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Biological Activity of SomeAlkaloid Compounds Isolated from *Conocarpus lancifolius* and *Calotropis procera* Plants

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بسم الله الرحمن الرحيم

(وفوق كل ذي علم عليم)

صدق الله العلي العظيم

سورة يوسف

الاية 76

Dedication To

The greatest Messenger of humanity, Mohammad

(peace to be upon him and his family)

The spring of kindness, love and support

My precious parents

And

My lovely husband.

My sons . . . Zaynab, Mustaffa and Abbas

Muna

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Muna

Summary

The present study revealed to extraction and purification of alkaloid compounds from two local plants, *Conocarpus lancifolius* Engl. and *Calotropis procera* (Aiton)R.Br. in Basrah governorate, souther of Iraq, tested their antibacterial activity against some gram positive and gram negative bacteria and tested their antioxidant, and anticancer activities against two important cell line cultures .

This study involved two different plant species: *C. lancifolius*, which belonged to the Combretaceae family, and the second *C. procera*, which belonged to the Asclepiadaceae family.

Both species were collected from different regions in Basrah governorate. Detection of Alkaloid compounds for each part of the two studied plants using two biochemical reagents, Dragendorff and Mayer reagent, which revealed the presence of alkaloid compounds in the leave of *C. lancifolius* only, whereas in *C. procera* was founded in leaf and root. Two extraction methods for alkaloid compounds were conducted that are; cold extraction (maceration) and hot extraction by using three different solvents (Methanol, Water, and Dichloromethane), which confirmed the efficiency of hot extraction to a cold one in alkaloids yield extraction for both two solvents methanol and water also,

percentage of yield alkaloid extracts appeared that Methanol solvent was superior to two other solvents (water and dichloromethane) in both plants with significant differences at a value reached 1.677 g\100g DW, in compared with water and dichloromethane values that reached 0.619 g\100g DWand 0.089g\100g DW) respectively.

GC-Mass qualitative analysis was done for alkaloid extract founded in both plant parts that revealed the presence of one alkaloid compound Methoxy Phenyl Oxime (MPO) ($C_8H_9NO_2$) in leaf alkaloid extract of *C.lancifolius* at RT=5.2min with M Wt =551.1 g/mol and area percentage 42.02% and one alkaloid compounds Colchicine (CO) ($C_{22}H_{25}NO_6$) in leaf alkaloid extract of *C.procera* at RT=12.8 min with M wt =399 g/mol and area percentage of about 7.1%. In contrast, root crude root alkaloid extract (CR) of *C.procera* was revealed of two types of alkaloid compounds that are 1H-Indol,2,3-dihydro-4-methyl atRT=21.9 with MWt =133.39g/mol and area percentage of about 42.42% and 1,3, benzene dicarboxylic acid,5-dimethylamino($C_{10}H_{11}NO_4$) at RT=23.7 with M Wt 209.2 and area ratio of 45%. Determination and purification of two alkaloid compounds MPO and CO were performed by the analytical High-Performance Liquid Chromatography (HPLC) method with some modifications.

These results were confirmed by Thin Layer Chromatography TLC, Fourier-Transform Infrared Spectroscopy FT-IR and Nuclear magnetic resonance ^{1}HNMR techniques.

The biological activity of the two purified MPO, CO, and root crude alkaloid compounds CR involved in vitro antibacterial, anticancer and antioxidant activity where the finding of antibacterial activity which tested against four different types of Gram-positive bacteria *Bacillus subtilis*, *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli* and *Klebsiella pneumoniae* by using three concentrations (50,150,200µg/ml) showed The significant value of antibacterial activity was for MPO compound against *B.subtilis* reached 22,66mm (at 200µg/ml) while the

lowest value was for CO compound against *K. Pneumoniae* reached 1.6mm at (50µg/ml).

The results of *in vitro* anticancer activity conducted on both MCF-7 and SKG-T4 cell lines revealed remarkable cytotoxicity of CO compound against MCF-7cell line with a value of IC_{50} reaching 55.33µg/ml compared with MPO and CR alkaloid compounds that have no cytotoxicity on that cell line. In contrast, MPO showed significant selective cytotoxicity against SKG-T4 of a value of IC_{50} reached 7.58µg/ml compared with CO compound that has cytotoxicity with a value of IC_{50} reached 522µg/ml and also there was no cytotoxicity of CR compounds on this cell line too. The finding of Antioxidant activity for Vit C (positive control), CR, MPO, and CO alkaloid compounds by using DPPH scavenging activity that arranged as Vit C> CR> MPO> CO with significant differences at all concentrations .

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List of Abbreviation

DMSO	Dimethylsulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
EDTA	Ethylene diamine tetraacetic acid
GC-MASS	Gas chromatography-mass spectroscopy
HBL	Human Blood Lymphocytes
HPLC	High-pressure liquid chromatography
IC_{50}	Half-maximal inhibitory concentration
M.P	Melting point
MCF-7	Michigan Cancer Foundation-7(breast cancer cell line)
MIC	Minimum Inhibition Concentration
Min	Minutes
MTT	(3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide
p.s.i	pounds per square inch

Rf	Retention factor: mobility relative to the solvent front
RT	Retention Time
SPP	Species
TLC	Thin layer chromatography

Chapter One

Introduction & Aim of the study

1. Introduction

Herbal medicine is an increasing area of health care that need attention for a medicinal plant that shares in maintaining human health and beneficent the quality of human life for thousands of years (AL-Mudhafar,.2009 and Khalil *et al*.,2020).

Medicinal plants and their derived compounds (Phytochemical) have been considered pharmacological significance since ancient times. The use of plants in medicine dates back to 60,000 years ago, before the birth of civilization. (Shen, 2015; Aldoghachi *et al*., 2021). As a result, up to 80% of the population relies solely on conventional medicine for primary health care (Jain *et al*., 2019).

The pharmaceutical industry relies heavily on medications and natural products as a source of raw materials for drug discovery and development in the current medical world. As a result of the large proportion of natural pharmacons to synthetic ones (Newman and Gragg , 2016; Sevindik ,2018).

It's possible that small amount of plant origin bioactive compounds could produce long-term adverse effects in medications. New chemical and pharmacological research can only now reveal the fundamental toxicological properties of plants used in traditional medicine for an extended period.

Medicinal plants considered as the gift of nature and are extensively used to treat several diseases, and their medicinal features came from their containing of secondary metabolites which

responsible for their therapeutic properties (Sharma *et al*., 2019 and Mehmood *et al*., 2020). In addition, due to the risk of using chemical drugs, there is a growing interest in using plant-derived medicinal products (Anwar *et al*.,2019).The only approach to determine whether or not molecules affect biological systems is to screen as many configurations as feasible (Firn and Jones.,2003). Natural product extraction and bioassay became the conventional method for drug discovery in the early twentieth century (Newman, *et al.*,2000; Sevindik, *et al*.,2018).

Secondary metabolites are defined as compounds that are created in minute quantities by a specialized biochemical pathway that is specific to a particular organism. In many instances, genetic and enzymatic evidence may be found to support this assertion (Pengelly,2004).In contrast, plants produce other compounds, such as carbohydrates and proteins, called primary metabolites. More than 50% of the new drugs are of natural origin, which plays a significant role in drug development in the pharmaceutical industry (Petrovska, 2012).

Plants synthesise bioactive compounds in medicinal herbs with pharmacological or therapeutic activities such as antibacterial, anticancer and antioxidants) (Ohno and Inuki, 2018). Phytochemicals are bioactive non-nutrient plant compounds that synthesise as secondary metabolites found in fruits, vegetables and grains such as alkaloids, tannin, flavonoids, phenols, glycosides, saponin and essential oils that exist in the plant share in reducing the danger of major chronic diseases. (Altameme *et al.*, 2015; Imad *et*

al., 2015). There is a renewal and urgent need to discover modern therapeutic phytochemicals agents with different chemical structures. Rare mechanisms of action are also needed for new and emerging infectious diseases (Nithya *et al*., 2016: Daley and Cordell, 2021). Alkaloids are organic compounds found naturally in large groups of organisms: Plants, bacteria, fungi and animals (Fester,2010).

However, this definition was not sufficiently broad to cover all substances that are readily considered to be alkaloids. The term "alkaloid" was first coined in 1819 by the German pharmacist Carl Fredrich Wilhelm Meissner, who defined it as "a substance derived from plants that react like alkalis. "Alkaloids are naturally occurring nitrogen-containing organic molecules with a greater or lesser degree of basic character, which has been redefined numerous times over the years" is the most widely recognised definition of the term today (Hesse,2002;Saxena,2007).

1.1Aim of the study

1- Isolation and Purification of alkaloid compounds from two medicinal plants *C. procera* and *C. lancifolius*, by using the HPLC technique with identification by using GC-MS, TLC, FT-IR, HNMR techniques.

2-Evaluation of bioactivity for purified alkaloid compounds as

A-Antibacterial activity against gram-positive bacteria including *Staphylococcus aureus*, *Bacillus subtilis*, and gram-negative bacteria including; *Escherichia coli* and *Klebsiella pneumoniae.*

B- *In vitro* Cytotoxicity on two cell lines (MCF-7and SKGT-4) compared with control tissue culture of normal cells HBL.

C-Antioxidant activity by using the method of DPPH (diphenyl-2-picrylhydrazyl) scavenging activity assay

3-Determination of the human RBCs hemolysis for purified alkaloid compounds .

Chapter two

Literatures Review

2- Literatures Review

2-1 Alkaloids

Alkaloids are the most diverse group of secondary metabolites defined as nitrogen-containing organic compounds in the Plantae; most have a more complex cyclic structure. The nitrogen atom is linked within the loop, mainly occurs alkaline, and is linked with acid to be salt. Many of them have significant physiological activity. In plants, a few weak alkaline alkaloids are found in an accessible form, such as an amide alkaloid. Alkaloids that have alkaline are primarily found in organic salt form, such as citrate, oxalate, tartrate, succinate and so on. Few of these compounds are inorganic salt forms, such as berberine and morphine sulfate (Fester, 2010;Yobin *et al*.,2014).

2-2 Classification of alkaloid compounds

Classification of alkaloids is based on their nitrogen-containing structural features. Hence they are divided into five main groups.

1-Heterocyclic alkaloids

These alkaloids contain nitrogen-atom inside the cyclic ring structure, which in nature, alkaloids are the most frequent, as shown in figure (2.1) (WHO,2006).

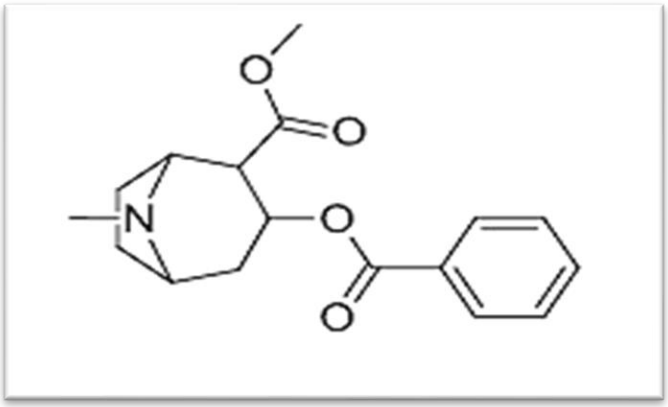

Figure (2-1) Heterocyclic alkaloids (WHO,2006).

2- Alkaloids with nitrogen, an exocyclic atom

These alkaloids, also known as "proto alkaloids" or "biological amines," contain a nitrogen atom found in the external form of the cyclic ring, and these types of alkaloids are less distributed in nature, such as ephedrine, as shown in figure (2.2), (Manske,1995).

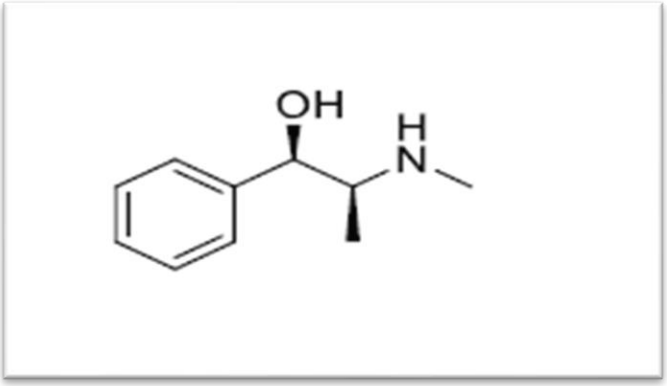

Figure (2-2) proto alkaloids (ephedrine) (Manske, 1995).

3-Polyamine alkaloids

These types of alkaloids are aliphatic compounds that have one or more amino groups, such as putrescine, as shown in figure (2.3), (Saxena,2007).

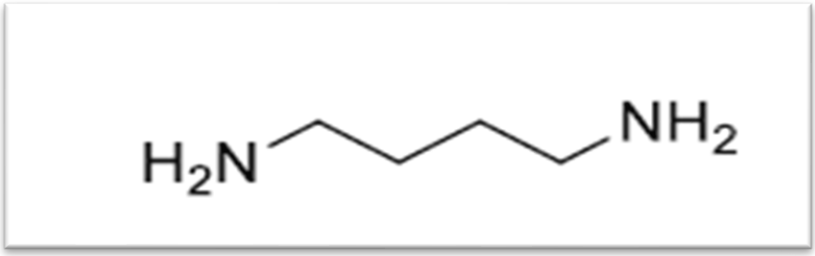

Figure (2.3) polyamine alkaloids (putrescine)
(Saxena,2007).

4-Peptide alkaloids

These kinds of alkaloids consist of amino acid monomers that are combined by peptide bonds, such as ergotamine, as shown in figure 2-4 (Hesse,2002).

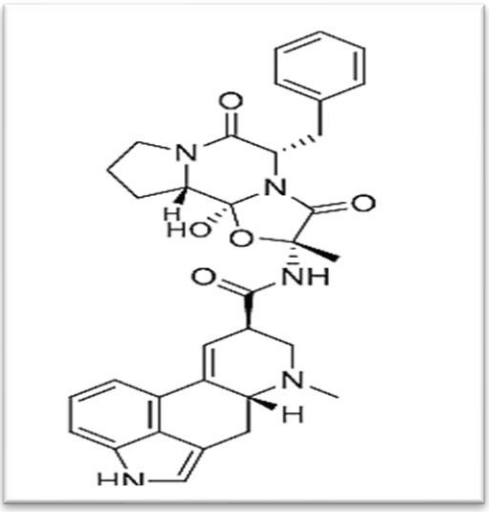

Figure (2-4) peptide alkaloids (ergotamine) (Hesse,2002).

5- Steroid and terpene alkaloid compounds

Terpene alkaloids have a mono-sesqui- di- and tri-terpene skeleton. In contrast, steroidal alkaloids have a terpene skeleton that includes tetraterpenes have a steroidal skeleton such as solasodine, as shown in figure (2-5) (Robert and Wink,1998).

