strain curve have provided good description on the material behavior during mechanical testing. Figure 3(a) presents stress versus strain curve for TSPC cured under aggressive environment (Industrial area) which consists of IND-12 sample. All of the curves (IND-12-1, IND-12-2 and IND-12-3) have shown consistency in shape except for IND-12-2 which behave differently during fracture. Here, IND-12-2 has regain strength after undergoes brittle damage and during the secondary strength increment (strain hardening), the specimen fracture with a little bit of softening behavior compared to IND-12-1 and IND-12-3 which fracture with the clear sign of brittle fracture. The average tensile modulus of IND-12 sample is 0.657 GPa representing the slope gradient of its elastic behavior and upon the application of compressive load, the sample only provide linear response without any sign of yielding. After reaching maximum strength, the specimens have shown rapid loss in strength up to fracture except for IND-12-2.

Figure 3(b) presents stress versus strain curve for TSPC cured under control environment (Inside laboratory) which consists of LAB-12 sample. The curve for LAB-12-1 and LAB-12-2 has shown almost perfect similarity in shape while there was a little bit deviation in LAB-12-3 specimen. The different in curve shape indicate that LAB-12-3 has more stiffness due to higher tensile modulus compared to others. However, the average tensile modulus for LAB-12 sample is 0.591 GPa which less then IND-12 sample indicating that in general IND-12 has higher stiffness compared to LAB-12 sample. Upon the application of compressive load, all of the three LAB-12 specimens behave linearly up to maximum load without any sign of yielding. Then, all of the specimens have shown a clear brittle fracture sign where fractured occur after all specimens experience rapid strength loss. Figure 3(c) presents stress versus strain curve for TSPC cured under control environment (Inside laboratory) which consists of OD-12 sample. OD-12-1 and OD-12-2 curve has shown linear relationship at the beginning of loading but at some point OD-12-2 indicate a minor sign of yielding where the curve has slightly bend down until both reach maximum load. Then, both specimens failed in similar mechanism which is brittle damage where sudden loss of strength without deformation has occurred until fracture. For OD-12-3, the testing load and deformation starts with a little sign of nonlinear behavior but regain linearity in the load to deformation relationship after a while. This condition usually happens due to surface defect of the specimen or misalignment in specimen placement on testing machine bottom pressure plate. After reach maximum load, the specimen OD-12-3 also has shown a brittle damage behavior through sudden loss in strength until fracture. The average tensile modulus for sample OD-12 is 0.672 GPa which is the highest tensile modulus compared to other samples 1ND-12 (0.657 GPa) and LAB-12 (0.591 GPa). Figure 3(d) presents the comparison of stress versus strain curve for all samples based on average curve shape for splitting tensile test. The average curves are plotted based on equivalent loading steps for each sample. Observation on all curve shows that TSPC failure mechanism under splitting tensile load is brittle fracture. In term of maximum splitting tensile strength, the peak of IND-12 sample has shown the highest value compared to LAB-12 and OD-12 sample. According to Table 2, the average splitting tensile strength of IND-12 is 10.36 MPa. After reaching maximum strength, during downward line, IND-12 sample behavior has shown a little strength regain before fracture but LAB-12 and OD-12 sample directly fracture after maximum strength has been reached. In general, it is observed that sample IND-12 behaved slightly different while sample LAB-12 and OD-12 has shown almost similar behavior as shown by the stress versus strain curve.

