# RESPONSE SURFACE DESIGN FOR ULTRASONIC ASSISTED EXTRACTION PROCESS FOR *CALOTROPIS PROCERA* SEEDS AND EVALUATION OF ITS ANTIOXIDANT ACTIVITY

BY

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# ABSTRACT

*Calotropis Procera* plant is a shrub commonly known as Sodom apple. It belongs to the Asclepiadaceae family (milkweed). *C. procera* is an original plant of North Africa and is widely dispersed in Asia. *C. procera* seed contains oil, protein, minerals, and soluble dietary fibers, and there are no reports on antioxidant concentration in the seeds. This study focuses on the development of the extraction conditions, namely, ultra-sonication power (percent amplitude), extraction time (minutes), and solvent to sample ratio (ml/g) to enhance antioxidant activity from *C. procera* seed. The defatting time screening was conducted to reduce processing time and energy. The best oil extraction time was one (1) hour using n-hexane (10 ml: 1 g), shaker (200 rpm) at room temperature. Screening of solvents was performed using chloroform, isopropanol, methanol, and water to maximize antioxidant activity using DPPH (1, 1-diphenyl-2-picrylhydrazyle) assay. The isopropanol extract gave the highest percentage inhibition in the DPPH assay, 57.731± 0.377 %, so it was used for optimization of the ultra-sonication-assisted extraction process. For the design of the optimization experiments, the response surface methodology (RSM) was adopted. With DPPH content as a response, three independent variables - ultra-sonication extraction time (10, 15, 20 minutes), solvent to sample ratio (10, 15, 20 ml/g), and ultra-sonication power (20, 60, 100 percentage amplitude), were optimized. The highest inhibition percentage of DPPH from *C. procera* seed extract was 68.81%, obtained at 20% amplitude of ultra-sonication, 10 minutes of ultra-sonication time, and 10 ml solvent per gram sample. The *C. procera* seed extracts were further investigated for their potential applications using *in-vitro* antioxidant assays like; total phenolic content (TPC) and ascorbic acid equivalent antioxidant potential (AEAC). The phenolic content of the seed extract was 26.324 mg of Gallic acid equivalent/g dry material. The AEAC antioxidant of *C. procera* seed extract was 28.707 mg of (Ascorbic Acid equivalent) AAeq/100 g. Gas chromatography mass spectrometry (GC-MS) was used to obtain a profile of the extracts and to identify potential bioactive compounds in the extracts. The GC-MS analysis indicated that β, β-carotene (21.94%), pentacontanoic acid propyl ester (14.93%), (E, E)-5-chloro-4-methyl-2,4-heptadiene (12.411%), (E)-2-nonenal (6.7174%), n-decanoic acid (6.1985%), pentanoic acid (5.9932%), (E)-2-octenal (1.6965%) and 1-methyl-4-nitroimidazole (1.3226%) were the major constituents of the *C. procera* seed extract. The antibacterial, antioxidant properties, anticancer, cardio-protective agents, skin protection from UV radiation, of phenolic compounds are well-studied. As such, this high phenolic content extract of *C. procera* seeds can be a promising source of alternative pharmaceutical products derived from natural sources to treat chronic diseases, decreasing future reliance on synthetic drugs.