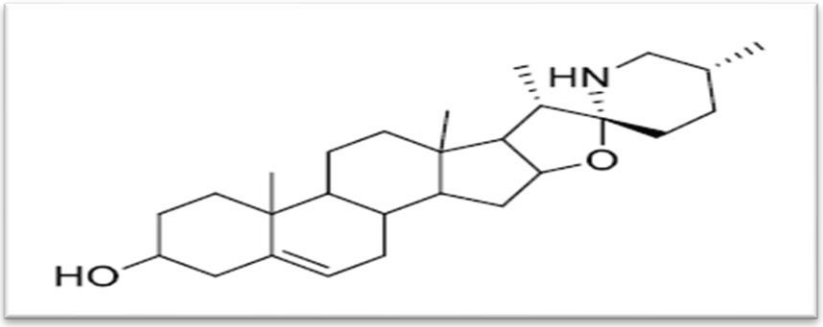

Figure (2-5) steroidal alkaloids (Solasodine)
(Robert and Wink, 1998)

2-3 Occurrence and Distribution

In the plant kingdom, alkaloid appearance is most common in higher plants (angiosperms including mono- and di-cotyledons). Flowers, roots, fruits, leaves, and seeds may contain alkaloids. Some lower non-flowering plants do produce alkaloids, such as paclitaxel compounds in *brevifolia Taxus* plant, (Pengelly, 2004). The Ergotamine and other alkaloids can also be synthesised by some fungi. Animals are also capable of producing alkaloids like the poisonous steroidal alkaloid samandarin by the fire salamander (Tuyen *et al*.,1998).

2-4 Properties of Alkaloids

Colourless crystals or amorphous alkaloids are the most common forms of alkaloids. Free-state, acid salts, and N-oxides are all states that they can take in plants. Even though they are rare, coloured alkaloids exist, such as the copper-red alkaloid sanguinarine.

In addition to carbon and nitrogen atoms, oxygen and sulfur atoms are less frequently found in alkaloids. Alkaloids must be soluble to be effective as a drug. A solution is the most common dosage form for alkaloid-based medications. The solubility of an alkaloid can be dramatically affected by a simple transition from the free state to the salt state or vice versa (Trease and Evans, 2009).

2-5 physiological importances of alkaloids in plants

Hypotheses about the physiological activities of alkaloids are numerous and varied; some claim that alkaloids are merely the outcome of metabolic processes that have no practical purpose or in plants as mineral replacements. Alkaloids, which have been shown to be beneficial in the species that make them, have largely discredited those earlier hypotheses.

1. They may act as a poisonous substance for the plants, protecting them from insects and herbivores.
2. They may act as nitrogen reservoirs in the plants.
3. They may act as plant stimulants or regulators responsible for their growth, mutation and reproduction.
4. They may act as reservoirs for protein synthesis. (Faster,2010).

2-6 Calotropis Procera

2-6-1 Classification of plant (Heywood, *et al*., 2007)

Kingdom: Plantae

Phylum: Spermatophyta

Class: Magnoliopsida

Order: Gentianales

Family: Asclepiadaceae

Genus: Calotropis

Species:Calotropis procera (Aiton) R.Br

Figure (2.6) *Calotropis procera* (Ait on R. Br)

2-6-2 Distribution of plant

Calotropis procera (Family: Asclepiadaceae) is a cultivable wild xerophytic shrub, as seen in figure (2.6), found across Africa, Asia and South America. *Calotropis spp* is a small genus of about six species of shrubs or small trees distributed in tropical and sub-tropical Asia, Africa, and central and south America (Ullah and Mohamed, 2018; Khalid *et al*., 2018; AL-Sulaibi *et al*., 2020; Hussain *et al*., 2020).

This plant is also represented in India by only two species, namely *C. gigantean* L. and *C. procera*. Both species closely resemble each other in structure and uses (Sameeh and mohamed, 2018; Sharma *et al*., 2019; CABI,2021).It has a tolerance to dry salt conditions (Rivas *et al*.,2020; Pompelii *et al*.,2019; Abeed *et al*.,2021).

2-6-3 Medical use of plant

C. procera can produce several bioactive compounds such as (alkaloid, calotropinmus, uskerin, and kalotaxein) that operate as a heart stimulant for healing ring warm and making milky white latex with many curative features (Iqbal *et al*., 2005; Saadabi *et al* ., 2012). Latex exists in special branching tubes called latex tubes (Mahajan and Badgujar, 2008) and has been the subject of interest because of its biological activities such as Antibacterial (Ishnava *et al*., 2012; Rani *et al*.,2019; ALRaowaily *et al*.,2020; Geraldo *et al*.,2021), Antifungal (Han *et al*.,2022), antiviral (Zheng *et al*.,2018) Anticandida (Sehgal *et al*.,2005), anti-inflammatory (Ramos *et al*.,2020), and anti carcinogenic activity (Silva *et al*.,2010; Samy and Chow.,2012; Wu *et al* .,20018).Dry latex of *C. procera* also has

anticancer activity due to differentiable targets and non-interference with common apoptosis pathways (Choedon *et al*., 2006).

Pharmacologically *C.procera* regard as a wound healing agent by traditional healers and as an abortifacient in folk medicines. (Abebe and Emare, 2020). The plants were regarded sacred, and the leaves were used in Vedic times in sun worship. Polysaccharides and four cardenolides were isolated from leaves and triterpenes from the root bark of *C. procera* (Bhaskar, and singh, 2009), that have insecticidal effect (Bader *et al*., 2021).

Direct exposure to the latex to the skin and the mucous membrane has been reported to cause contact dermatitis, keratitis, and toxic iridocyclitis (Almezaine *et al*., 2005). Leaf extracts show antimicrobial activity and Roots can be used as a digestive agent, healing malarial fever , Eczema, leprosy, Elephantiasis, Diabetes, Asthma, Cough and Rheumatism (Nadeem *et al* .,2019), and the Roots are linked with the help of a red thread on the affected part to relieve filarial (Jain *et al.*, 2019).

Moreover, the root powder is mixed with butter, and this ointment is used for the bite of rabid dogs and on paralysed limbs. In addition, the Root was given with black pepper during protracted labour and used for spleen complaints, elephantiasis, and rheumatism. The paste of root bark is locally applied in elephantiasis and used to treat Diarrhea and dysentery. In the case of diarrhoea changes, the faecal matter into a semisolid mass within the first day of treatment (Kumar *et al*.,2009).

2-7 *Conocarpus lancifolius*

2-7-1 Classification of plant (Heywood,*et al*.,2007)

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Sub class: Rosidae

Order: Myrtales

Family: Combretaceae

Genus: Conocarpus (L)

Species: Conocarpus lancifolius Engl.

Figure 2-7 *Conocarpus lancifolius Engl*.

2-7-2 Distribution of plant

C. lancifolius, a common riverine tree native to Somalia, is a species of *Conocarpus*. There are two species of flowering plants in *Conocarpus* genus. Both are indigenous to tropical regions of the world and are members of the Combretaceae family. *Conocarpus erectus*, a widely distributed mangrove species, and *Conocarpus lancifolius*, a seasonal river species only found along the southern Red Sea shores, are only the two species of this genus. It is also a dicot, multiple evergreens and branching, as shown in figure (2.7) (Marcar *et al*., 1999; Malik *et al*., 2020).

The ability to thrive in harsh environments has allowed it to extend from its original confines along Iraq's southern and central coasts along the Red Sea over the past decade (daily temperature more than 40 C, water inadequacy and salinity. As a result, *C.lancifolius* is currently Iraq's most giant evergreen ornamental tree. These evergreen trees can produce a substantial amount of biomass in a short period, provided they receive adequate irrigation (Suleman et al., 2013).

2-7-3 Medical use of plant

C.lancifolius is utilised as a medicinal plant for the treatment of diabetes, anaemia and a host of other ailments, including flu, conjugative inflammation, diarrhoea and fever (El-Sayed *et al*., 2012). As dosed at 200 mg/kg of body weight, the *C. lancifolius* extract possesses alpha-glucosidase inhibitory activity, lowering blood glucose levels in diabetic rabbits and lowering plasma total cholesterol, triglycerides and LDL. Additionally, in treated diabetic rabbits, the activity of SGOT and SGPT decreases,

increasing HDL levels (Saadulla *et al*.,2014). Additionally, *Conocarpus* extract is toxic to numerous parasitic protozoa species (Al-Musayeib *et al*.,2012). Fruit extracts from the *C. lancifolius* plant also have anticancer activity against the MRC-5 cancer cell line (Al-Taweel *et al*.,2016).

C. lancifolius extract also demonstrates effectiveness against bacteria, showing a high inhibition zone by disc diffusion method against different bacteria species at concentrations of extracted alkaloid 10 to 200 mg/mL (Ali *et al*., 2013). As a result, *C.lancifolius* has the ability to combat a variety of pathogens, including fungi and bacteria (Hussein, 2016). There are also eight total phenolic compounds found in *Conocarpus* species leaf extract detected by thin layer chromatography (TLC), including nine alkaloids and five saponin compounds (Touger *et al.*,2015).

Anaemia, catarrh, conjunctivitis, diabetes, diarrhea, vomiting, hemorrhages, orchitis, skin ulcers, and syphilis can all be treated with C. *lancifolius* (Liogier, 1990; Asfaw and Fentahun, 2020).

2-8 Antibacterial activity of alkaloid compounds

Infections caused by bacteria are widely recognised as a significant cause of illness and death. As a result of multi drug resistance, these tumours become more critical and dangerous. Thus, there is an urgent need for novel natural substances to combat antibiotic-resistant bacteria, which is where plants come in. Extracts of three alkaloids from the bittersweet plant, *Solanum dulcamara* (Solanaceae), have shown significant antibacterial action against the *S. aureus* bacterium (Kumar *et al*., 2009; Hagaggi and Mohamed, 2020) . Both *S. aureus* and multi drug resistance *S.aureus*

(MRSA) were inhibited by bis-indole alkaloids found in marine crustaceans (Zoraghi *et al*.,2011).

It was discovered that *Epinetrum villosum* (Exell) contains a coastline alkaloid, which has a powerful antibacterial effect against *Shigella* strains, *Campylobacter jejuni* and *Campylobacter coli* bacteria. (Otshudi *et al*.,2005).

2-9 Antibacterial Mechanism of Action of Alkaloids

Most alkaloids are found to be bactericidal more than bacteriostatic. For example, squalamine was found to have potent bactericidal properties that killed Gram-positive and Gram-negative bacterial pathogens by ≥99.99% in about 1–2 h (Alhanout *et al*., 2010). The mechanism of action of alkaloids, as antibacterial agents, has been found to be different between kinds of alkaloids. The antibacterial action for alkaloids, pergularinine and tylophorinidine from the indolizine class refers to the inhibition of the enzyme dihydrofolate reductase, which causes the inhibition of nucleic acid synthesis (Rao and Venkatachalam, 2000). Two mechanisms of bacterial inhibition were shown to occur Within the isoquinoline class; Ungereminea phenanthridine isoquinoline by the inhibition of nucleic acid synthesis, while benzophenanthridine and protoberberine isoquinolines alkaloids were found that agents act by the troubling of the Z-ring and inhibition of cell division, and this was further proved by many studies (Boberek *et al*., 2010 ; Casu *et al*., 2011). On the other hand, synthetic quinolones inhibit the type II topoisomerase enzymes, while the natural quinolone alkaloids lacking the 3-carboxyl function act as respiratory inhibitors by reducing oxygen consumption in the treated bacteria (Heeb *et al*., 2011; Tominaga *et al*., 2002). Agelasines alkaloids exert their antibacterial activity by inhibiting the

dioxygenase enzyme BCG 3185c, causing a disturbance in the bacterial hemostasis. This result was revealed from overexpression and binding affinity experiments on the anti-mycobacterial alkaloid azelastine D (Arial *et al*., 2014). Squalamine from the polyamine alkaloid class acts by disturbing bacterial membrane integrity (Salmi *et al*., 2008).

2-10 Anticancer activity of alkaloid compounds

Phytochemicals are rich sources for the treatment of different health problems. Anticancer medicinal plants possess cancer healing properties because of their bioactive secondary metabolites. More than 60% of anticancer remedies are of natural origin or are produced by modifications of natural products. Anticancer medicinal plants show cancer healing properties due to their many active secondary metabolites (Viana *et al*.,2017).

The discovery and development of vinblastine and vincristine alkaloids from the *Catharanthus roseus*, Etoposide (VM 26) and Teniposide (VP 16-213) from *Podophyllum* spp., irinotecan or Camptothecin from *Camptotheca acuminate*, Paclitaxel from *Taxus* spp.

and many other natural compounds from different sources as anticancer agents gave persuasive evidence that plants' secondary metabolites could be a prospective source of anticancer agents and prophylactic cancer medicine. Many medicinal plants have been specified to have anticancer activity (Adiguzel *et al*., 2009; Korkina and Kostyok, 2012; Tanih and Ndip, 2013; Ouedraogo *et al* .,2020). Considering the importance of natural products in cancer therapy, many anticancer designate medicinal plants subjected to phytochemical screening for their secondary metabolites, reported in published literature Five alkaloid detection and

Flavonoids extracted Kainsa *et al*., (2012). Also, studies by Kinghorn, (2000) and Malika *et al*. (2011) reported anticancer activity for different phytochemical secondary metabolites from various medicinal plants..

2-11Antioxidant activity of alkaloid compounds

Living organisms are armed with a defence system to treat free radicals and other reactive oxygen species (ROS). This defence system has an enzyme such as catalases, superoxide dismutase, glutathione peroxidase, and glutathione reductase, or compounds such as glutathione, vitamins E and C, etc. If free radicals are neutralised by the body's anti-oxidative defence system, the body is in healthy conditions.

However, depletion or loss of antioxidant levels may lead to free radical-caused oxidative stress. Oxidative stress can cause cellular and tissue damage, DNA mutation, cancer etc. (Nithya *et al.*, 2016). Besides, in the current world, the human body is significantly exposed to external sources of free radicals. Therefore, the body's anti-oxidative defence system might not be convenient to avoid oxidative-caused damages altogether (Ghani, 2003). In this regard, antioxidant complements, or foods containing antioxidants, could help the body's defence system to reduce or neutralise oxidants. Medicinal plants used in traditional medicine are familiar significant sources of natural antioxidants. Therapeutic plant-derived natural antioxidants, which are in the form of raw extracts or chemical constituents, are very active in blocking the oxidation process by neutralising free radicals (Yusuf *et al.*, 2009).

The use of medicinal plants in traditional medicine has not yet been evaluated for its medicinal properties, including antioxidant activity (Hanachi and Kostyouk, 2009).

Several studies have been published concerning many plant extracts, which included the study of biologically active properties such as antibacterial, antitumor, antifungal and antioxidant of medicinal plants shown in study of Remy *et al.* (2013), which revealed antioxidant activity of alkaloid compound (luminarine) isolated and purified from the root of *Napoleona imperialis*. Another study performed by Sharareh *et al*., (2015) showed antioxidant potency *in vitro* for a bioactive compound isolated from the leaves of seven Iranian medicinal plants.

2.12 Previous studies

Many studies were done for phytochemical screening of medicinal plants to discover a new bioactive compound with strong pharmacological action and less cytotoxicity that are; the study by Benabdesselam *et al* . (2007) on alkaloid extract (total quinolizidine alkaloid) of two species of *fumaria* plant *(F. capereolata, F. bastardii*) that occurred a strong antioxidant activity of both species, however alkaloid extract of *F. bastardii* was more strong than *F. capereolata*.Besides, the *F .bastardii* was more alkaloid content (521mg/100g). The antibacterial activity of methanolic leaf extract of *Alstonia scholaris* (L.) R. Br.by varied phytochemical composition,GC-MS analysis, and several solvents were investigated study by Swamy *et al*.(2019). In this study, researchers found alkaloids, coumarins and flavonoids as well as phenols, quinines, saponins and tannins in the leaves, , which showed significant antimicrobial activity against some

bacteria *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *S. aureus* and *E. coli.*

The antibacterial activity of the methanolic leaf extract was superior to that of the ethyl acetate extract. The UV-Vis, FTIR, and GC-MS methods have been used to identify the bioactive chemicals.Results from the GC-MS analysis indicated the existence of nine phytochemical substances with various medicinal properties included The alkaloid compound oxime-methoxy-phenyl.