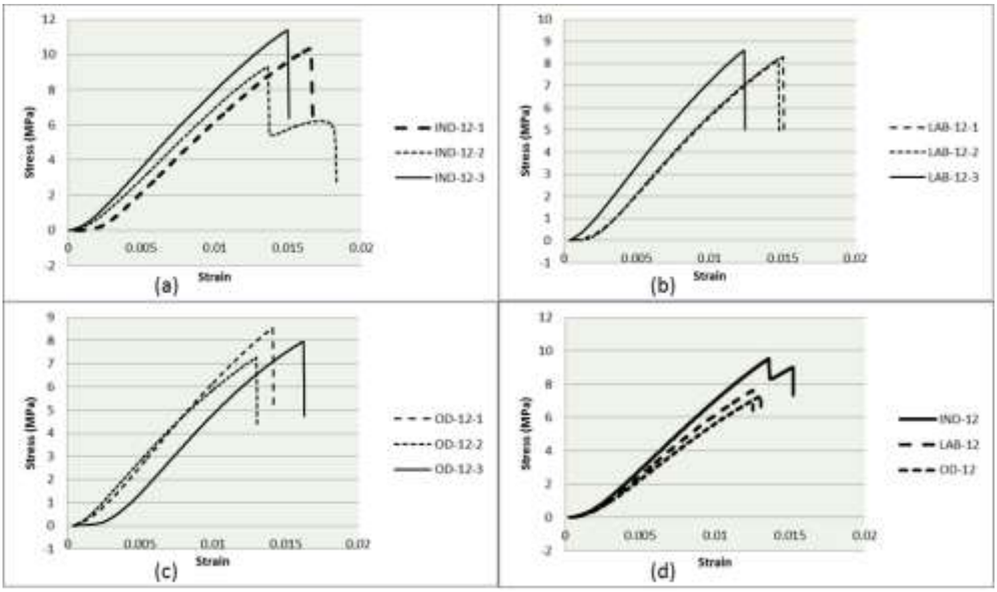

Figure 3: Stress versus strain curve (a) IND-12. (b) LAB-12. (c) OD-12. (d) Average of all samples.

4. CONCLUSIONS

At a glance, TSPC has shown similar behavior as other concrete material where under compressive test, failure occur with strain hardening and softening behavior before crushing. Meanwhile, under splitting tensile test, TSPC and other concrete material failed with brittle fracture mechanism. In addition to that, the splitting tensile strength value of concrete material always lowers than their compressive strength (Chhorn et. al, 2018). In this study, the average splitting tensile strength for 12 months aged TSPC are 10.36 MPa (Industrial exposure), 7.92 MPa (Indoor control exposure) and 8.97 MPa (Tropical climate exposure) while in previous study, Amirnuddin et. al (2021) has revealed that 18 months aged TSPC crushed at 71.7 MPa (Industrial exposure) , 62.2 MPa (Indoor control exposure) and 56.6 MPa (Tropical climate exposure). Previously reported on polyester based polymer concrete states that longer curing time has further enhanced the strength (Cakir, 2022), meanwhile long term exposure to elevated temperature and high humidity has reduced its mechanical properties (Gao et. al, 2019). In accordance with those previous findings, splitting tensile strength of long term cured TSPC has increased but with different rates according to exposure condition for all samples employed in this study with 10.36 MPa (Industrial exposure), 7.92 MPa (Indoor control exposure) and 8.97 MPa (Tropical climate exposure). In comparison to that, study by Manda et. al (2022) has presented that short term cured of TSPC in room temperature has an average splitting tensile strength of 6.08 MPa. Besides that, according to Manda et. al (2022), failure mode for splitting tensile strength of TSPC specimen began with the formation of primary crack followed by secondary crack before the specimen break into two sections which split along the diametrical line. The experiment has shown similar pattern where the TSPC specimen has been split into left and right section with some of the middle section peeling out from either left or right section. The stress strain curve allowed the observation of material behavior under static loading for better characterization of the material property under evaluation. The study revealed that all specimen has shown linear trend up to maximum load and after that the specimen continue to fracture without further strain gain indicating a perfect brittle damage behavior.

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Influence of Brine Composition and pH on the Wettability of Sub-Bituminous Coal for Carbon Dioxide Sequestration