# خلاصة البحث

نبات العشر أو (العشار) *Calotropis procera* هو شجيرة معروفة باسم تفاح سدوم. ينتمي إلى عائلة *Asclepiadaceae* (الصقلاباوات). يعتبر *C. procera* نباتًا أصليًا في شمال إفريقية وهو منتشر على نطاق واسع في آسيا. تحتوي بذور *C. procera* على زيت وبروتين ومعادن وألياف غذائية قابلة للذوبان، ولا توجد تقارير عن تركيز مضادات الأكسدة في البذور. تركز هذه الدراسة على تطوير ظروف الاستخراج، وهي قوة الموجات فوق الصوتية (السعة المئوية)، ووقت الاستخراج (بالدقائق)، ونسبة المذيبات إلى العينة (مل/جم) لتعزيز نشاط مضادات الأكسدة من بذور *C. procera*. تم إجراء فحص لوقت إزالة الدهن بغرض تقليل وقت المعالجة والطاقة. أفضل وقت لاستخلاص الزيت كان ساعة (1) باستخدام n-hexane ( مل 10: 1 جم)، shaker (200 دورة في الدقيقة) في درجة حرارة الغرفة. تم إجراء فحص المذيبات باستخدام الكلوروفورم والأيزوبروبانول والميثانول والماء لتحسين نشاط مضادات الأكسدة باستخدام إختبار (DPPH (1, 1-diphenyl-2-picrylhydrazyle. أعطى مستخلص الأيزوبروبانول أعلى نسبة تثبيط في اختبار DPPH، 57.731± 0.377 %. لذلك تم استخدامه لتحسين عملية الاستخلاص بمساعدة الموجات فوق الصوتية. لتصميم تجارب التحسين، تم اعتماد منهجية سطح الاستجابة (RSM) مع محتوى DPPH كاستجابة، هناك ثلاثة متغيرات مستقلة تم تحسينها، وقت الاستخلاص فوق الصوتي (10، 15، 20 دقيقة)، نسبة المذيبات إلى العينة (10، 15، 20 مل/جم)، وقوة الموجات فوق الصوتية (20، 60، السعة المئوية 100). أعلى نسبة تثبيط لـ DPPH من مستخلص بذور *C. procera* كانت 68.81٪، تم الحصول عليها عند 20٪ سعة فوق صوتية، 10 دقائق من وقت فوق صوتية، و 10 مل مذيب لكل جرام عينة. تم فحص مستخلصات بذور *C. procera* لتطبيقاتها المحتملة باستخدام إختبارات مضادات الأكسدة في المختبر مثل؛ إجمالي المحتوى الفينولي (TPC) وإمكانات مضادات الأكسدة المكافئة لحمض الأسكوربيك (AEAC) . كان المحتوى الفينولي لمستخلص البذور 26.324 مجم مكافئ حمض جاليك/جرام مادة جافة. كان مضاد الأكسدة AEAC

لمستخلص بذور *C. procera* 28.707 مجم من (مكافئ حمض الأسكوربيك) AAeq /100 جم. تم استخدام مقياس الطيف الكتلي اللوني للغاز ($^{GC-MS}$) للحصول على ملف تعريف للمستخلصات ولتحديد المركبات النشطة بيولوجيًا المحتملة في المستخلصات. أشار تحليل $^{GC-MS}$ إلى أن (21.94%) β, β-Carotene، واستر Pentacontanoic acid propyl (14.93%)،(12.411%) (E, E)-5-Chloro-4-methyl-2,4-heptadiene)، (E)-2-Nonenal (6.7174%)، Pentanoic acid (5.9932%)، (E)-2-Octenal (1.6965%) و 1-Methyl-4_nitroimidazole (1.3226%) كانت المكونات الرئيسية لمستخلص بذور *C. procera* تمت دراسة الخصائص المضادة للبكتيريا، ومضادات الأكسدة، ومضادات السرطان، وعوامل حماية القلب، وحماية الجلد من الأشعة فوق البنفسجية، والمركبات الفينولية. على هذا النحو، يمكن أن يكون هذا المستخلص عالي المحتوى الفينولي لبذور *C. procera* مصدرًا واعدًا للمنتجات الصيدلانية البديلة المشتقة من مصادر طبيعية لعلاج الأمراض المزمنة، مما يقلل الاعتماد على الأدوية الاصطناعية في المستقبل.

# APPROVAL PAGE

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# DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| *C.procera* | *Calotropis Procera* |
| ROS | Reactive Oxygen Species |
| UAE | Ultrasonic-Assisted Extract |
| S.A. | Synthetic Antioxidant |
| BHU | Butylated Hydroxyanisole |
| BHT | Butylated Hydroxytoluene |
| TPC | Total Phenolic Content |
| DPPH | 2, 2-Diphenyl-1-Picrylhydrazyl |
| GCMS | Gaschromatography Mass Spectrophotometry |
| UV | Ultra Violet |
| $O_{2^-}$ | Superoxide Anions |
| $OH^{\cdot}$ | Hydroxyle Radical |
| $H_2O_2$ | Peroxyle Radical |
| DNA | Deoxyriboneucleic Acid |
| SFE | Supercritical Fluid Extraction |
| MAE | Microwave-Assisted Extraction |
| NMR | Nuclear Magnetic Resonance Spectroscopic |
| FTIR | Fourier Transform Infrared |
| MS | Mass Spectrophotometry |
| HPLC | High Performance Liquid Chromatography |
| NIST | National Institute of Standard and Technology |
| LCMS | Liquid Chromatography Mass Spectrometry |
| AR | Analytical Reagent |
| RSM | Responce Surface Methodology |
| FCCD | Face-centred Composite Design |
| ANOVA | Analysis of Variation |
| AEAC | Ascorbic Acid Equivalent Antioxidant Potential |
| CCD | Central Composit Design |
| CV | Coefficient of Variation |
| 3D | Three Dimintional |
| 2D | Two Dimentional |
| SE | Standard Error |
| Ppm | Part Per Milion |