Study by Alhazmii *et al*. (2018) included leaf ethanolic extract of *C.procera* that appeared to have significant antimicrobial activity against *S. aureus*, *E. coli* in all concentrations, and mild antibacterial activity was observed with *Bacillus subtilis* and *streptococcus pyogenic* in high concentration.

The study of Dalimounthe *et al*. (2018) included extraction of crude alkaloid by using the maceration method from plant *Litsea cubeba*, and ethanol extract was fractionated with liquid-liquid extraction using n-hexane, chloroform at pH (3,7,9,11) to obtain alkaloid fractions, alkaloid compounds were isolated by preparative thin layer chromatography(TLC) the results appeared a strong antioxidant potential for fractions and isolates where determined by using ATBS and DPPH assay.

Many studies were carried out to discover new anticancer drugs from plants, especially alkaloid compounds like the study by Minhajur *et al*.(2017) in which Alkaloids, flavonoids, and sterols were screened for in 23 anticancer medicinal plant species from 17 family that found alkaloid compounds present in all studied plant species, and the study of Mohsen .(2021) that revealed anticancer activity of tropane alkaloids (Atropine and scopolamine) on MCF-6 and AMG-13 cell lines which separated and

identified from two Iraqi and Iranian species of *Datura stramonium*, determination and purification of alkaloids were performed by HPLC technique.

Also, a study by Ismaili *et al*.(2017) , that included extraction of crude alkaloid compounds from four species of papaver plants. It evaluated the antibacterial and antifungal activity of these four crud alkaloid extracts, and the results indicated their activity as antifungal more than antibacterial activity. In the study of Erdemuglu *et al*.,(2009), capillary GC-MS was used to examine the alkaloid composition of aerial portions of *Genista vuralii* (Fabaceae). that has detected ten quinolizidine alkaloids, including N-methylcytosine, cytisine, tetrahydrorhombifoline,17-oxosparteine,5,6-dehydrolupanine, lupanine, 17-oxolupanine, anagyrine, baptifoline, and 13-tigloyloxylupanine. Anagyrine (93.04 per cent) was the most prevalent alkaloid in this group.

Additionally, the alkaloid extract of *G. vuralii* was examined for antibacterial and antifungal activity against typical bacterial strains including *E. coli, P. aeruginosa, Bacillus subtilis, and S. aureus) (Candida albicans, Candida krusei).* alkaloid extract showed good efficacy against *S. aureus, B. subtilis, and C. krusei, C. albicans*, with MIC of 62.5 g/ml. Other *Datura stramonium* alkaloid compounds, the remaining MIC values were between 125 and 500 g/ml.

In study by Altameme *et al*.,(2015) alkaloid compounds extracted from the *D. stramonium* leaves and tested for in vitro antibacterial activity against *E. coli, Proteus mirabilis, S. aureus, Pseudomonas aerogenosa and K. pneumoniae* by the diffusion method in agar were found by GC-MS analysis of the leaves ethanolic extract of *D. stramonium*. Various conventional antibiotics were tested to see how their zones of inhibition different.

Astudy by Giare *et al* .,(2011) Conducted methanolic extract of *Ficus auricularis* stem bark were subjected to qualitative phytochemical analysis and found to contain a wide range of compounds, including alkaloid and carbohydrate compounds as well as saponin and glycoside compounds. This extract's antioxidant activity was found to be 84.088 % at 0.1 mg/ml, whereas chloroform extract's was found to be 83.864 % at 0.029 mg/ml. Both extracts had IC_{50} values of 0.042 mg/ml.

On the other hand, hexane extract had a 42 % higher free radical scavenging activity than conventional ascorbic acid. the methanol extract was more effective than hexane extract in inhibiting the growth of S. aureus, which had a zone of inhibition of 7.8 ± 0.36 mm.The antibacterial activity of the plant extract was found to be lower than that of the usual antibiotics utilised, but it exhibited potential antioxidant action.

Moreover , study by EL-Sheikh et al.(2018) detected alkaloid compounds in 26 plants used in Ethno veterinary medicine in Sudan. The crude extracts for the plant samples were obtained using Five solvents (Ethanol, petroleum ether, chloroform, ethyl acetate and aqueous). Phytochemical screening method on the ethanolic and aqueous extracts revealed that alkaloids were abundant in most candidate plants except only five, namely, Maerva crassifolia (Stem park), Pennisetum orientale (Seeds), Balanites aegyptiaca (Fruit), Cucurbita moschata (Seeds) and C.erectus (Leaves). Thin layer chromatography (TLC) used to detect alkaloids in those plant extracts has shown good results.

Many studies on the medicinal plant *Calotropis spp* was done include the study of Shirsat *et al*.(2013) that conducted the quantitative of GC-MS analysis and phytochemical screening for medicinal plant *Calotropis gigantea* that concluded a chloroform extract of whole plant extract which

has 15 constituent and the preliminary phytochemical screenings revealed the presence of Alkaloids ,steroids ,triterpenoids .,resins and carbohydrates.

Also the study of Gajare *et al*. (2012) that conducted ethanolic root extract of *C. procera* that appeared antibacterial activity against the pathogenic bacteria whereas it doesn't show antifungal activity and this quantitative study revealed that ethanol root extract of this plant was the richest source of alkaloid compounds .

Also the study of Morsy *et al*.(2016) showed the presence of alkaloid compounds in crude ethanolic extract 70% of *C. procera* of stem and root only that showed antibacterial activity on *K. pneumonia* with inhibition zone diameter 20 mm. On the other hand the study by Sameeh, and Mohamed. (2018) appeared that , methanolic crude extract of leaves and fruit of this plant showed DPPH antioxidant activity and GCmass analysis showed the presence of many bioactive compounds that playing an important role in inhibition progress of several diseases.

Also, the study of this plant conducted by Naser *et al*.(2019) included chemical screening and GC-MS analysis of leaves ethanolic crude extract that appeared the presence of three types of alkaloid compounds, colchicine 0.8% pseudojervine1.55% and (Thebaine) which is found in highest percentage (7.6 in 100% ethanolic extract) and that crude extract of leaves exhibited antibacterial activity of each *B.subtilis*,17 mm, *E. coli* 6mm, and *S.aureus* with the highest diameter of inhibition zone 18 mm).

Beside the study of Mohamed *et al*.(2014)) that showed a high antibacterial activity of latex extracted from leaves of *C.procera*. Alkaloid chemicals were discovered in ethanolic and chloroform leaf extracts of *C.*

procera in various research, including chemical screening and qualitative and gas chromatography-mass spectrometry (GC-MS) analysis.

Astudy by Verma et *al.* (2013).Also, performed a comparative GC-MS analysis of ethanolic leaves and latex of *Calotropis gigantean* which showed the presence of a total of 46 bioactive compounds (24 from leaves and22 from latex most of them were similar in both parts.

Afew studies that carried out to isolation and purification of alkaloid compound colchicine that has important biological activity such as the study of Kumar *et al* .(2016) that included isolation and purification for colchicine from *Gloriosa superb* plant by performing High-Performance Liquid Chromatography and it was observed that the recovery of colchicine from extracts prepared by soxhlet method was greater than that of sonicator likewise, the colchicine content in the extract of microwave vacuum dried tuber was greater as compared to the hot air oven dried tuber.

Another study by Krishnasamy *et al*.(2016) included isolations and purification of colchicine compounds from *Indigofera aspalathoid* by using column chromatography and detecting the anticancer activity on Hep3Bcell lines of IC_{50} =344.25 that found colchicine induces apoptosis inHep3B cell lines.

Few studies detected the presence of alkaloid compound methoxy phenyl oxime in the plant, the most important study about this compound was carried out by Chipps *et al.*(2012) which included extracted bitter melon (*Momordica charantia*) seeds in water: ethanol (1:1) It was shown that methoxy phenyl oxime was the must active component in the crude extract when tested against human embryonic kidny293T (HEK293T) and human colon tumor116 (HCT116) cell lines.

Also, the study of Dahpour *et al*.(2012) showed the presence of methoxy phenyl oxime compound in the leaves ethanolic extract of *sedum pallidum* analysed by GC-mass spectrometry of about 4.34% of the total crude extract and the results showed that crude ethanolic extract of *sedum pallidum* showed moderate antibacterial activity against three types of pathogenic bacteria (*B. subtillus, S aureus*, *K. pneumonia*) at400 mg\ml disk.

Methoxy phenyl oxime was identified by Huda *et al*.(2015) in methanolic leaves extract of *Urtica dioica* by Gas chromatography-mass spectrometry and Fourier Transform Infrared (FT-IR).

Also, the study by Saddeq *et al*.(2019), evaluated against six harmful microorganisms using cold aqueous extraction of *C. lancifolius* leaves through sonication also, methoxy phenyl oxime compound was extracted from bacteria that studied by Barghouthi *et al*.(2017) that include isolation and identification of the novel antibacterial agent methoxy phenyl oxime from *Streptomyces pratensis* QUBC97 Isolate.

A few local studies on alkaloid compounds were represented by;

A study by Raheema and Shoker (2020) was designed to evaluate the aqueous extracts of *C. lancifolius* leaves revealed the presence of tannin, Saponins, Comarins, phenols, alkaloids, flavonoids, glycoside and terpenes compounds. Results showed a yield of phenols extracts of C. lancifolius were 46.1%, while the extracts were 22.2% alkaloids. AgNPs were proved by Atomic Force Microscopy. The average diameter of 75.50 nm. HPLC analysis indicated the presence of four phenolic compounds: Rutin, Epigenen, Kamferol and Catechine while containing two alkaloids, Scopolamine and Hyoscine. Results showed that C. lancifolius extracts and

AgNPs, possess higher antibacterial activity against both gram-positive and gram-negative pathogenic bacteria

A study of Alsmail and Hussein (2019). That includes isolating and identifying alkaloids and glycosides active compounds from the *Geranium lucidum* and *purpureum* species using the HPLC technique. Phytochemical analysis of the studied plant revealed the presence of four alkaloids compounds (palmatine, columbamine, pseudocolumbamine, geraniin) and Five glycosides compounds (geranioside A, geranioside B, quercetin 4-O-β -glucopyranoside, quercetin 3-O-β -glucopyranoside, kaempferol- β-glucopyranoside, These compounds showed differences in their concentrations between and within species studied where Geraniin showed the highest concentration 96.05 ppm in *G.purpureu*A study by Saddeq *et al*. (2019) that included cold aqueous leaves extraction of *C. lancifolius* by sonication method, the extract appeared to have antibacterial activity against six pathogenic bacteria at different concentrations, and the biggest inhibition zoon was at 200 µg/ml

A study by Gasiem *et al*. (2016) including preliminary phytochemical screening of the *cordia myxa* extract, revealed the presence of alkaloids, Flavonoids, saponins, tannins, Glycoside, steroids and coumarin by using different solvents. Antimicrobial activity of alkaloid extracted from leaves and mucilage from fruits was done by well diffusion method, and Mucilage extract showed an Inhibition effect on gram-negative bacteria including *E. coli* and *K. pneumonia* isolated from urine at concentrations 100,500,250,125,63.5 mg/ml respectively, while no effect of alkaloids extracted from leaves against all pathogenic bacteria.

A study by Altameme *et al*. (2015) conducted a phytochemical analysis for methanolic leaf extract of *Urtica dioica* by GC-MS and FTIR technique that appeared the existence of many bioactive compounds, including the alkaloid compound methoxy phenyl oxime.

Chapter Three

Materials and Methods

3-1 Instruments

Instruments and chemicals used in this study are listed with their suppliers in tables (3-1) and (3-2).

Table 3-1: instruments and their suppliers

Instrument	**Supplier**
Centrifuge	Hettich Germany
CO2 incubator	Cypress Diagnostics Belgium
GC-Mass spectrometry	Agilent Technologies / USA
Hot Plate Magnetic Stirrer	SELECTA /SPAIN
HPLC apparatus	LC-W100A / USA
microtiter reader	Thermo Fisher Scientific
Rotary Vacuum Evaporator	Buchi Rot: Vapor RE
Soxhlet apparatus	Schott Duran /Germany
Spectrophotometer	Spectrophotometer sp8-100 Pye unicum
TLC jar	(23x22x8 cm) Schott Duran Germany
U.V Lamp	Ultra viole-Comage Cat-No 29010
Ultraviolet Lamp(ENF-260)	Spectripline \ USA
UV-detector	CECIL: Aquarius England

Table 3-2: Chemicals that are used and their supplier

Materials	**Company**	**Origin**
Acetone	BDH	England
Acetonitrile(HPLC grade)	Sigma-Aldrich	Germany
Ammonia (25%)	Sigma –Aldrich	Germany
Chloroform	ROMIL,Ltd	Cambridge
Colchicine Standard	Sigma-Aldrich	Germany
DPPH	Sigma-Aldrich	Germany
Dragendorff reagent	Sigma-Aldrich	Germany
Ethanol(99.8%)	BDH	England
Ferric chloride	Merk	Germany
HCL	Merk	Germany
Mayer reagent	Sigma-Aldrich	Germany
Methanol	Sigma –Aldrich	Germany
Muelar Hinton Agar	Sigma –Aldrich	Germany
Nutrient Agar	Sigma –Aldrich	Germany

3-2 Plants collection

The two plants of *C. procera*, and *C. lancifolius* were collected in a period between (March- Jone ,2020) from Basrah governorate, Southern Iraq. The first plant, *C.procera* was collected from, Assad-Babel square, while collections of the second plant, *Conocarpus lancifolius*, were from gardens of the University of Basrah. Identification of the field-ordered plants

wasAuthenticated as *C.procera* and *C. lancifolius* plants by plant Taxonomist Prof. Dr. Sahar Abd Al-abbas Malik, College of Science, Department of Biology, University of Basrah.

3-3 Preparation of selected plants for extraction

After the plants were classified, the whole plants were thoroughly washed using tap water to remove any contaminates and then shade dried for several days at room temperature. After separating all parts of the plant, the dried pieces are ground to a fine powder through the mechanical grinder and then stored in tight plastic bags labelled for study.

3-4 Preliminary Biochemical test for alkaloid compounds detection

Two Biochemical tests were done for methanolic extract (Hot continuous extraction) of each part alone (Leaf, root, fruit, and flower) of two studied plants *(C. procera* and *C.lancifolius*) for detection of alkaloids.

3-4-1 Dragendorff reagents

Two stock solutions were prepared. The first one was prepared by dissolving 0.6 g of bismuth sub-nitrate ($Bi5O(OH)9\ (NO3)_4$) in 2 ml conc.HCL 37%- and 10-ml water. Liquid potassium iodide (KI) in water (10 ml) was used to make the second solution. These stock solve were assorted with each other, with 7 ml conc HCL and 15 ml water, then diluted with 400ml water.

The extract was diluted with 1ml of reagent, and a reddish-brown or orange precipitate formed as +ve test (Harborne,1984).

3-4-2 Mayer reagent:

The first solution was prepared by dissolving 0.5 g of (Hg_2CL_2) in 30 ml water, While the second solution was prepared by dissolving 2.5 g (KI) in 5 ml water, then Both solutions were mixed, and the volume made up to 50 ml with water.1 ml of reagent is added to the extract. When alkaloids are present, a creamy precipitate forms as a positive test. (Evans, 1972)

3-5 Solvent Extraction

Two methods of extraction were done, cold (maceration) and hot continuous extraction Soxhlet by using three solvents (Methanol , water and Dichloromethane) for *C. procera* (Leaf and root) and *C. lancifolius* (Leaf) to evaluate the percentage of presence of alkaloid within two studied plants..(Salem *et al*. 2012) .