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ABSTRACT

Carbon Capture and Storage (CCS) is a revolutionary technology to reduce carbon emissions into the atmosphere by capturing the CO_2 and injecting it into a deep reservoir. This paper focuses on the storage part by analysing coal reservoir properties. Coal is chosen as the reservoir of study due to its wetting characteristics that make CO_2 preferentially adsorb to it. One aspect that affects the CO_2 geological storage is the wettability of the CO_2/rock/brine system, which correlates to the contact angle. Previous studies showed that pH has an impact on the wettability of the system. The organic acid commonly found in the petroleum basin is mainly acetic acid. Therefore, the experiment varied the pH from 5 to 9 using acetic acid and varying salinity from 0 ppm to 30000 ppm. Coal reservoir enable trapping and storage of CO_2 within the rock matrix and pore spaces through a combination of physical and chemical mechanisms, including adsorption trapping, solubility trapping, and mineral trapping. Sub-bituminous coal is made of polar oxygen-containing groups, such as carboxyl (-COOH), hydroxyl (-OH), and methoxyl ($-OCH_3$) groups. The experiment was conducted under elevated supercritical pressures and temperatures of 10 MPa, 15 MPa, and 305 K, respectively. The contact angles are measured by the Vinci IFT 700 machine. Wettability of the sub-bituminous coal is also measured through the zeta potential values observed from the interaction between the brine and coal interface. The results showed that sub-bituminous coal generally shows gas-wet nature, especially under supercritical CO_2 conditions under acidic and neutral environments with high salinities due to the effect of low pH on surface charge and salt screening effect, which prove to be the most favourable conditions for CO_2 to wet the coal surface.

Keywords: CCS, coal, CO_2, wettability, zeta potential, supercritical

1. INTRODUCTION

This paper discusses various aspects related to carbon dioxide (CO_2) emissions and environmental issues caused by it, highlighting the potential solutions for addressing these challenges. Carbon capture and storage (CCS) technologies are identified as a promising approach to reduce CO_2 emissions. One of the prime geological locations for CO_2 sequestration are coal reservoirs that cannot be mined. Coal comes in four main varieties, or "ranks. The four ranks of coal are anthracite, bituminous, subbituminous and. In the process of transformation (coalification), peat is altered to lignite, lignite is altered to sub-bituminous, sub-bituminous coal is altered to bituminous coal, and bituminous coal is altered to anthracite (PMF IAS, 2016). Coal reservoirs are selected as the reservoir of study due to coal's wetting characteristics make CO_2 preferentially adsorb to it. Zeta potential represents the difference in potential between a particle's surrounding medium and its ionic environment, measured in the plane of shear (Syed Taha Taqvi & Ghada Bassioni, 2019). When small particles are dispersed in solutions, they can form charged contacts with the bulk liquid. This interaction depends on the balance of positive and negative electrolytes. Wettability in the coal/brine/CO_2 system can be assessed using contact angle measurements and zeta potential analysis. This study addresses the limited research on how varying pH and brine concentrations affect the wettability of sub-bituminous coal. This study will measure the wettability of sub-bituminous coal through two methods, contact angle and zeta potential, at varying pressures and constant temperature. To assess wettability, the contact angles will be determined using the IFT 700 interfacial tension machine, and zeta potential measurements will be obtained with the Zetasizer Nano-ZSP.

2. LITERATURE REVIEW

2.1 Carbon Capture and Storage (CCS)

CCS is the process in which CO_2 generated at the end of life is captured back, returned to the lithosphere where the fossil-C originally had come from, and safely stored in suitable underground geological structures. There are many CCS projects around the world, here are some of the biggest: CNPC launched research and pilot test of CCS-EOR in Jilin Oilfield in 2007 and has put CCS-EOR into industrial application at 69 well groups (Industrial CCS-EOR in CNPC's Jilin Oil Eld Energize • Harmonize • Realize, n.d.). ExxonMobil's Shute Creek CCUS facility was commissioned in 1986 with the principal economic driver to sell captured CO_2 from its gas processing plant to oil companies for the purpose of pumping it into depleted wells to recover more oil – or enhanced oil recovery (EOR) (Shute Creek – World's Largest Carbon Capture Facility Sells CO_2 for Oil Production, but Vents Unsold, 2022). In the case of Sinopec's Qilu Petrochemical CCS Project, 1MtCO_2/year and a total of 10.68MtCO_2 from Sinopec's Qilu refinery most likely from a coal/coke gasification plant, which will be used for enhanced oil recovery (EOR) over a 15-year period. By the mid-2030s, the project aims to enhance oil production by around 2.97Mt (oil).