# LIST OF SYMBOLS

| | |
|---|---|
| ℃ | Celsius Symbol |
| g | Gram |
| µ | Micro |
| % | Percentage |
| x | The Value of Unknown Concentration |
| y | The Absorbance |
| Δ | Delta |
| $R^2$ | Coefficient of Determination |
| *Y* | Predicted Response |

# CHAPTER ONE

# INTRODUCTION

## 1.1 BACKGROUND OF THE STUDY

Medicinal plants represent a magnificent source of economic value, and the extraction and characterization of bioactive compounds from medicinal herbs contribute to the discovery of new pharmaceuticals with high therapeutic value. Plant extracts with bioactive properties such as antioxidant, antimicrobial, or antiviral activity are considered superior to synthetic medicinal compounds (Li, Yang, Yang, & Zu, 2018). Similarly, the consumption of natural sources of antioxidants are a trend in alternative medicine and health. The global market for antioxidants, which is mainly driven by the food, cosmetics, and pharmaceutical demands, is expected to be $6.4 billion in 2022 (BCC Research, 2017).

Antioxidants are substances that protect cells of organisms from oxidation caused by free radicals produced by chemical reactions and chain reactions. Antioxidants are essential in food for many reasons, including protection of the human intestines because they are easily absorbed by the body. Using antioxidants can protect the human body from chronic ailments, such as cancer, aging, and diabetes, which are due to continuous exposure to free radicals from outside sources, including sunlight, radiation, and pollution (Wipatanawin, Phongsawanit, Maneeratprasert, Lertsiri, & Deetae, 2015). In addition, they protect the food from deterioration and therefore guarantee a higher quality of nutrition. For example, the oxidation of lipid and protein lead to degraded food quality by generating off-tastes and degraded colors and nutrient losses (Hellwig, 2019; Vieira, Zhang, & Decker, 2017).

Antioxidant may be classified into two types, i.e. natural and synthetic antioxidant. Natural antioxidants are safer and have better antioxidant activity, while the consumption of synthetic antioxidants may have dangerous side effects (Asif, 2015). Though natural antioxidants are harmless to health and have superior antioxidant activity, their application is still deficient (Asif, 2015). To develop the applications, it is necessary to screen an adequate number of plant sources for their antioxidant potential. In this study, the seeds of *Calotropis procera*, also known as Sodom apple,

were selected as a potential source of antioxidants upon analysis of the related literature. *C. procera*, belonging to the Asclepiadaceae family of milkweeds is a widespread plant that is known for its many therapeutic characteristics and usage to treat a variety of ailments in traditional medicine. Studies have shown that *Calotropis procera* contains active biological compounds, such as phenols, terpenoids, and flavonoids which usually have antioxidant properties. It grows easily and wildly, and *C. procera* plant parts are generally not consumed as food or fodder. Therefore, it will provide a natural, cheap, and sustainable source of bioactive therapeutics once its bioactive compounds are extracted and thoroughly characterized. The bioactive compounds may thus be used as natural therapeutics from a plant extract or in their pure form (Wipatanawin et al., 2015).

Simultaneously, advanced developments have been made in the extraction processes itself which is essential for the study of phytochemicals. The extraction represents the initial step in medicinal plant research, and it has a large impact on the ultimate yield and quality of the product. There are some traditional methods of extraction as well as some modern methods of extraction. Maceration, percolation, and soxhlet extraction approaches are conventional extraction method. While, ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE), and microwave-assisted extraction (MAE), are some examples of unconventional extraction approaches. Ultrasonic-Assisted Extraction (UAE) as a novel method, offers a simple, rapid and effective extraction technique compared to traditional extraction methods (Chen et al., 2012), which have been used to extract natural antioxidants from various medicinal plants. Due to advantages such as efficacy, short extraction time, ease, a substantial reduction in solvent ratio, low temperatures, and low energy consumption (Fan et al., 2012; Yan et al., 2011), ultrasound has been used in several specific sectors, such as the food industry, the chemical industry, and the materials industry (Leonelli & Mason, 2010).