3-6 Extraction of total Alkaloids

3-6-1 Hot continuous extraction

According to Harborne (1984), a total alkaloid extracted Briefly 20 g of dry plant powder was extracted with n-Hexane in a constant extraction by soxhlet apparatus 250 ml volume solvent for defatting. Then the filtrate was re-extracted with 80% Methanol for 24 h in a continuous extraction by soxhlet apparatus 250 ml volume, this extract filtered and then the filtrate was concentrated by a rotary evaporator under vacuum at 45C until the solution reached 10 ml and transferred to separating funnel and 2 N HCl added gradually to make the pH value come to (pH=2) then the extract washed with 10 ml chloroform three times, the pH value of the extract reached to(pH=10) by adding NH4 OH and partitioned with 10 ml

chloroform 3times.the chloroform portion dried to obtain the total alkaloid extract,the dried extract weighted and preserved in clean container at 4 C for further investigation.

3-6- 2 Cold extraction (maceration)

The air-dried powder (100 g) was soaked with 200 mL of methanol (80%). After one week of soaking, the solution was passed through activated charcoal to remove the chlorophyll and filtrate. A similar amount of solvent was used to treat residue after filtration (Salem *et al*. 2013). A rotary evaporator at a decreased pressure and 45°C dried the crude methanol extract. When not used, the extract was lyophilised and kept at a temperature of 4°C in the refrigerator. Alkaloids were precipitated by drop-wise addition of 10% NH_4 OH to samples of the chloroform fraction of lyophilised, methanol extract, which were subsequently diluted in 50 mL of 99.9%-ethanol and treated with an equal volume of 1 % aqueous HCl.. Using a centrifuge (5000 rpm at four C^o for 30 minutes) and 1 % NH_4 OH, the precipitated was recovered and rinsed with NH_4. The $CHCl_3$ fraction containing the precipitated alkaloids was obtained by dissolving the residue in a few drops of $CHCl_3$. The extracts were concentrated under a vacuum at 40-60°C while the solvents were removed at decreasing pressure (Harborne, 2005).

3-7 Preliminary screening for alkaloid compounds by using Gas chromatography-mass spectrum analysis

The screening of plant extracts by GC-.MS analysis was done in Basrah oil company –Nihranbin-Omar Laboratory. For methanolic alkaloid extract for both *C. procera* (Root, leaves) and *C.lancifolius* (Leaves) that appeared

with existing alkaloid compounds for detecting their alkaloid types and structure by using a modified method (Zaher *et al* .,2020) by GC-.MS analysis. An Agilent gas chromatograph with a mass detector attached to Agilent 5977A spectrometer with an HP- 5MS (5% Phenyl methyl siloxane), 30m ×0.25mm × 0.25 mm ID of the capillary column. The injector temperature was 40 ^{0}C maintained for 5 min, then raised gradually to 300^{0}C at an increment of 10\min. Helium gas 99.99% used as mobile phase at a flow rate of 1ml\min. An injection volume of 1 uL. It was taken at 70 EV, with a scanning interval of 4 min, and fragments ranging from 45 to 450 Dalton for mass spectrometry. There was a solvent delay of 4 minutes, and the GC-MS operation lasted 45 minutes. A split injection was used to administer the samples (50:1).

3-8 Identification separation and purification of alkaloid compounds by using the High-Performance Liquid Chromatography (HPLC) technique

3-8-1 Identification of alkaloid compounds by using (HPLC) technique

HPLC analysis was carried out using a modified method (Shinde and Laddha, 2014) LC-W100A HPLC (USA) system connected to LC-UV100 plus UV detector with manual injectors. Data interfered using PC with (les (x86) \HPLC SYSTEM). Separation was performed through Exformma technologies Column Arcus EPC18 5um, 4.6 x 250mm, isocratic mobile phase used for the analysis of leaf methanolic alkaloid extract for *C.lancifolius* consists of (acetonitrile: water50:50), and (acetonitrile: water75:25) isocratic mobile phase for both leaf and root methanolic

alkaloid extract for *C.procera* with 1ml/min flow rate at 25 ^{0}C, and pressure of 100 p.s.i, injection volume was 10 µl. 0.05 mg\ml standard solution of colchicine and sample of leaf methanolic alkaloid extract for two studied plants were filtered by (125mm) filter paper before injection, run time was 10 min for each run, and detection was conducted at (300nm) and (280) wavelength.

3-8-2 Separation and Purification of alkaloid compounds by using+ HPLC technique

The modified method was used to separate methoxy phenyl oxime and colchicine alkaloid compounds by HPLC (Bishr *et al*.2016). The eluted mobile phase during the appearance of the identified peak of alkaloid compound from the crude methanolic extract with recorded retention time compared to standard was collected. Isolated mobile phase portions then undergo HPLC analysis to confirm the purity of the isolated compound using the same conditions. The collected mobile phase was transferred to a giant petri dish, allowed to dry overnight, and the yellow crystal was obtained.

3-8-3 Thin Layer Chromatography (TLC)

For more identification, Separation of leaf methanolic alkaloid extract of two plants *C.procera* and, *C.lancifolius.* Thin-layer chromatography was used TLC(10*10) cm ,TLC silica gel 60 F254 merk plate dried and was activated using hot air for 15 minutes before use. Baseline and solvent front line was drawn before application of the sample was developed with chloroform**:** methanol: formic acid (20:1:0.2) solvent system (Rathod *et al*.2021) for separation of Colchicine compound founded in leaf methanolic

alkaloid extract in *C. procera*, while, Hexane: ethyl acetate : methanol: water (7:3:5:5) solvent system (Friesen and Pauli,1996) was done for separation of methoxy phenyl oxime founded in leaf methanolic alkaloid extract in *C. lancifolius*, the prepared solvent system was transferred to (20 cm x 15cm x 8) Glass jar covered with a glass cup. Filter paper is immersed in the solvent system to ensure saturation of the atmosphere with the solvent vapours before the separation process of the sample begins after standing for 30 minutes.

3-9 Identification for purified MPO alkaloid compound

More than one instrumental analysis was used to identify the purified alkaloid compound methoxy phenyl oxime purified from leaves of *C.lancifolius*. Its standard compound hasn't been found. Thus, there must be precise detection for that rare alkaloid compound.

3-9-1 Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR spectra were carried out at the University of Basrah, Polymer Research Center, on FT-IR Stuart shiatsu corporation spectrophotometer, using KBr disc.

3-9-2 Nuclear magnetic resonance spectroscopy (NMR)

H NMR spectra at 500 MHz were recorded with INOV. A spectrometer at Iran, University uses DMSO-d6 as solvent at a temperature of 25 C0 with chemical shifts expressed in ppm and coupling constants in Hz.

3-9-3 Melting point

The melting point was measured in a capillary tube using the electrothermal melting point apparatus model Stuart SMP.30 apparatus (UK) at the University of Basrah, College of Sciences, Department of Chemistry.

3-10 *In vitro* antibacterial activity assays

The antibacterial activity was carried out on the crude precipitated alkaloids from *C.procera* root and on the two purified alkaloid compounds colchicine and methoxy phenyl oxime that were purified from *C. procera* and *C. lancifolius* respectively with three concentrations reached 200,150,50 µg/mL against two Gram-positive bacteria; *B. subtilis* and *S.aureus* and two Gram-negative bacteria: *E. coli* and *K.pneumonia*, that used as human pathogenic bacteria. Nutrient agar (NA) medium was used to maintain the tested bacterial organisms, while Mueller Hinton agar (MHA) was used in all bioassays applying the disc diffusion method (Eloff, 1998).

3-10-1 Bacterial test

All of the bacteria used in this study were collected and identified at microbiology laboratory of Basrah Hospital of women and Children by Vitec Apparatus, as shown in the table (3.3).

3-10-2-Bacterial culturing media

1- Nutrient Broth. 2- Mueller Hinton Agar.

Table 3.3 Bacterial species used in this study

Bacterial species	Source
Escherichia coli	Stool
Klebsiella pneumoniae	Blood
Staphylococcus aureus	Ear swap
Bacillus subtilis	Blood

3-10-3 Kirby-Bauer disc diffusion method

Susceptibility to Kirby-Bauer disc diffusion (NCCLS, 2002) method was used to measure the sensitivity of the bacterial strains against the two purified alkaloid compounds colchicine, methoxy phenyl oxime and the crude alkaloids of *C. procera* root, where a tested bacteria with a suspension of 1 mL of 10^5 CFU/mL were spread on the surface of solid media plates (Mueller Hinton. Agar). Filter paper discs of 5 mm diameter were loaded with 20 µL of the alkaloids and placed on the inoculated plates. The plates were incubated at 37°C for 24 h. The diameters of the inhibition zones (IZs) were measured in millimetres. The biological activities of the alkaloids were carried out at the concentration of 50,150,200µg/mL against the tested bacterial strains. Positive and negative controls (Gentamycin 30 mg/disc and DMSO saturated discs) respectively were used to compare the inhibition zone obtained. The experiment was carried out in three separate instances.

3-10-4 Minimum Inhibitory Concentrations (MIC)

Minimum inhibitory concentrations (MICs) were determined by serial dilution of two purified alkaloid compounds colchicine and methoxy phenyl oxime that were purified from *C. procera* and *C.lancifolius* respectively and crude precipitated alkaloid extracts of *C. procera* root, and these serial dilutions were (15,35, 55, 75,95,115,135,155,175)µg/ mL) Using 96-well microplates 50µ L sterile Mueller Hinton Broth (MHB) was used to fill all wells, with a few modifications. The sterility control included solely Oxoid MHB (Sigma-Aldrich), whereas the growth control contained both MHB and the tested bacteria. The microplate was covered and incubated overnight at 37°C and at 100% relative humidity with 50µ L of the bacterial suspension (10^5 CFU/mL) added to each row. As an indicator of growth inhibition, each well was incubated for 30 minutes with a solution of piodonitrotetrazolium violet (Sigma-Aldrich) (Eloff, 1998).

3-10-5 *In vitro* hemolysis test

In the laboratory, hemolysis was used to test the cytotoxicity of alkaloid chemicals isolated from the two plants on human erythrocytes (RBCs) investigated *in vitro*. According to (Bouma,2002) healthy and nonsmoking blood was utilised in this test, with certain adjustments as follows: Concentration of alkaloid compounds in blood ranged from 30 to 50 g/ml, and the mixture had to be centrifuged for 10 minutes at 3000 RPM to avoid severe hemolysis, according to the standard procedure. In the following step, the optical density of the mixture optical density (O.D.) was determined using the 540 nm wavelength. To evaluate how much hemolysis is caused by

plant extracts, this test uses a percentage of hemolysis to 100% percent hemolysis as a metric.

The O.D. of the combination was measured at 540 nm after it was diluted with tap water and centrifuged, achieving complete hemolysis (100 %). Following the completion of the absorption measurement, the following equation was used to compute the percentage of hemolysis:

Hemolysis%= (AT–AS) / (A 100% H–AS) x 100%

AT: Absorbance of test solution, AS: Absorbance of normal saline :

A100% H: Absorbance of 100 % hemolysis.

3-11 *In vitro* anticancer activity assay on cell lines

The cancer cell lines of MCF-7 (breast adenocarcinoma derived from metastatic site: pleural effusion) and SK-GT--4 (esophagus adenocarcinoma derived from metastatic site: pleural effusion) were provided by (the IRAQ Biotech Cell Bank Unit in Basrah), And maintained by method of (Al-Ali *et al*., 2022).

3-11-1 Cytotoxicity Assays

To determine the cytotoxic effects for the two purified alkaloid compounds colchicine and MPO and crude precipitated alkaloids (CR) of *C. procera* root the MTT cell viability assay was conducted on 96-well plates. different concentrations (1, 5, 10, 50,100,250,500,750) µg/ml at 37C^{o}) of alkaloid compounds was used to evaluate cell viability by using method of Al-Shammari *et al*. (2019).

Viability rate as (PR) = B/A*100 A is the mean optical density of untreated wells, and B is the mean optical density of treated wells. IR= 100- PR (Freshney, 2010).

3-11-2 Acridine Orange/Ethidium Bromide (AO/EB) staining

Ethidium bromide and Acridine Orange 100µg/ml both were added to the cells and kept in dark at room temperature. The morphological changes were observed using a fluorescence microscope (Liu ,*et al* .,2015).

3-12 *In vitro* antioxidant activity by Free Radical Scavenging Activity of -diphenyl-β-picrylhydrazyl (DPPH)

Radical scavenging activity effect for crude precipitated alkaloids of *C. procera* root and on the two alkaloid compounds colchicine and methoxy phenyl oxime that were purified from *C.procera* and *C.lancifolius* respectively were carried out as described by (Jamuna *et al*., 2012) 1 ml of concentrations (200,100,50,25,12.5) of alkaloid methanol solution was mixed with 1 ml of DPPH methanol solution (0.04mg). An equal amount of methanol and DPPH served as control. After incubation in the dark for 20 min, absorbance was recorded at 517 nm. The percentage scavenging was calculated according to the following equation:

% Scavenging = Ac – AS\Ac × 100%

Ac: absorbance of control AS: absorbance of the sample.

3-13 Statistical analysis

Based on the type of data, statistical analysis tests were employed. To report the statistical significance of the differences between the means of various groups at ($P \leq 0.05$), one -way and two -way ANOVA analysis (ANOVA) utilizing programs (SPSS version-24) was used to calculate the mean and standard error of the mean. Also Graph pad prism software 6.04 was used for calculation of IC50 values of cell lines.

Chapter Four

Results

4-1 Preliminary Biochemical tests for alkaloid compounds

The results of biochemical tests of methanolic extracts for each part of the two studied plants by using two chemical reagents for detecting alkaloid compounds represented by Dragendorff reagent & Mayer reagent appeared the presence of alkaloid compounds in leaf only in *C. lancifolius* that gave positive test for both reagents, while gave a negative test for other parts of this plant, whereas, the second plant *C. procera* showed the presence of alkaloid compounds in leaf &root while non-presence in other parts of this plant that summarized in table (4-1).

Table 4-1: Biochemical tests for alkaloid compounds detection.

Plant	Part of plant	Alkaloid test	
		Dragendorff reagent	Mayer reagent
C. lancifolius	Fruit	-	-
	Flower	-	-
	Leaf	+	+
	Root	-	-
C. procera	Fruit	-	-
	Flower	-	-
	Leaf	+	+
	Root	+	+

4-2 Solvent extraction

The results for three tested solvents, as shown in figure (4.1) for the percentage of yield alkaloid extracts appeared that Methanol solvent was superior to two other solvents (water and dichloromethane) with significant differences at a level ($p \leq 0.05$) at a mean value reached 1.677 g\100g DW, in comparision with water and dichloromethane mean values for a yield alkaloid extracts that reached (0.619 and 0.0892) g\100g DW respectively.

The finding of two tested methods used in alkaloid extraction of *C. lancifolius* was the Hot continuous extraction method (soxhlet), and cold extraction (maceration), which revealed that hot methanol extraction was superior to cold one with a significant difference at a level ($p \leq 0.05$) of mean values reached (1.60 and 0.96 g\100g DW) respectively.

Also, hot water extraction was superior to cold one with a significant difference at a level ($P \leq 0.05$) of mean values reached (0.96 and 0.40 g\100g DW), respectively. At the same time, there was no significant difference between hot dichloromethane with a cold one of yield alkaloid extracts of a value reached (0.14 and 0.03 g\100g DW) respectively. besids, in *C. procera*, the hot methanol extraction was superior to the cold one, with a significant difference of values reached (2.76 and1.33 g\100g DW), respectively. Still, there were no significant differences between the hot and cold extraction methods for both water and dichloromethane solvents, as shown in table (4.2).