2.2 CO_2 Sequestration Behaviour

CO2 sequestration behaviour is heavily governed by its critical point. The critical point for CO2 is $T_c = 31.1$˚C and $P_c = 7.38$ MPa (equal to 738 m hydrostatic column of water) (Chen et al., 2022). A supercritical fluid (SCF) is a material that can be either liquid or gas, used in a state above the critical temperature and critical pressure where gases and liquids can coexist.

2.3 Coal Reservoir

A coal reservoir is a dual structural system composed of pores and fissures. Coal's pore system is generally accepted as the combination of the pores that are in coal matrix and the cleats that separate matrix, both of which form the dual-porosity structure of coal

(Chen et al., 2015). As with the pores in coal matrix, there are several methods to categorize such pores, the most frequently used classification method is to categorize them into three groups in terms of sizes: macro, meso, and micropores. Coal permeability describes the transportability of water and gas to flow through interconnected pores and cleats of coal formations and plays a key role in achieving economic methane flow and production rates (Zhao et al., 2019). Absolute permeability can be further divided into matrix permeability and cleat permeability due to the dual-porosity system of coal.

2.4 Trapping Mechanisms

The mechanism for CO_2 trapping in coal seams is primarily adsorption through micropores in the coal matrix. CO_2 is stored in coal predominantly as adsorbed molecules on micropore surfaces (adsorption trapping) that allow higher densities and greater volumes of CO_2 at shallower depths than in sandstone and carbonate reservoirs. (Golding et al., 2011). At larger time scales, the injected CO_2 will be either dissolved in formation fluids, which is called solubility trapping, or precipitated in solid minerals, which is called mineral trapping (Chen et al., 2022). For solubility trapping, the dissolution of CO_2 in the liquid phase affects the CO_2 storage capacity (Abba et al., 2019).

2.5 Zeta Potential and Wettability

Zeta potential is the difference in potential between a particle and its surrounding ionic atmosphere, typically measured in the plane of shear (Hodne *et al.*, 2007. When solid particles are dissolved in water, their surface charge leads to the formation of counterions, creating two layers: the Stern layer and the diffuse layer, collectively known as the electric double layer (EDL) (Syed Taha Taqvi & Ghada Bassioni, 2019). High surface charge particles have a larger double layer, leading to electrostatic repulsion that prevents them from coming into close proximity. Zeta potential is influenced by salinity and pH, with an isoelectric point typically occurring between pH 7 and 8 (Salgın *et al.*, 2012). As salt concentration increases, the absolute zeta potential value decreases due to reduced charge density and electrostatic potential (Ibrahim & Nasr-El-Din, 2016).

2.6 Contact Angle and Wettability

Wettability, a crucial factor in CO_2 geological sequestration, is often assessed using contact angle measurements. The sessile drop method is typically preferred for determining these angles.

Table 1: Contact angle values and its relation to wettability

Contact Angle (θ)	**Wettability**
0° to 70°	Water-wet
70° to 110°	Intermediate-wet
110° to 180°	Oil-wet