Natural antioxidant extraction from plants should be increased to provide a safe, viable, and sustainable source of natural therapeutics. In order to do so, the extraction process must be optimized for maximum antioxidant activity. This can be achieved by the use of statistical and mathematical design and analysis of experiments, using response surface methodology (RSM), for example.

## 1.2 PROBLEM STATEMENT AND ITS SIGNIFICANCE

The health challenges associated with a synthetic antioxidant (S.A.) are increasing annually across the globe. According to toxicologists and nutritionists, Ames (1983) and Baardseth (1989) have confirmed that several synthetic antioxidants such as butylated hydroxyanisole (BHU) and butylated hydroxytoluene (BHT) are of concern. For example, such products can have carcinogenic effects on living organisms ( as cited in Tepe et al., 2005). Harmful residual results from S.A. are generally detrimental to human cells and health (Caleja, Barros, Antonio, Oliveira, & Ferreira, 2017). Synthetic antioxidants and natural antioxidants have comparable efficiencies in their extraction and characterization when appropriate processes are followed. There is a need to identify potent, natural antioxidants and extract them.

Similarly, there is a lack of strategic research activities to determine the optimum methods of extraction of the antioxidants from *Calotropis procera* seeds. Various conventional techniques for extracting antioxidants from plant materials and other foodstuffs have been reported, such as agitation by shaking or stirring, homogenization at high speeds, and maceration. However, a comprehensive experimental comparison of these techniques has not been reported for the extraction efficiency of antioxidants compounds. In addition, some studies have highlighted the drawbacks, citing low product quality, safety hazards, and a prolonged extraction time (Jannatul Azmir et al., 2013). Nowadays, novel extraction techniques such as ultrasonic-assisted and microwave-assisted extraction techniques have also been developed to extract antioxidants from plant materials, and they have been used to overcome some of the shortcomings of conventional extraction methods (Ara & Sarah, 2017). The current extraction methods for *C. procera* are conventional, and they fail to consider efficiency in terms of yield, antioxidant activity, extraction time, and costs from *C. procera*. It is necessary to determine a more efficient extraction technique and optimize it for maximum antioxidant activity. Furthermore, available literature on *C. procera* seeds lack characterization of the crude extract from *C. procera*, and the antioxidant activity of *C. procera* seeds has not been studied.

Defatting seeds for the antioxidant study is important because seeds are rich in fats and oils, which interferes with the optical density readings and may produce a false result. Furthermore, the antioxidant assays for the defatted extract were higher than those of the undefatted ones. Thus, the removal of fat could facilitate the antioxidants

extraction (Bravo, Monente, Juániz, Peña, & Cid, 2013). Therefore, this study has embarked on an investigation of the extraction and characterization of the antioxidant activities of *C. procera* to contribute to the fulfillment of globally increasing demand for natural antioxidants with low cost and solve the health problems associated with synthetic antioxidants.

## 1.3 RESEARCH OBJECTIVES

The overall objective of this research is to study the antioxidant properties of *C. procera* seed extract:

1. To screen for optimal defatting time for oil removal using hexane and shaker, and then screen the solvents for the ultrasonic-assisted extraction to obtain the *C. procera* seeds extracts with the highest antioxidant level.
2. To optimize the extraction parameters namely, extraction time, solvent to sample ratio, and ultrasonication power to enhance the antioxidant properties.
3. To identify the compounds responsible for the antioxidant activity in *C. procera* seed crude extract by gas chromatography-mass spectrometry (GCMS).

## 1.4 RESEARCH SCOPE

The scope of this research includes the defatting process to remove lipids from the *C. procera* seeds. Firstly, screening was done to determine optimal defatting time. Next, the research also involved the screening of solvents of various polarities using chloroform, distilled water, isopropanol, and methanol to extract the antioxidant compounds from the *C. procera* seeds. The ultrasonic-assisted extraction parameters (extraction time, solvent to sample ratio, and ultrasonication power) were optimized to maximize the antioxidant activity. The antioxidant activities of the extracts were determined using two antioxidant assays i.e. total phenolic content (TPC) using the Folin-Ciocalteu method, and ascorbic acid equivalent antioxidant potential (AEAC). Finally, the crude extract was characterized and the active compounds were identified using gas chromatography-mass spectrometry (GCMS).