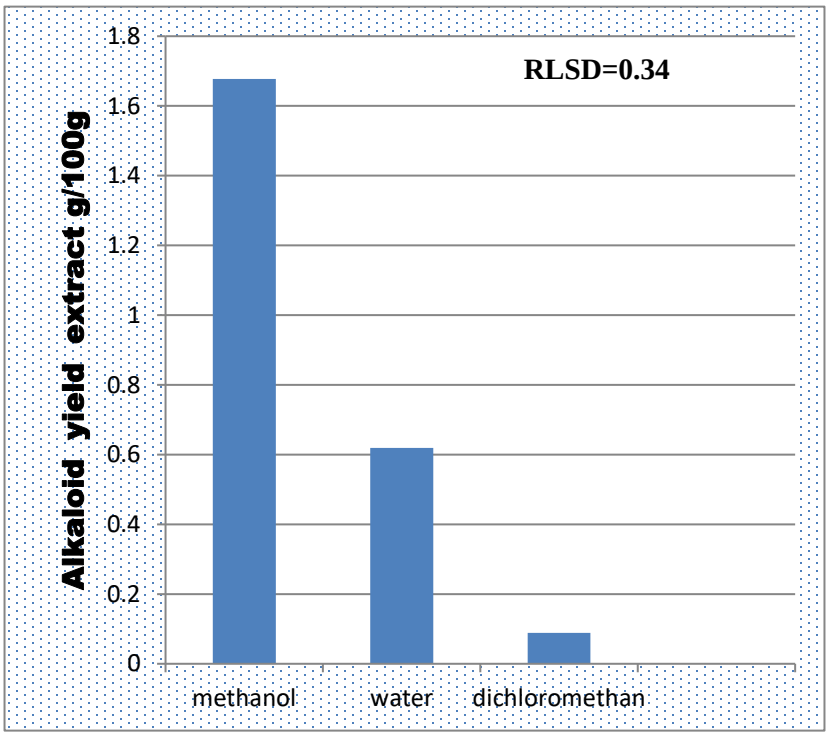

Figure (4-1): Comperatave Alkaloid yield extracts from two studied plants by different solvents

Table (4-2): Percentage of alkaloid yield extracts for two studied plant by using three solvents (Methanol ,water and dichloromethane) with hot (soxhlet) and cold (Maceration) extraction methods.

	% of Alkaloid yield extracts g/100g						
Plant Species	**Methanolic Extraction**		**Water Extraction**		**Dichloromethane Extraction**		
	Hot	Cold	Hot	Cold	Hot	Cold	**RLSD**
C. lancifolius	1.60	0.96	0.96	0.40	0.14	0.03	0.369
C. procera	2.76	1.33	0.77	0.34	0.13	0.06	0.462

4-3 GC-MS analysis for crude alkaloid compounds in two studied plants

Finding of GC-MS analysis for leaf methanolic crude alkaloid extracts of *C. lancifolius* revealed the presence of one type of alkaloid compound that is Methoxy phenyl oxime (MPO) at R.T reached 5.2 min with area percentage reached 42.02% and molecular weight 551.16g/mol as seen in the table (4.3), figures (4-2, 4-3,4-4), moreover the results of leaf methanolic crude extract of *C. procera* revealed the existance of one type of alkaloid compound that was Colchicine (CO) at RT reached 12.8 min with area percentage reached (7.1%) with molecular weight (399.4g/mol) as illustrated in table (4-4) and figures (4.5)and (4.6). On the other hand, GC-MS analysis for methanolic root alkaloids extracts of *C. procera* revealed the presence of two types of alkaloid compounds that are;1H-Indole,2,3-dihydro-4-methyl ($C_9H_{11}N$) at R.T (21.9 min), with area percentage reached (42.4%) with molecular weight (133.19g/mol) and 1,3, benzene dicarboxylic acid,5-dimethylamino ($C_{10}H_{11}NO_4$) at R.T (23.7min) with area percentage reached (45%) with molecular weight (209.2g/mol) as shown in table(4-5) and figures (4-7,4-8, and 4-9).

Table 4-3: GC-MS analysis for leaf Methanolic alkaloid extract of *C. lancifolius.*

Name of alkaloid compound	Retention. Time (min)	Formula	M Wt. g/mol	Area percentage %
methoxy phenyl oxime	5.2	$C_8H_9NO_2$	151.16	42.02%

Table (4.4): GC-MS Analysis for leaf Methanolic alkaloid extract in *C.procera.*

Name of alkaloid compound	Retention.Time Min	Formula	M wt. g/mol	Area percentage %
Colchicine	12.8	$C_{22}H_{25}NO_6$	399.4	7.1%

Table (4.5): GC-MS Analysis for Root Methanolic alkaloid compounds in *C. procera*

Name of alkaloid compound	**Retention.Time (Min)**	**Formula**	**M wt. g/mol**	**%Area**
1H-Indole,2,3-dihydro-4-methyl. (4-Methylindoline)	21.9	$C_9H_{11}N$	133.19	42.4%
1,3,benzene dicarboxylic acid,5-(dimethylamino)	23.7	$C_{10}H_{11}NO_4$	209.2	45%

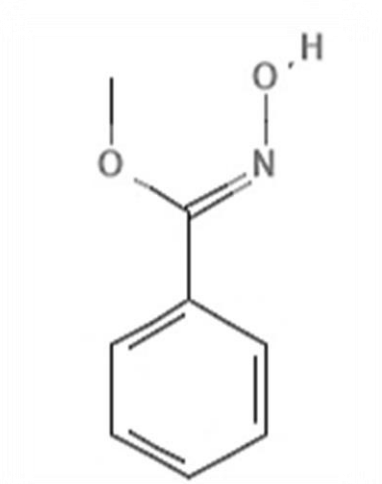

Figure (4-2) Chemical structure of alkaloid compound Methoxy Phenyl Oxime

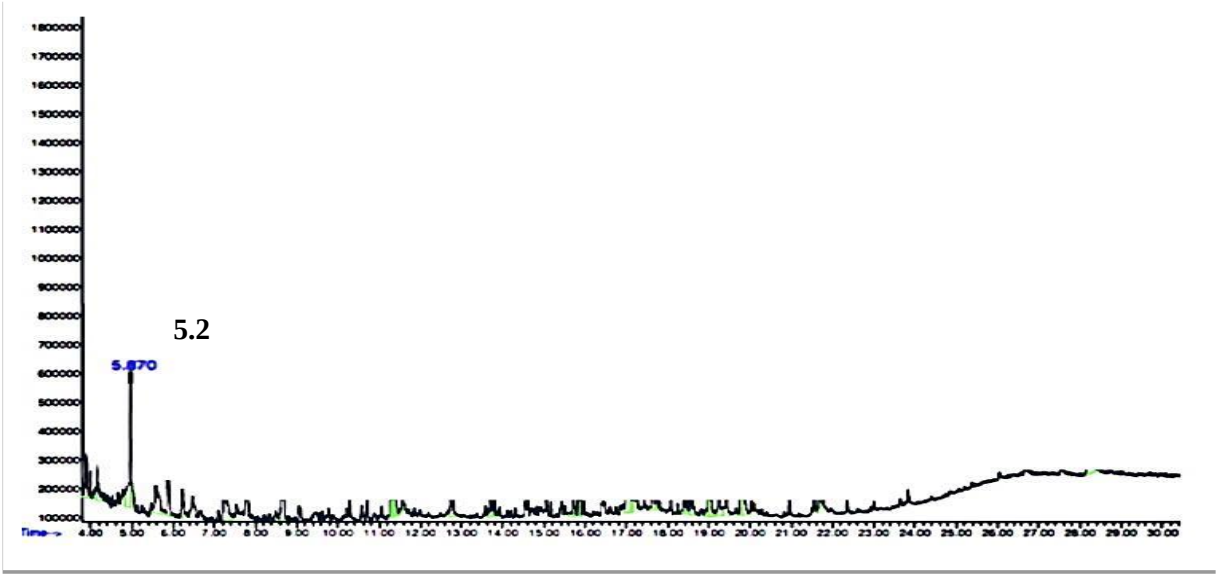

Figure (4-3): GC-MS chromatogram for leaf methanolic alkaloid compounds in *C. lancifolius.*

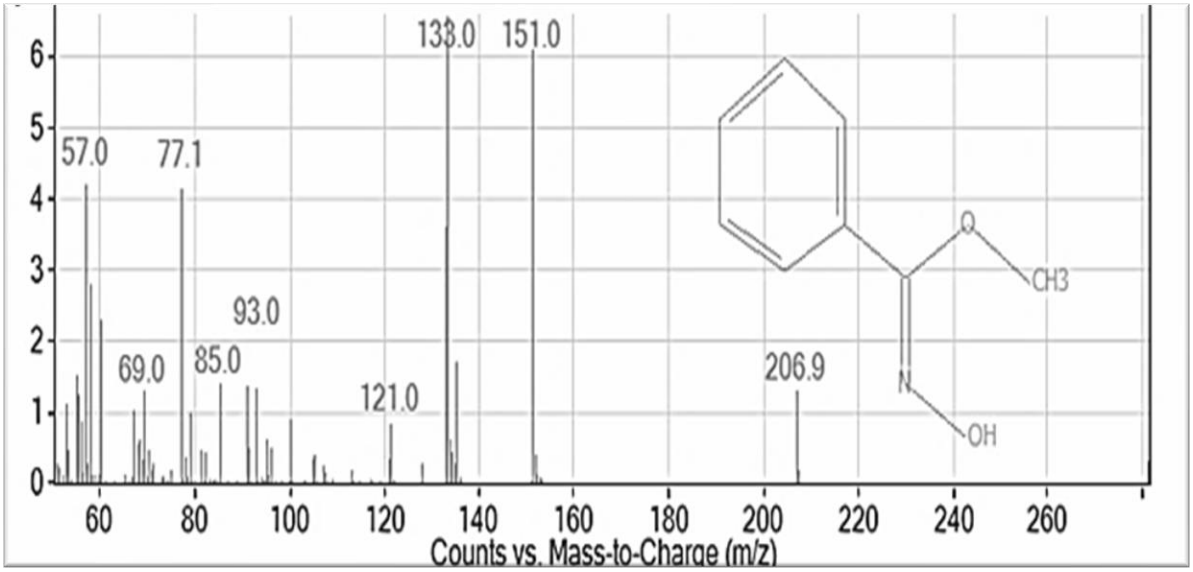

Figure (4-4) Mass-spectrum of alkaloid compound Methoxy Phenyl Oxime $C_8H_9N0_2$ that existing in leaf methanolic extract of *C.lancifolius*.

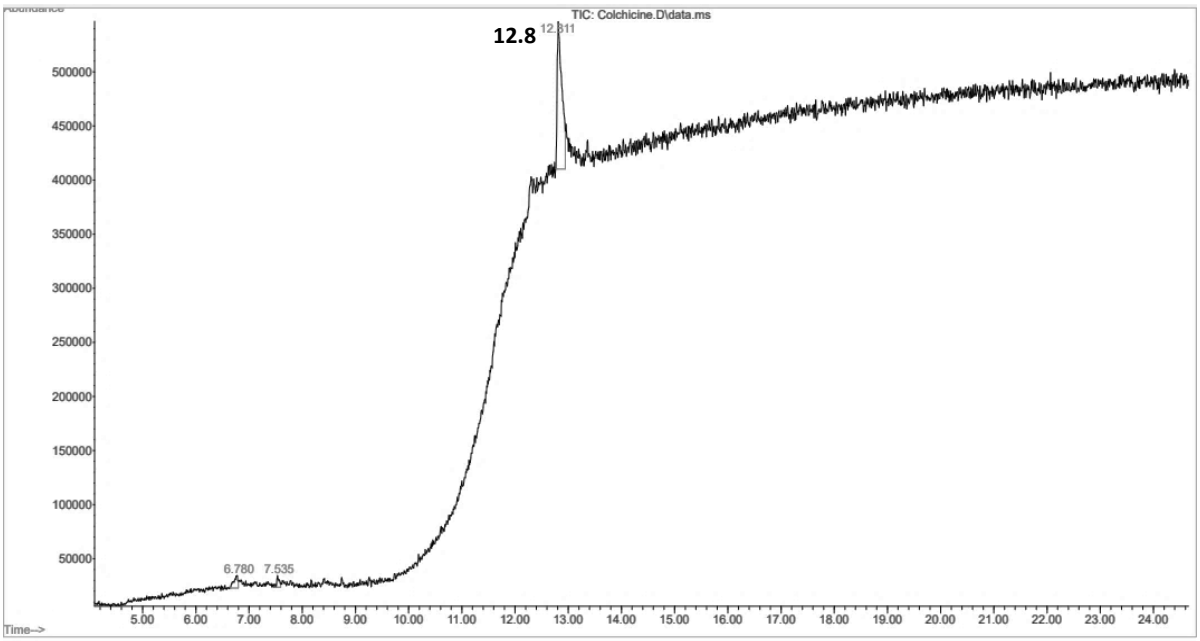

Figure (4-5) GC-MS Chromatogram for leaf Methanolic alkaloid compound in *C. procera*

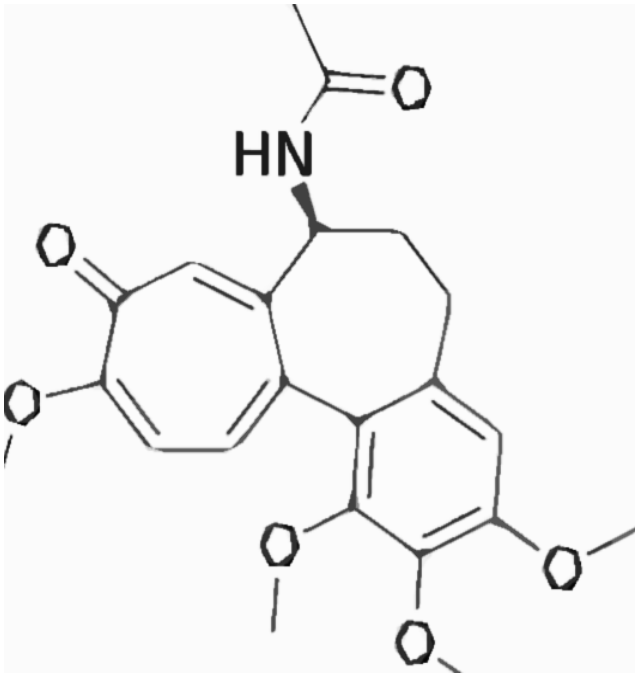

Figure (4-6) Chemical structure of colchicine alkaloid compound

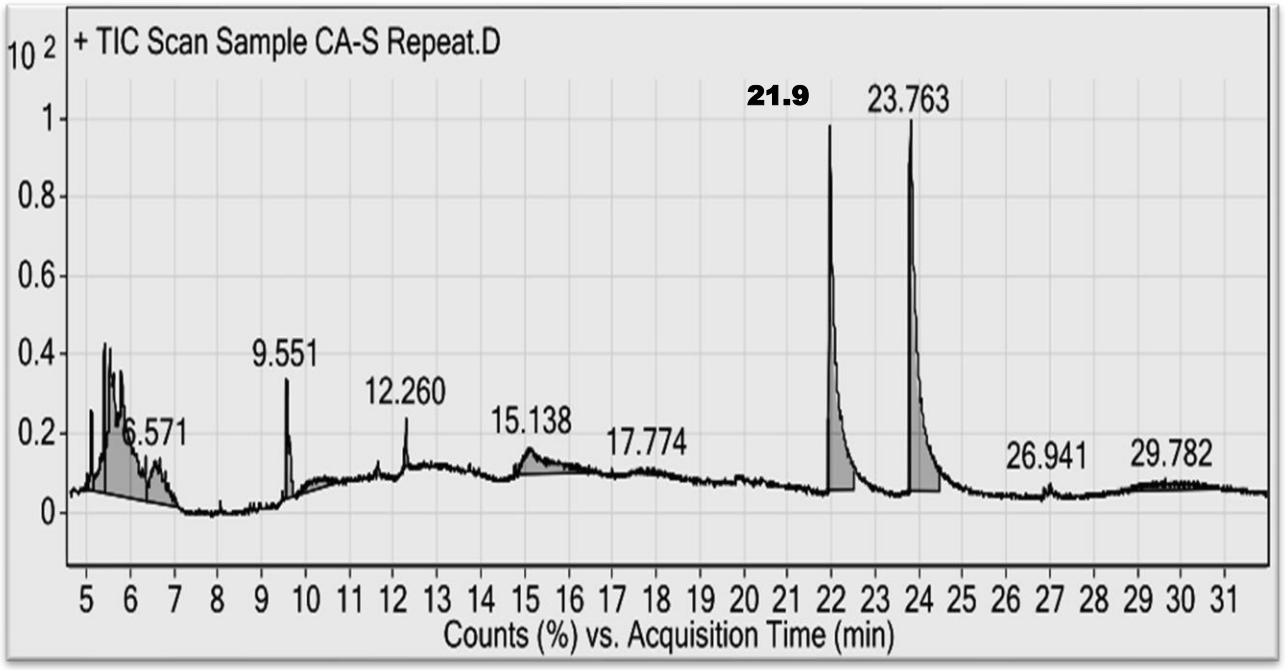

Figure (4-7) GC-MS chromatogram for Root methanolic crude alkaloid compounds of *C.procera.*