3. METHODOLOGY

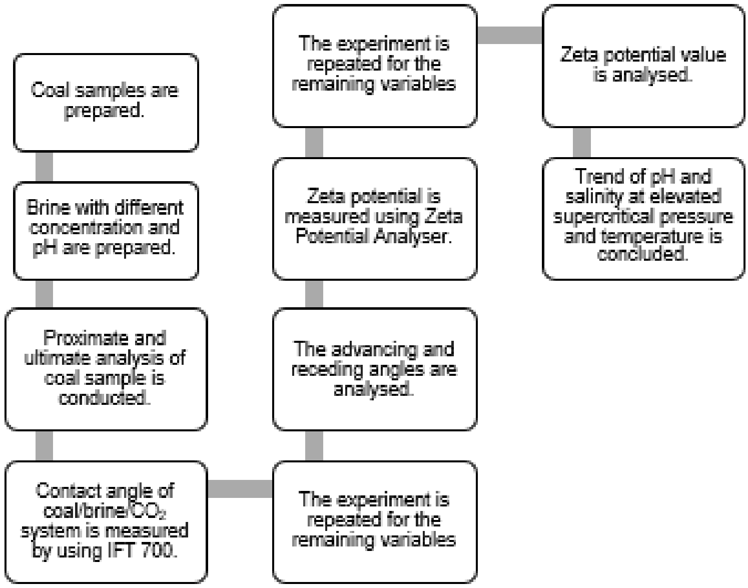

Figure 1: Experiment Workflow

4.RESULTS & DISCUSSION

Table 2: Summary of Contact Angle Results

pH	Salinity (ppm)	Pressure (MPa)	Contact Angle	Wettability	pH	Salinity (ppm)	Pressure (MPa)	Contact Angle	Wettability
5	0	10	98.5	Gas-Wet	7	0	10	91	Gas-Wet
		15	102.5	Gas-Wet			15	91.5	Gas-Wet
	10000	10	96	Gas-Wet		10000	10	93.5	Gas-Wet
		15	100.5	Gas-Wet			15	110.5	Gas-Wet
	20000	10	93	Gas-Wet		20000	10	101	Gas-Wet
		15	93.5	Gas-Wet			15	101.5	Gas-Wet
	30000	10	70	Water-Wet		30000	10	65	Water-Wet
		15	86.5	Gas-Wet			15	69	Water-Wet

Table 3: Results from Contact Angle Test at Standard Conditions

pH	Salinity (ppm)	Pressure (MPa)	Contact Angle	Wettability
9	0	10	100.5	Gas-Wet
		15	88	Gas-Wet
	10000	10	85	Gas-Wet
		15	103	Gas-Wet
	20000	10	99	Gas-Wet
		15	89	Gas-Wet
	30000	10	47.5	Water-Wet
		15	42.5	Water-Wet

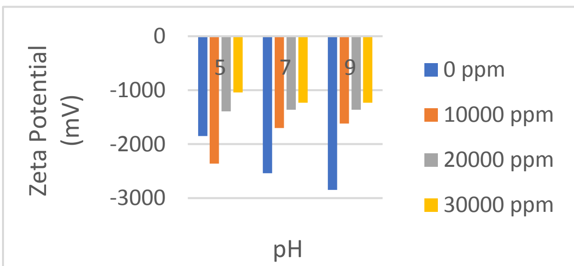

Figure 2: Graph of Zeta Potential vs pH vs Salinity

a) pH affects wettability significantly under supercritical temperature and pressures (above supercritical temperature and pressure of CO_2), as pH of a solution typically relates to the concentration of H^+ ions (acidic) or OH^- ions (basic) and the potential for proton transfer reactions. Supercritical CO_2 ($ScCO_2$), on the other hand, is a nonpolar and chemically inert fluid.
b) Acidic environments under supercritical conditions show high contact angle readings (gas-wet). For acidic conditions, the net positive charge of sub-bituminous coal results in an increased affinity for non-polar groups, which in this case is $scCO_2$, thus larger contact angle readings and more gas-wet nature.
c) Neutral environments under supercritical conditions show high contact angle readings (gas-wet). For neutral conditions, an increase in salinity results in a decrease of the EDL of sub-bituminous coal particles. This can be proven by the reduction in magnitude of zeta potential under the standard temperature and pressure tests. A low EDL and zeta potential reduces electrostatic interactions, which reduces any affinity to polar or non-polar groups. Contact angle readings are high due to the higher density of $scCO_2$ at supercritical conditions, which results is better adsorption of $scCO_2$ onto the coal surface, thus more gas wet.
d) Alkaline environments show lower contact angle readings, but still in the gas-wet region. Under alkaline conditions, the sub-bituminous coal surface becomes negatively charged due to the deprotonation reaction between the surface

functional groups and the OH^- ions. The net negative surface charge increases affinity for polar groups like aqueous NaCl (water-wet nature). However, the effects of salinity and density (explained in the previous point), the alkaline conditions still show gas-wet nature of sub-bituminous coal.

e) Under standard temperature and pressure conditions, lower pH and higher salinity result in larger contact angle measurements, indicating gas-wet behaviour. Higher salinity is associated with a lower zeta potential value, while higher pH leads to a higher zeta potential value, resulting in water-wet behaviour.

f) The increase in salinity enhances the screening effect, while a higher pH causes deprotonation of surface functional groups in sub-bituminous coal, increasing the magnitude of the zeta potential value.