## 1.5 DISSERTATION OUTLINE

This research investigates the antioxidant activity on the *C. procera* seed after optimization of the ultrasonic-assisted extraction. The thesis content is organized into five chapters; each chapter begins with an introduction section and ends with a summary. Chapter One explains the background of the study and highlights the problem statement, research objectives, and research scope. Chapter Two examines the available literature on *C. procera*, phytochemical analysis methods related to the antioxidant activity, the ultrasonic assisted extraction technique, and gas chromatography-mass spectrophotometry. Chapter Three illustrates the method of screening for the defatting time and solvent screening to obtain the *C. procera* seed extracts with the highest antioxidant level. In addition to that, it details the optimization of the parameters of extraction time, solvent to sample ratio, and ultrasonication power, in the ultrasonic-assisted extraction. Chapter Three also includes the methodology for the gas chromatograpy-mass spectrometry for profiling the antioxidant compounds in *C. procera* seeds extract and identifying the potential antioxidant compounds. Chapter Four presents and discusses the results of the experiments conducted in this research. Finally, chapter five concludes on the findings of this research, and presents some recommendations for future work.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 INTRODUCTION

This chapter focuses on various researches and studies that have been conducted on the *C. procera* plant and its phytochemical analysis, chemistry, and medicinal uses of its various parts and demands new antioxidants derived from *C. procera* seed. In addition, it discusses chemical profiling gas chromatography-mass spectrometry (GC-MS). It also discussed extraction methods like conventional and unconventional methods, their advantages and disadvantages—especially the modern method of extraction ultrasonic-assisted extraction method. Moreover, it discusses optimization by response surface methodology (RSM).

## 2.2 CALOTROPIS PROCERA PLANT

*Calotropis procera* is a weed plant commonly known as Sodom apple. The plant belongs to the Asclepiadaceae family, which includes latex bearing plants. *C. procera* is known for its various medicinal properties in the traditional medicinal system and uses to cure various diseases. The Asclepiadaceae family includes about 2,000 species divided into 280 genera found throughout the tropical and subtropical continents (Ara & Sarah, 2017). Asclepiadaceae's species are spread in warm, tropical climates up to about 1050 meters above sea level. As a member of the Asclepiadaceae family, *Calotropis procera* has an excellent value as a medicinal plant of prominence in folk medicine (Boulos, 1999).

The plant is known in Arabic as oshar, usher, debaj or kisher; Some English names for this plant are Sodom apple, giant milkweed, calotrope, rubber tree, calotropis, rubber bush, dead Sea fruit, mudar fiber, desert wick, swallow-wort.; and in Hindi as madar, akada, akdo, aak (Gupta, Gupta, Kapoor, & Sharma, 2012).

### 2.2.1 Taxonomical Study of Calotropis Species

Systematic classification of *Calotropis procera* was done by three taxonomists Bentham Hooker, Engler Prantl, and Hutchinson (R. K. Verma & Yadav, 2017). (Table 2.1) below shows the taxonomy of *Calotropis procera*. It belongs to the family of Asclepiadaceae.

Table 2.1 Systematic Classification of Calotropis procera

| **Classification** | **Bentham and Hooker** | **Engler and Prantl** | **Hutchinson** |
|---|---|---|---|
| **Kingdom** | Plantae | Plantae | Plantae |
| **Class** | Dicotyledones | Dicotyledones | Dicotyledones |
| **Division** | Gamopetalae | Sympetalae | Lignosae |
| **Order** | Gentianales | Asclepiadaceae | Asclepiadaceae |
| **Family** | Asclepiadaceae | Asclepiadaceae | Asclepiadaceae |
| **Genus** | Calotropis | Calotropis | Calotropis |
| **Species** | Procera | Procera | procera |

Source: (Rahman & Wilcock, 1991)

### 2.2.2 Origin of Calotropis Procera

It is an original plant of North Africa, and it is wildly-dispersed throughout Sudan, but mainly in central Sudan. West Africa, such as Angola, is one of the habitats of *C. procera*. Moreover, it is widely distributed in Asia: Saudi Arabia, Yemen, United Arab Emirates, Oman, Iraq, Kuwait, Palestine, Iran, Afghanistan, India, Pakistan, Nepal, Vietnam, Indochina and Malaysia (Rahman & Wilcock, 1991; Ramos et al., 2006; S. Verma, 2016).