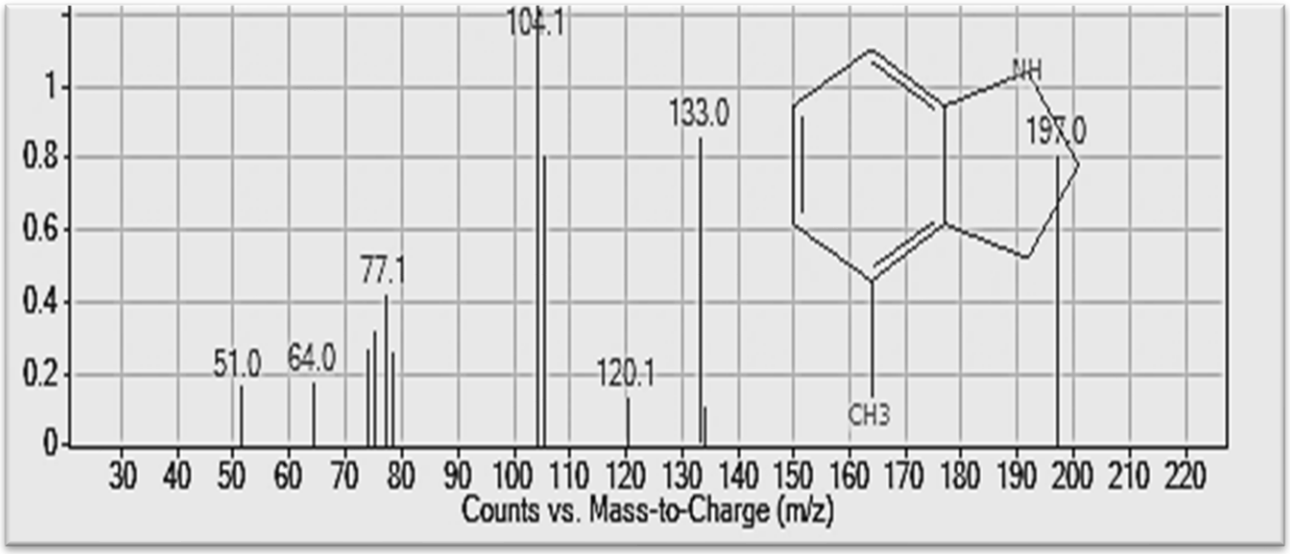

Figure (4-8) Mass Spectrum of alkaloid compound 1H-Indole,2,3-dihydro-4-methyl $C_9H_{11}N$ founded in root of *C. procera* .

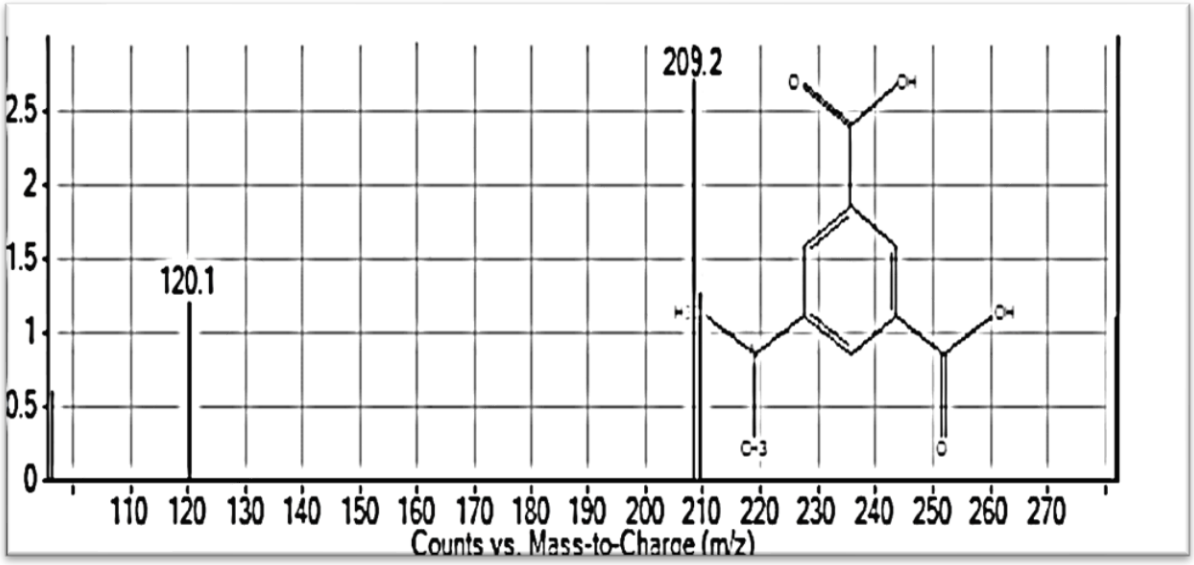

Figure (4-9) Mass spectrum of alkaloid compound 1,3,benzenedicarboxylic acid,5-dimethylamino($C_{10}H_{11}NO_4$) founded in root of *C.procera.*

4-4 Isolation, Purification and Identification of alkaloid compounds by HPLC technique

The HPLC analysis for methanolic leaf alkaloid extract of *C. lancifolius* resulted in the separation and purification of a rare alkaloid compound, Methoxy phenyl oxime. Identification of the alkaloid compound methoxy phenyl oxime compound was determined at an R.T reached (10.6min) as shown in figure (4.10). While, HPLC analysis for leaf methanolic alkaloid extract of *C. procera* resulted in separation and purification of colchicine that was identified by comparison of its actual retention time to the retention time of colchicine standard reference (Sigma Aldrich) at chromatographic conditions, where the two peaks for colchicine and its standard corresponded in their retention time that was reached 2.5, min, as shown in figure (4.11),(4.12). Furthermore, HPLC analysis for methanolic root alkaloid extract of C.*procera* resulted in the separation and identification of two main

peaks at retention time (4.4min and 6.1min) at 300 wavelengths as shown in figure (4-13).

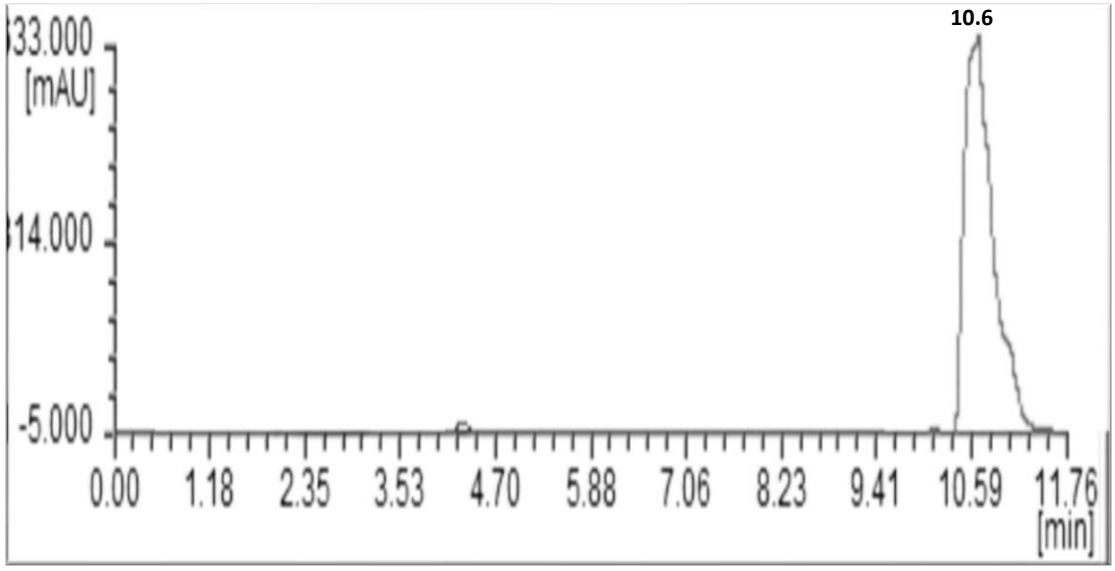

Figure (4-10) :HPLC chromatogram for purified MPO in leaf methanolic extract .of *C.lancifolius* plant

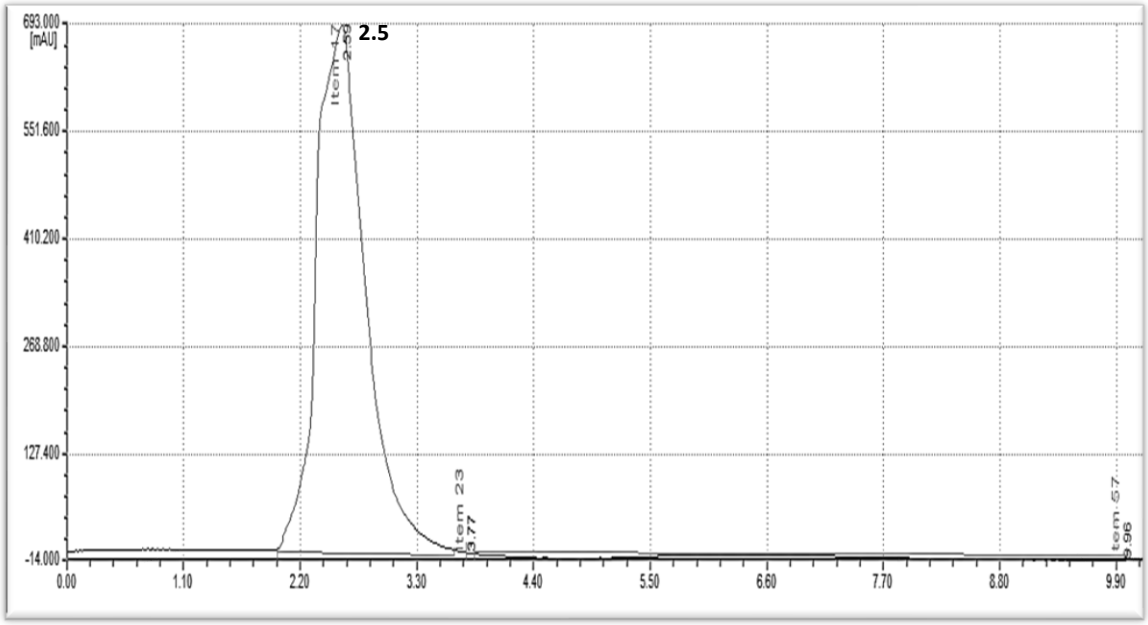

Figure (4.11) HPLC chromatogram for standard of colchicine

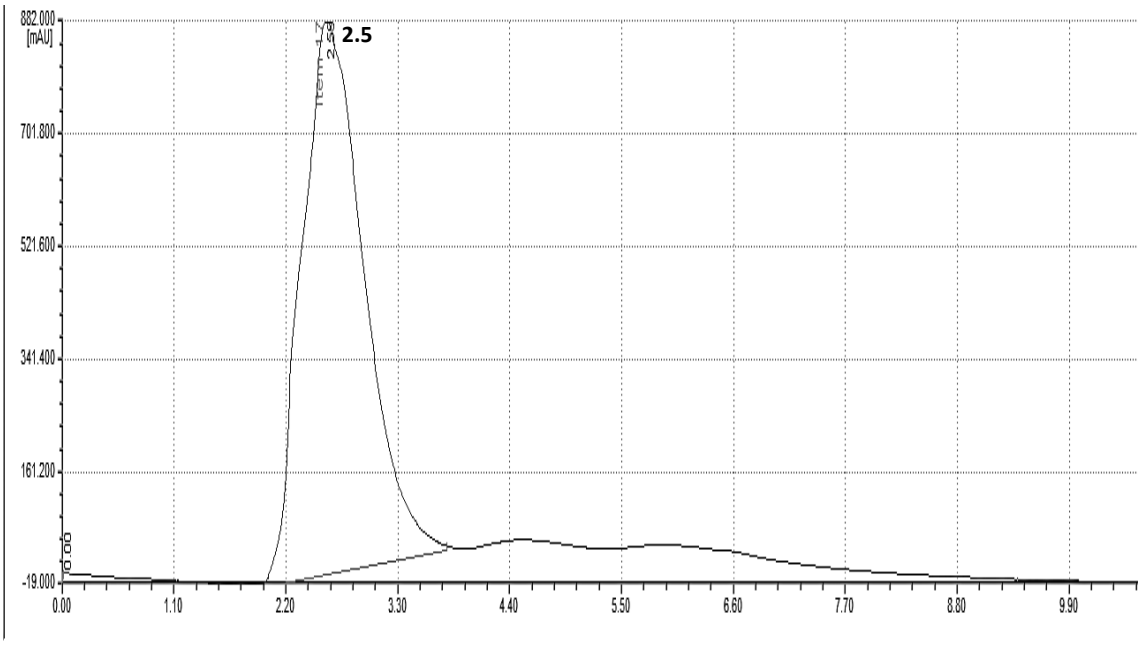

Figure (4.12)-HPLC chromatogram for purified Colchicine founded in leaf methanolic alkaloid extract in *C. procera.*

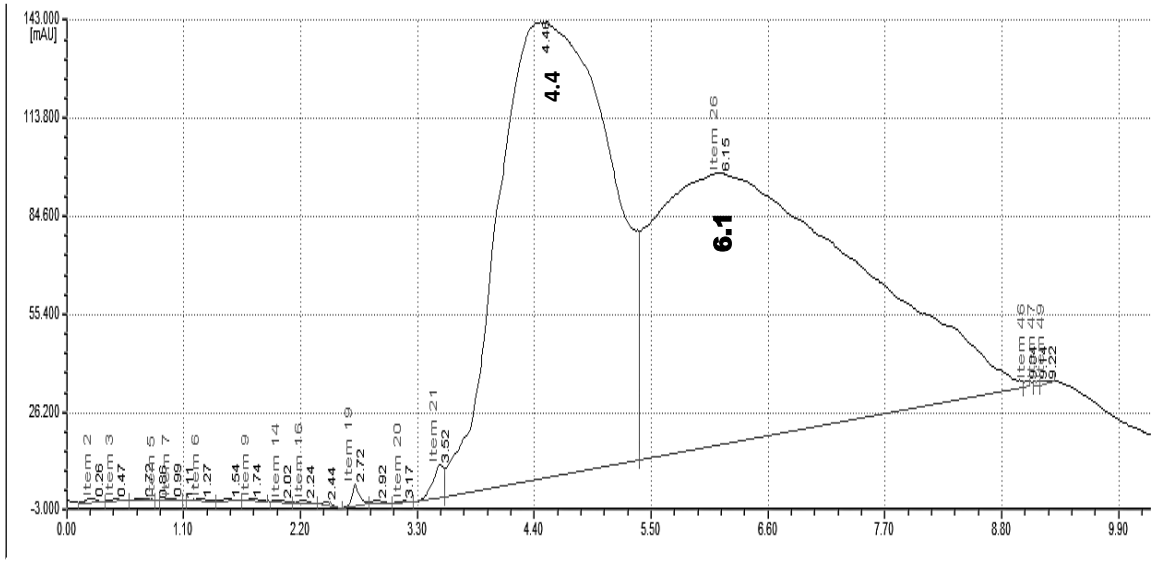

Figure (4.13) HPLC chromatogram for root methanolic alkaloid extract in *C. procera*

4-5 Thin Layer Chromatography (T L C) Results

As shown in table (4.6), the results of TLC separation for a sample of purified colchicine by using chloroform: methanol: formic acid in portion (20:1:0.2) solvent system revealed an appearance of one clear spot that was identical to standard colchicine spot of a value of *Rf* reached 0.22 in compared with 0.24 for the standard compound as shown in figure (4-14-A). Also, separation for a sample of purified MPO compound resulted in the appearance of one clear spot by using Hexane: ethyl acetate : methanol: water (7:3:5:5) solvent system with a value of *Rf* reached 0.71 as shown in figure (4-14-B).