5.CONCLUSION

pH affects wettability significantly above CO_2 supercritical temperature and pressures. Acidic and neutral environments under supercritical conditions show high contact angle readings (gas-wet). Alkaline environments show lower contact angle readings, but still in the gas-wet region. Under standard temperature and pressure conditions, lower pH and higher salinity result in gas-wet behaviour. Sub-bituminous coal is generally gas-wet in CO_2/brine/coal system.

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Determination of Commonly Abused Drugs in Cough-Cold Medicines using Thin Layer Chromatography

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ABSTRACT
Easy access to over the counter (OTC) drugs leads to misuse and abuse among Malaysians. Due to this matter, the study aims to identify and quantify the active components in cough-cold medicines using thin-layer chromatography (TLC). Four standards comprised dextromethorphan hydrobromide (DXM), phenylephrine hydrochloride (PHED), chlorpheniramine maleate (CHPH), and paracetamol (PCM) together with five cough-cold medicines were selected. Ten solvent systems were examined from which ultraviolet radiation worked best with solvent system A (methanol:ammonia in a ratio of 100:1.5) while iodine fuming worked best with solvent system G (cyclohexane:acetone:chloroform in a ratio of 60:30:20), which provided the best separation and differentiation for cough-cold samples. Detected spots assisted the calculation of retention factors (hR_f) for comparison between samples and their respective standards. Similarities of hR_f values help validate the compositions stated on the packaging. TLC can be used as a screening technique at the initial stage of the analysis process.

Keywords: Thin layer chromatography (TLC), Cough-cold medicines.

1. INTRODUCTION

Drugs have the potential to influence a person's physical and mental state, and in Malaysia, drug abuse is a concerning issue, especially among young individuals. The country has a high rate of drug use, and drug traffickers often exploit loopholes to make drugs more accessible, including to teenagers. OTC medications, which can be purchased without a prescription, serve various purposes, from pain relief to treating colds and congestion. Some OTC drugs have active ingredients that can be misused when taken in high doses, and cough-cold medicines, readily available and affordable, are frequently misused. For instance, DXM commonly found in cough syrups, is a common target for misuse. CHPH is an antihistamine commonly found in cold and allergy medications. PCM is used for pain relief, but taking too much can lead to liver failure. PHED is a combination of an antihistamine and a decongestant used to relieve congestion. To address this issue, TLC was utilized to identify and measure the active ingredients in OTC cough-cold medicines in Malaysia, which can enhance our understanding of these products and aid in preventing drug abuse.

2. LITERATURE REVIEW

High-performance liquid chromatography (HPLC) is a common analytical technique for determining the composition and concentration of compounds in pharmaceutical products. Researchers use it for various studies, like Sagathiya and Bagada (2014) who employed HPLC to analyze cough and cold medications, offering simple, precise, accurate, and rapid results. Another study by Darwish et al. (2015) used HPLC to determine the concentrations of specific compounds in oral liquids, while Mahesh et al. (2014) developed an HPLC approach to identify multiple components in OTC tablet formulations. HPLC has several challenges including high costs, complex operation, time-consuming sample preparation, potential waste generation, and sensitivity to environmental conditions (Dong, 2013). Furthermore, it may not provide a complete chemical profile of all compounds and not all compounds are compatible with HPLC. TLC is an alternative method for separating non-volatile substances. It uses an adsorbent-coated sheet and a solvent to carry the sample. TLC is durable, quick, and cost-effective, making it a valuable separation method, especially for limited samples. This paper describes a simple method based on TLC to separate active components in cough-cold medicines.

3. METHODOLOGY

All samples were purchased from local pharmacies in Malaysia. Table 1 shows the list of samples. Two tablets from each sample were pulverized and dissolved in a 4 mL mixture of 2:2 v/v chloroform and methanol. After a few minutes, the test tube contents were filtered. The supernatant was transferred to another clean test tube for TLC.