### 2.2.3 Morphology of Calotropis Procera

*Calotropis procera* is a perennial shrub or small tree that grows with a height of 5.4m and rigidly upright high with gigantic and plentiful branched. The plant's whole parts have milky latex (Sharma, Kharb, & Kaur, 2011). The fruit has many brown seeds, which are light, flattened with big white silky hair named (pappus) at the top of the seeds to spread the plant (Mariod & Mirghani, 2017) see (figure 2.1).

Figure 2.1 *C. procera* Plant, Fruits, and Seeds
Source: (Mariod & Mirghani, 2017)

#### *2.2.3.1 Root*

*C. procera* with little to no near-surface lateral roots has an extensive, robust taproot. The roots have little branches in Indian sandy desert soil and attain depths of 1.7 to 3.0 m. (R. K. Verma & Yadav, 2017).

#### *2.2.3.2 Bark & Branches*

The bark is dense, coarse, and corky and the color is yellow-brown; the branches are green and fleshy, and tomentum may cover them (white fur-like hair). It is also herbaceous with a woody, aerial, upright, cylindrical, branched, stable, milky latex lower section. (R. K. Verma & Yadav, 2017).

#### *2.2.3.3 Leaves*

The leaves of *C. procera* are opposite-decussate, flat, and ovate, with 4-6 pairs of sub-opposite nerves prominent on the abaxial layer, acute apex, and sessile (almost decurrent) foundation, pale green and quite broad, about 30x25 cm long. The leaves are slightly thick, fleshy and coriaceous. They are 10 to15 cm long and 4.5 to 6.5 cm in breadth. They are described as comprehensive, broadly cuneate, obovate or oblong, slightly cordate, and auricle at the base with short, simple hair on the upper side near the petiole's attachment. *C. procera* fragile leaves are covered with dark ashy pubescence. It is possible to characterize the mature leaves as almost smooth or even glabrous and pale green (Sharma et al., 2011).

#### *2.2.3.4 Inflorescences and Flowers*

Inflorescences are produced from the leaves' base in the cymes of 3-20 cm (c.7 cm) Verma & Yadav, 2017). The flowers consist of 5 thin, triangular, dirty white sepals, five (5) large, ovate petals (c1 cm x 1 cm) which are white at the base and purple at the tips, and five (5) purple stamens that surround a white five (5) lobed stigma (Verma, 2016).

#### *2.2.3.5 Fruits*

The *C. procera* fruits are green, oval-shaped mushy fruits (follicular) up to 15 cm in length and 10 cm in diameter. To release plumed, papery, light brown seeds with white filaments up to 6 cm long on one side see (Figure 2.2), they are partitioned in half (Al-Taweel et al., 2017).

Figure 2.2 C. procera Seed Silky Hair (Pappus) and Seeds

### 2.2.4 Phytochemical Analysis

Phytochemicals are naturally occurring substances found in plants that provide health benefits. Alkaloids, flavonoids, tannins, phenols, saponins, hormones, glycosides, terpenes. They shield plants from infection and add to the color, fragrance, and taste of plants. Therefore, they have a role to play in conserving human health because their food consumption is essential. Dietary phytochemicals are present in berries, vegetable legumes, whole grains, nuts, seeds, mushrooms, herbs, and spices (Silva & Abeysundara, 2017). These have antioxidant, anti-inflammatory, anticancer, and antibacterial effects.

#### *2.2.4.1 Terpenoids*

Terpenes belong to the leading group of secondary metabolites, consisting of five-carbon isoprene units attached by thousands of means to each other (many isoprene units). Terpenes are pure hydrocarbons. A grouping of terpenes forms terpenoids with different functional groups and oxidized methyl groups are transferred or added to different positions. terpenoids are classified into monoterpenes, sesquiterpenes, diterpenes, sisterpenes, and triterpenes, based on their carbon units. Many terpenoids with differences in their composition are biologically active and are used globally to treat many diseases, for example, eugenol, terpinen-4-ol, thujone, and camphor (Shagufta, 2018). It has been reported that *C. procera* latex has terpenes, namely, β-Amyrin, germanicyl, and α-Amyrin (Farooq, Nisar, Merzaia, & Azeem, 2017). According to the standard protocol of phytochemical analysis, the salkowski test is used for terpenoids detection (Ramachandran et al., 2019).