Table 4.6: *Rf* value for two purified alkaloid compounds.

Purified alkaloid compounds	**Solvents system**	***Rf***
Standard Colchicine	Chloroform : methanol : formic acid (20:1 :0.2)	0.24
Colchicine	Chloroform : methanol : formic acid (20:1 :0.2)	0.22
Methoxy phenyl oxime	Hexane: ethyl acetate : methanol: water (7:3:5:5)	0.71

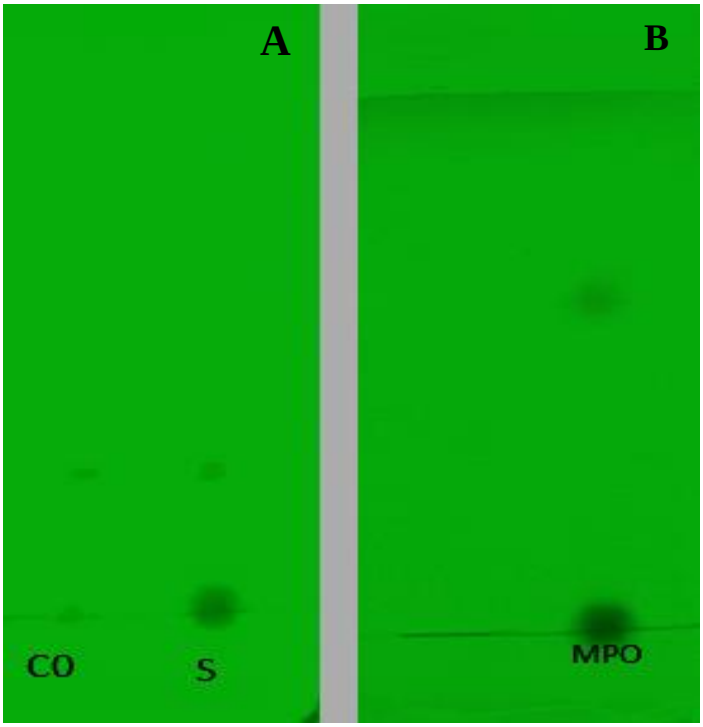

Figure (4-14); Analytical TLC under UV light at 254 nm for(A) ; Colchicine (CO) with its standard (S) and.(B) Methoxy Phenyl Oxime (MPO).

4-6 Identification and characterization of purified alkaloid compound Methoxy phenyl oxime

4-6-1 Physical properties

Some physical properties of the purified alkaloid compound (MPO) were illustrated in table (4-7), represented by Melting point (M. P), Color and appearance.

Table 4.7: Some physical properties for purified alkaloid compound (M P O) represented by Melting point (M. P), Color & Appearance

Alkaloid compound	M.P.(^{0}C)	Colour	Appearance
MPO	63-65	White	Crystals

4-6-2 Fourier transforms infrared spectroscopy (FT-IR)

FT-IR analysis for alkaloid compound MPO showed the O-H groups that were interpreted by the band at (3351.58 cm^{-1}). Also, the band appeared at (2928 cm-1) belonging to the CH- stretching aliphatic C-CH_3 group. The absorption band appeared at 3000 cm-1 that was overlapping with broad band of (OH) group attributed to C-H (Aromatic) group stretching vibration as illustrated in the table (4-8) and figure (4-15).

Table 4-8 the characteristic IR absorption bands in (cm-1) of purified alkaloid compound MPO.

Functional group	Frequency wave number in cm^{-1}
OH	3351.58
CH (aliphatic)	2928
CH (aromatic)	3000

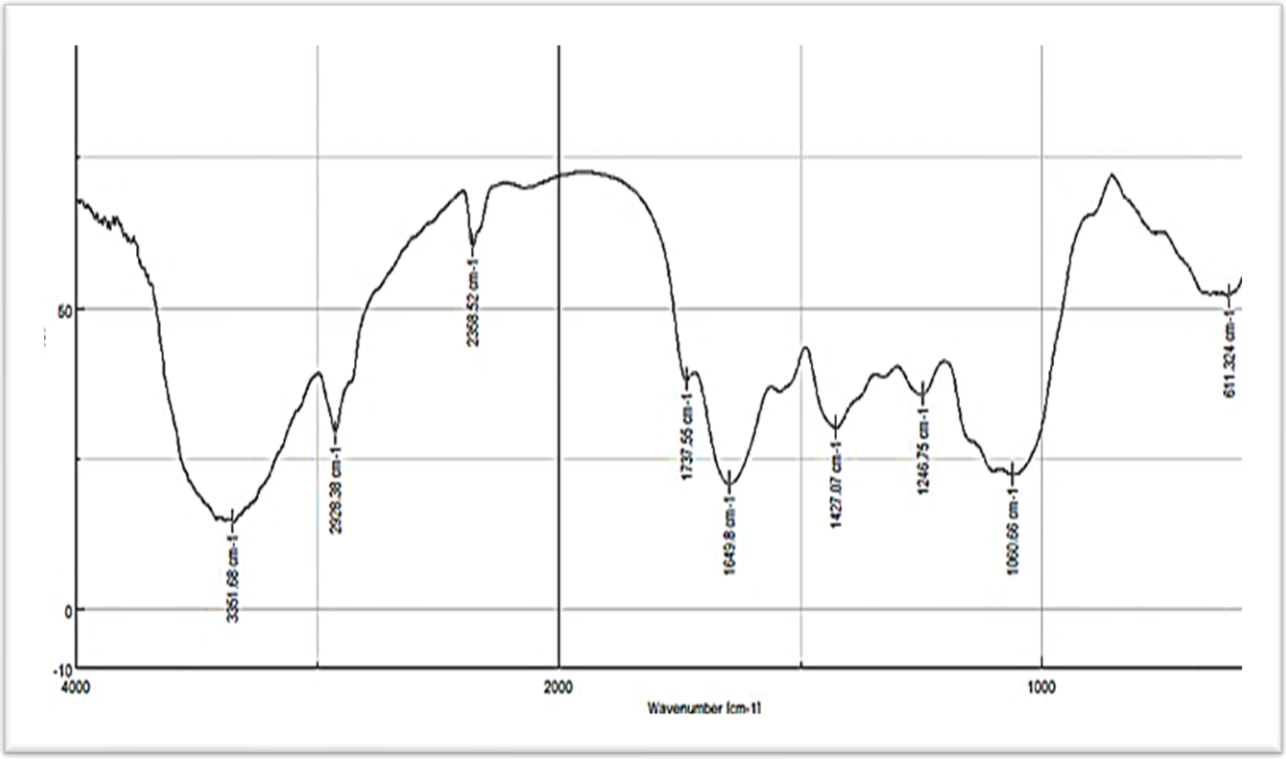

Figure (4-15): FT-IR spectrum of alkaloid compound MPO purified from *C. lancifolius*

4-6-3 The HNMR spectra

Results of the HNMR interpretation of the MPO alkaloid compound spectrum were reflected in table (4.9), and spectra were shown in figure (4.16). Assay performed for MPO alkaloid compound using DMSO-d_6 as a solvent, so one single to this solvent was seen at 2.509 ppm, multiple signals between(7.27-7.28) belong to aromatic protons also, there is signal appeared at 2.09 ppm belong to OH-group. Besides, the singlet signal at 3.33 belongs to -CH_3 group.

Table (4.9): Results of H Chemical Shift of Methoxy phenyl oxime compound MPO purified from *C.procera*

Chemical Shift (ppm)	Integration	Type of H-Bond	Splitting type
2.09	1H	-OH	Singlet
2.509	6H	DMSO	-------
3.33	3H	-CH3	Singlet
7.27-7.28	5H	Aromatic (C-H)	Multiple

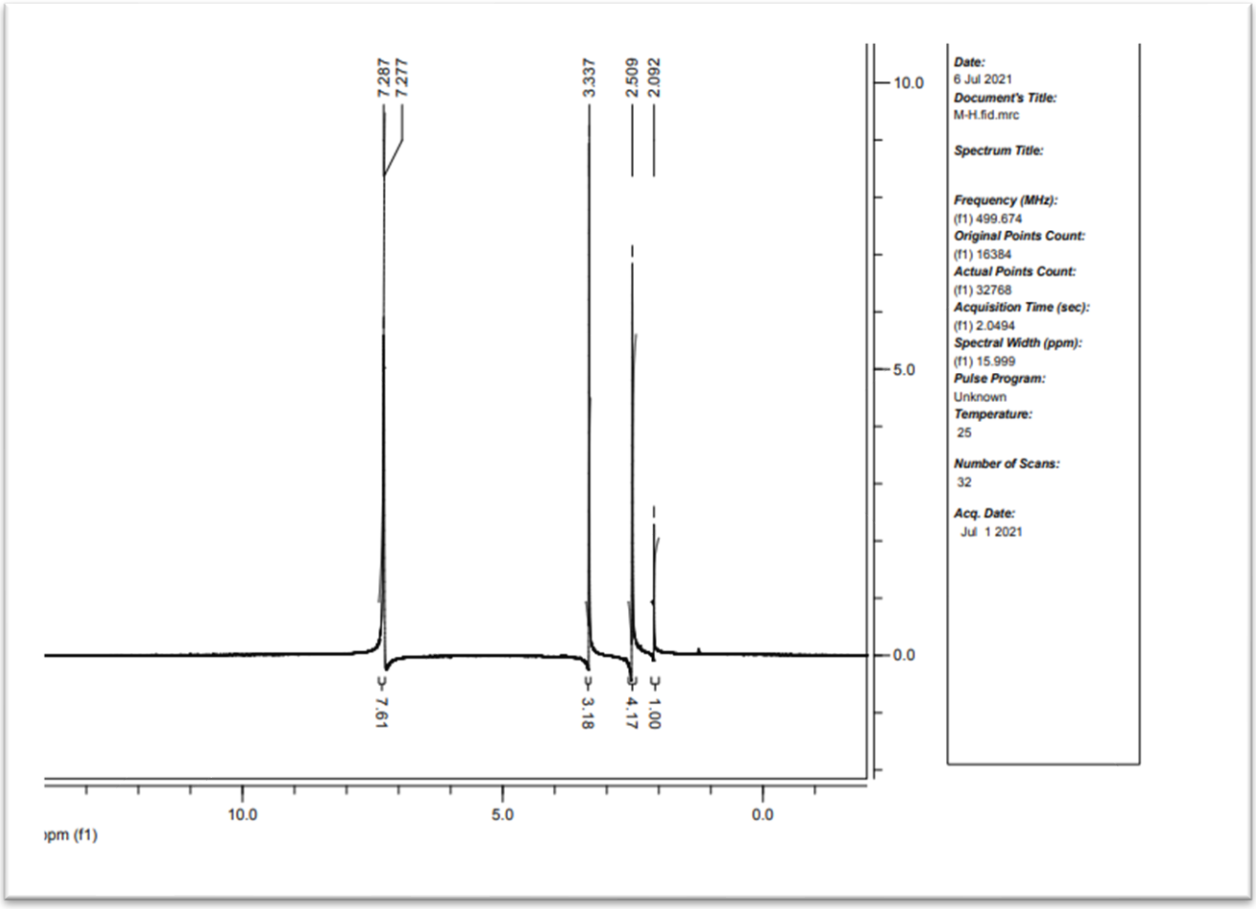

Figure (4.16): The HNMR of Methoxy phenyl oxime compound MPO purified from *C.lancifolius*

4-7 Antibacterial activity of alkaloid compounds purified from two studied plants

Finding of antibacterial activity for two purified alkaloid compounds, methoxy phenyl oxime (MPO), colchicine(CO) and crude alkaloid compounds(CR), against each studied gram-positive bacteria *S. aureus* and *B.subtilis* and gram-negative bacteria *K.pneumoniae* and *E.coli* revealed that MPO has a significant diameter of inhibition zone at a concentration of (200µg) on *B.subtilis* of a value reached 22.6mm as compared with inhibition zone diameter against both *E.coli, K. pneumonia* at the same concentration that reached 20.6mm,18.3mm respectively.

Still, there were no significant differences with inhibition zone diameter against *S.aureus* that reached 22.3mm. as seen in figure (4.17). Whereas, the finding of antibacterial activity for (CR) against four studied bacteria appeared to have a significant inhibition zone diameter at a concentration of (200µg) against *E.coli* reached 17mm as compared with inhibition zone diameter against *B.subtillus*, *S. aureus* and *K.pneumoniae* that reached 16mm,13.3mm,10.6mm respectively as seen in figure (4.18).There was a weak antibacterial activity of Colchicine at all concentrations that appeared at about 4.3mm on average, as shown in figure (4.19).

The results of sensitivity of four studied bacteria towards the alkaloid compound MPO, CO, crude alkaloid extract CR and positive control Gentamycin antibiotic(GEN) showed that *B.subtilis* bacteria has a significant sensitivity at conc 200 towards MPO alkaloid compound at a value reached (22.66mm) as compared to GEN, CR and CO at the same conc that reached (20,16,5.6mm) respectively.

Also, *S.aureus* sensitivity was significant at 200 con, towards MPO alkaloid compound at a value reached (22.33mm) as compared to GEN, CR and CO that reached (21.4mm, 13.3mm, 5.7mm) respectively. Besides, *E.coli* bacteria also has a significant sensitivity at conc 200 towards MPO alkaloid compound at a value reached (20.6mm) compared to GEN, CR, and CO (18mm,17mm,6.3mm), respectively.