Each drug sample was dissolved in ten different solvent systems. Table 2 shows the solvent system that was employed in this study. TLC plates measuring 20 cm x 20 cm applied with a uniform silica gel G were activated in an oven at 110°C for 30 minutes. To apply the sample, a fine capillary tube was used by placing them 1 cm above the plate's base. The spots were air-dried for 1 minute at room temperature. Different solvent solutions were used to saturate the solvent chamber. Then, the plates were placed at a 45-degree angle in a chamber filled with saturated solvent, and covered with a lid. The solvent front migrated to a height between 5.5 and 7.5 cm from the origin. After the process, the plates were removed and air-dried at room temperature. The separated spots were visualized using iodine fuming before calculating the hR_f values.

Table 6 Description of samples

Sample number	Sample name	Code	Form	Composition	Standard present	Manufacturer
1	Dextromethorphan hydrobromide	S1	Powder	Dextromethorphan hydrobromide	-	Pharmaniaga Dextromthorphan Bhd
2	Chlorpheniramine maleate	S2	Powder	Chlorpheniramine maleate	-	Kotra Pharma
3	Phenylephrine	S3	Powder	Phenylephrine	-	Alcon
4	Paracetamol	S4	Powder	Paracetamol	-	Pharmaniaga Manufacturing Bhd
5	Horamine	HOR	Tablet	Chlorpheniramine maleate-4mg	S2	HOVID Bhd
6	Uphamol	UPH	Tablet	Paracetamol-500mg	S4	Upha Pharmaceutical Mfg. (M) Sdn Bhd
7	Febricol-RX	FEB	Tablet	Paracetamol-500mg; Phenylephrine HCl-5mg; Chlorpheniramine Maleate-2mg	S2, S3, S4	Xepa Soul Pattinson (M) Sdn Bhd
8	Tussidex forte	TUS	Liquid	Dextromethorphan hydrobromide-15mg	S1	Xepa Soul Pattinson (M) Sdn Bhd
9	Antamin	ANT	Liquid	Chlorpheniramine maleate-4mg	S2	Xepa Soul Pattinson (M) Sdn Bhd

Table 2 Solvent systems for TLC analysis

Code	Solvent system	Ratio	Code	Solvent system	Ratio
A	Methanol:Ammonia	100:1.5	F	Benzene:Ethanol	60:40
B	Chloroform:Methanol	90:10	G	Cyclohexane:Acetone:Chloroform	60:30:20
C	Ethyl acetate:Methanol:Ammonia	85:10:5	H	Dichloroethane:Methanol:Water	95:5:0.2
D	Isopropyl alcohol:Chloroform:Ammonia	45:45:10	I	Cyclohexane:Toluene	50:50
E	Chloroform:Acetone	80:20	J	Acetone:Methanol	60:40

4. RESULTS AND DISCUSSION

The TLC plates were placed in ten different solvent systems. The spots were marked after visualization under ultraviolet radiation and iodine fuming. The hR_f values were then compared with their respective standards to validate their presence as tabulated in Table 3. The FEB sample contained S2, S3, and S4 in its drug composition, and it was expected to have three spots on the plate, but only one showed up on each plate. The similarity of the FEB sample with either S2, S3, or S4 was reflected in the hR_f values. The UV viewing of the sample in solvent system A had a similar hR_f value of 88 to the S2, S3, and S4 standards, creating difficulty in identifying which standard appeared on the sample's plate. The same similarity occurred in systems E and G, where the hR_f values for S2, S3 and S4 matched that of the sample. In system H, all of them had identical hR_f values of 88 when viewed under the UV lamp. In system J, the sample and S4 had similar hR_f values of 83.

The hR_f values may not be identical across all systems, but they must be similar for the comparison to be successful.

Table 3 TLC analysis of selected OTC cough-cold medicines using different solvent systems

Solvent system codes	Visualization techniques	Standard samples (hR_f)				Pharmaceutical samples (hR_f)				
		S1	S2	S3	S4	FEB	UPH	HOR	ANT	TUS
A	UV	96	87,93	65,87	87	88	77	33	87	98
	Iodine	96	-	65	87	88	77	-	87	98
B	UV	57	30,49	47	36	5	25	62	35	36
	Iodine	-	-	47	36	5	25	-	35	36
C	UV	-	-	-	42	68	62	70	93	93
	Iodine	-	-	-	42	68	62	-	93	93
D	UV	90	-	-	56	80	72	-	86	88
	Iodine	-	-	-	-	80	72	-	86	88
E	UV	-	18,21	-	25	13	15	16,45,60	70	88
	Iodine	-	14,18,21	16	25	13	15	16,45,60	70	88
F	UV	-	-	16	5	-	-	-	-	-
	Iodine	-	-	89	5	-	-	-	-	-
G	UV	45	-	52,55	10	5	9	-	46,55	41,46
	Iodine	45	-	52,53,55	10	5	9	7,24	46,55	41,46
H	UV	90	79,88	-	88	88	82	96	87	91
	Iodine	-	-	79,84	77	88	82	-	87	91
I	UV	27	-	-	5	-	-	-	-	-
	Iodine	-	-	-	5	-	-	-	-	-
J	UV	80	78	-	78,83	83	76	93	55,78	84
	Iodine	-	-	-	78	83	76	93,96	55,78	84

(-) Not detected

Sample UPH had S4 in its composition when viewed under both UV and iodine fuming. The similarity was evident in solvent system A, where the standard and sample had hR_f values of 87 and 77, respectively. The hR_f values were also close in system G, with hR_f 10 for the sample and hR_f 9 for S4, in system H with hR_f 88 for the sample and hR_f 82 for S4, and in system J with hR_f 78 for the sample and hR_f 76 for S4. However, there was a significant difference in hR_f values in system E, with the sample having a value of 15 and the standard at 25. In other solvent systems, the differences in hR_f values were too distant to establish a correlation.

The HOR sample contains S2 as a standard in its pharmaceutical preparation. When comparing the sample with S2 under different conditions, it was observed that under ultraviolet radiation with solvent system E, S2 had a value of 18, while HOR had a value of 16. Similar comparisons were made using solvent system H, where the sample had a value of 96 while S2 had 88 in hR_f value. Unfortunately, the HOR sample did not show many spots under both UV radiation and the iodine fuming method, even though other solvent systems were tried. This suggests that the HOR sample may not be suitable for the visualization techniques, or the composition of the product is complex and may require other methods, such as HPLC. Furthermore, with only one apparent standard present in the sample's composition, multiple spots were visible on the sample plate. This suggests that other ingredients could be analyzed using the same technique. However, despite differences in hR_f values in various systems, there was a significant dissimilarity between the sample and the standard, making the spots of no use.

The ANT sample also had the same standard, S2, in its composition as the HOR sample. Interestingly, only plates in solvent system F did not have detectable spots, and there were only five spots found like the standard when viewed under ultraviolet radiation. The hR_f values for both the standard and the sample were the same in systems A and J, but they varied slightly in system B. in systems D and G, the spots were more like S1, which suggests the presence of S1 in the ANT sample, although it is not claimed by the manufacturer.

For the TUS sample, it had a standard component called S1 in its composition. Under both UV radiation and iodine fumes, most of the samples had similar or identical hRf values in various solvent systems. The hR_f values for TUS and S1 were quite close in systems A, D, G, H, and J, indicating similarities between the sample and the standard.
The spots showed closer hR_f values when viewed under UV radiation than with iodine fuming. This suggests that some samples might not react well with iodine fumes, leading to significant differences in hR_f values compared to the standards. However, the retention factor of the samples was generally distinguishable from their standards. In some solvent systems, there were discrepancies in hR_f values, possibly due to experimental errors.

5. CONCLUSION

The study had two main goals; first, to find the best way to see the active ingredients in each sample, and second, to confirm the presence of these components by comparing their retention factors (hR_f) values) with known standards. For the first goal, different techniques were tested to visualize the spots on silica gel-G plates after TLC analysis. Ultraviolet radiation worked best, followed by iodine fuming. The second goal, comparing hR_f values with standards. Samples FEB, UPH, ANT, and TUS matched their standards in some cases, but not consistently in all solvent systems. Moreover, the study aimed to find the best solvent system for separating components in its pharmaceutical preparations. Ultraviolet radiation worked best with solvent system A (methanol:ammonia in a ratio of 100:1.5). Iodine fuming worked best with solvent system G (cyclohexane:acetone:chloroform in a ratio of 60:30:20), which provided the best separation and differentiation for cough-cold samples.

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