On the other hand, *K pneumoniae* bacteria sensitivity has a significant difference at 30 conc towards GN antibiotic with a value reached 19mm as compared to MPO, CR and CO that got values (18.3mm,10.6mm, and 4.3mm) respectively, also negative control of DMSO solvent was tested as illustrated in a figure (4.20) and table (4.10).

Table 4-10 Antibacterial activity of two purified alkaloid compound MPO, CO and CR

		Inhibition zone /mm					
Plants	**Alkaloid compound**	**Con µg/ml**	***S.aureus***	***B.subtilis***	***E.coli***	***K.pneumonia***	**RLSD**
C.lancifolius (Leaf)	Methoxy phenyl oxime	200	22.33	22.66	20.66	18.33	Bacteria =1.34 Conc.= 1.64
		150	21	21.3	19.3	18	
		50	18	20.3	18.3	18	
C.procera (Root)	Crude alkaloids	200	13.3	16	17	10.6	Bacteria =0.80 Conc.= 0.79
		150	12	15.3	16	9	
		50	11	13.3	15.3	7.3	
C.procera (Leaf)	Colchicine	200	5.7	5.6	6.3	4.3	Bacteria = 0.94 Conc.= 0.82
		150	5.7	3.6	5.3	2.6	
		50	5	2.6	4	1.6	
Positive control	Gentamycin	30	21.4	20	18	19	
Negative control	DMSO	0	0	0	0	0	

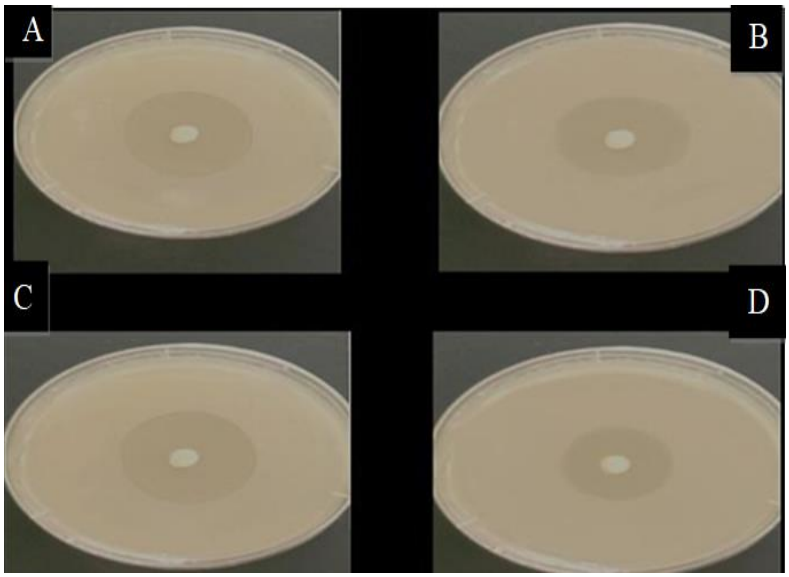

Figure (4-17) : Antibacterial activity of MPO alkaloid compound at 200 µg/ml against *S.aureus*(A) ; *B.subtilis* (B) ; *E.coli* (C) ; *K.pneumoniae* (D).

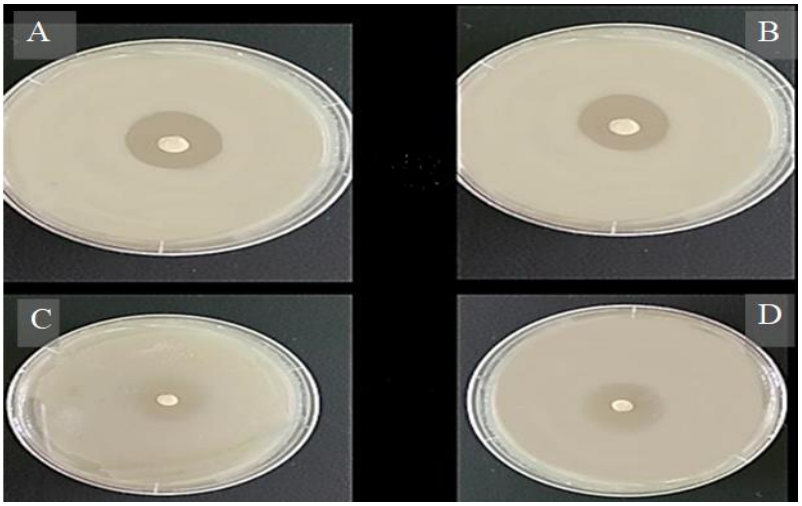

Figure (4-18): Antibacterial activity of Crude alkaloid extracts at 200 µg/ml against *S.aureus* (A) ; *B.subtilis* (B) ; *E.coli* (C) ; *K.pneumonia* (D).

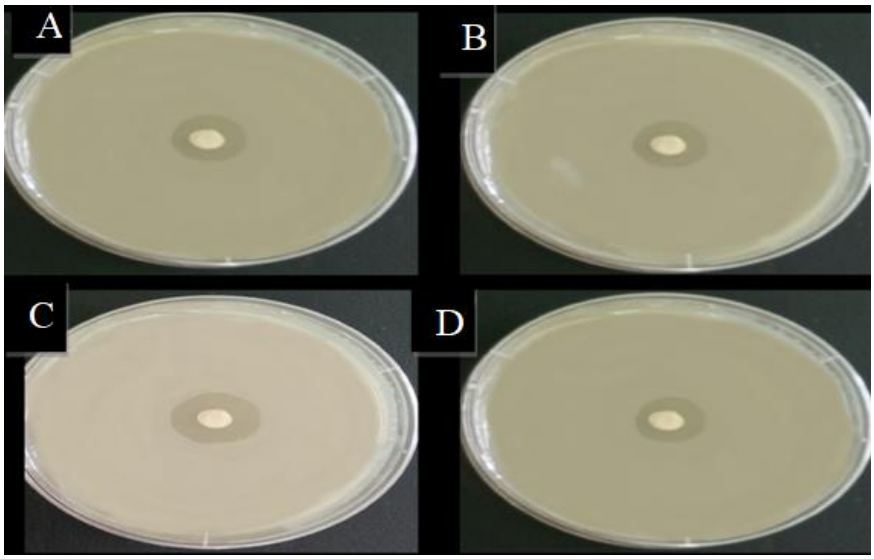

Figure (4-19): Antibacterial activity of Colchicine alkaloid compound against at 200 µg/ml *S.aureus* (A) ; *B.subtilis* (B) ; *E.coli* (C) ; *K.pneumonia*e (D)

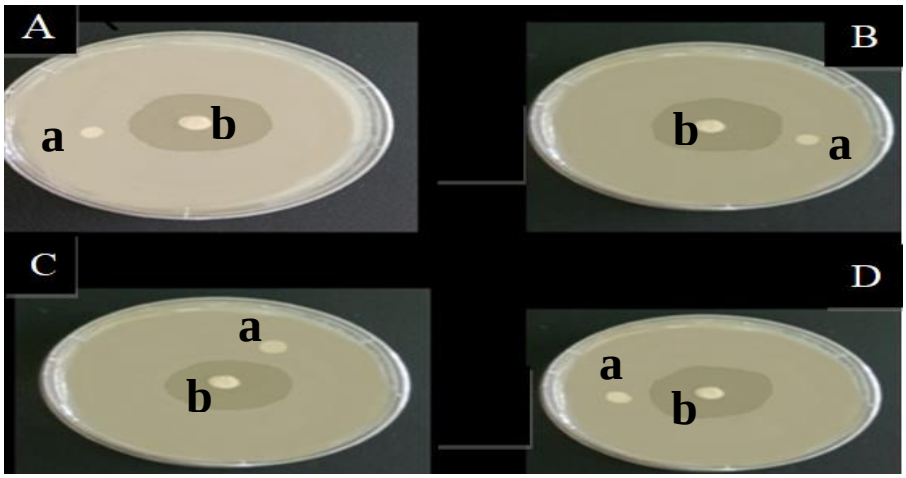

Figure (4-20) : Antibacterial activity of **–b-**Gentamcin antibiotic at 30µg/ml (positive control) and **-a-**DMSO compound as (negative control) against *S.aureus* (A) ; *B.subtilis* (B) ; *E.coli* (C) ; *K.pneumoniae* (D).

4-7-1 Minimum Inhibition Concentration (MIC) Results

The results of MIC of the purified compounds MPO and crude alkaloid extracts, as indicated in table (4.11), appeared that Methoxy phenyl oxime has the lowest value of MIC against B.subtilis that reached 35 µg/ml while the biggest value was against K. *pneumoniae and S. aureus that* reached 95 µg/ml, and against *E coli* reached 55µg/ml respectively. Also root crude alkaloid compounds have the lowest MIC value against *B.subtilis*, which reached 55 µg/ml, and the biggest value was against *K. pneumoniae,* which reached 135 µg/ml, and MIC values against both *S. aureus and E. coli was* reached 75 and 115 µg/ml respectively. Whereas colchicine compound has the bigest value of MIC against four studied bacterial species that reached 175 µg/ml.

Table (4.11): MIC of purified alkaloid compound MPO,Co and CR against four studied bacteria

		MIC% µg/ml			
Plant	**alkaloid compounds**	***S.aureus***	***E. coli***	***K.pneumoniae***	***B. subtilis***
C.lancifolius (Leaf)	Methoxy phenyl oxime	55	95	95	35
C. procera (Root)	Crude alkaloid extract	75	115	135	55
C. procera (Leaf)	Colchicine	175	175	175	175

4-7-2 Hemolysis test for two purified MPO , Colchicine and root alkaloid extracts on human RBCs.

Percentage of human RBC hemolysis for each alkaloid compound Methoxy Phenyl Oxime, root alkaloid extracts and Colchicine that were reached (1.22%, 1.54%, 2.31%) respectively. Thus, there was weak cytotoxicity for each alkaloid compound compared to a positive control (tap water) that caused 100%hemolysis for human RBCs.

Table (4.12): Human erythrocytes hemolysis percentage for alkaloid compounds, Methoxy Phenyl Oxime, root crude alkaloid extracts of *C. procera and* Colchicine at 450nm.

Alkaloid compounds	Absorbance at 450nm	Hemolysis percentage %
Methoxy Phenyl Oxime (50 µg/ml)	0.027	1.22%
Root alkaloid extract in *C.procera* (50 µg/ml)	0.032	1.54%
Colchicine (50 µg/ml)	0.044	2.31%
Normal saline(Negative Control)	0.008	0%
Tap water (Positive Control)	1.562	100%

4-8 In *vitro* anticancer activity of studied alkaloid compounds

4-8-1 Cytotoxicity study of the studied alkaloid compounds against (MCF-7) and (SK-GT-4) cell lines.

Cytotoxicity effect of the two purified alkaloid compounds Colchicine, Methoxy phenyl oxime and root methanolic crude alkaloid extract was tested by studying their ability as anti-proliferative against human cancer cells (MCF-7&SK-GT-4) that showed colchicine compound has a cytotoxic activity against both of two cell lines while MPO compound appeared to have a significant selective cytotoxic activity against SK-GT-4 cell line only. In contrast, Root methanolic crude alkaloid extract has no cytotoxic activity against the two cell lines (MCF-7&SK-GT-4). As seen in figure (4-21) Results of CO compounds showed significant mean of percentage of viability on MCF-7 cells at Con (500) that reached 22.8 % as compared with values for Con of (5 ,10,50,100 and 250) that reached 100% ,98.6%, 84.9% ,23.7% and24.8% respectively.Also, the IC50 value was calculated to CO compound on MCF-7 and found to be 55.33 µg/ml.

While as reflected in fgure (4.22) CO compound showed a significant mean of percentage of viability on SKG-T4 cells at Con (1000) that reached 32.74 % as compared with values for 250 and 500 that reached 56.4% and 46.7% respectively. And there were no significant differences with 750 Con that reached 33.72%. Also, the IC50 value was calculated to CO compound on SKG-T4 and found to be 522 µg/m

whereas , results for MPO compound Showed significant mean of percentage of viability on SKG-T-4 cells at Cons (250) that reached 19.2% as compared with values for 1 , 5 and10 Consentrations that reached(82.2%

,78.9% and 27.3)respectively. And there was no significant differences with 250 Conc that reached 19.4% ,also, the IC50 value was calculated to MPO compound on SKG-T4 and found to be 7.59 µg/ml,as seen in figure (4.23). On the other hand, Results of antiproliferatave activity of two purified MPO, andCO at Conc (1000) on normal cell HBL showed increasing viability effect of MPO compound on HBL cells with value reached 132% in compared with CO compound that has a low percentage of viability on HBL cells that reached 96.4%. as compared with control (0 conc) as seen in figure(4.24) and (4.25) respectively.

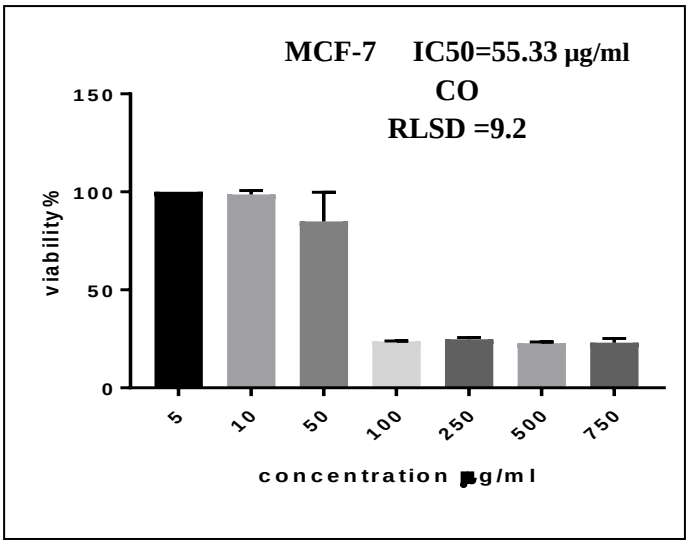

Figure (4.21): Cell viability percentage of (MCF-7) cell line treated with Colchicine compound concentrations

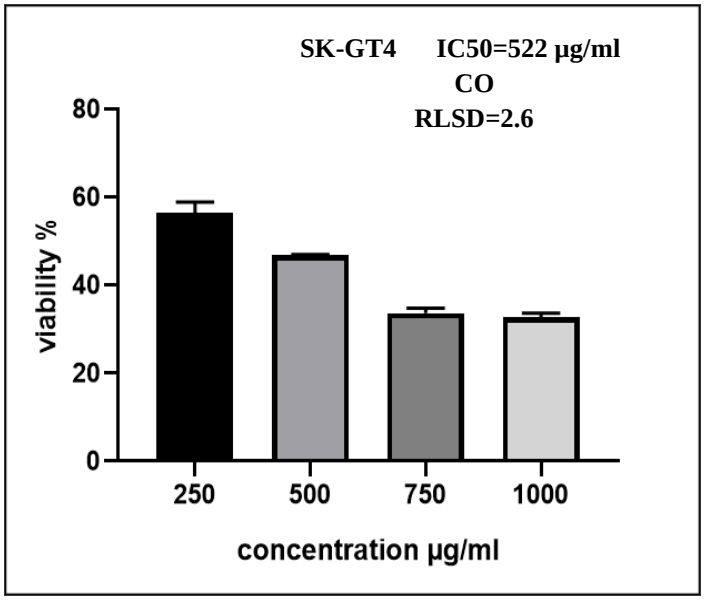

Figure (4.22): Cell viability percentage of SKGT-4 cell line treated with Colchicine compound concentrations

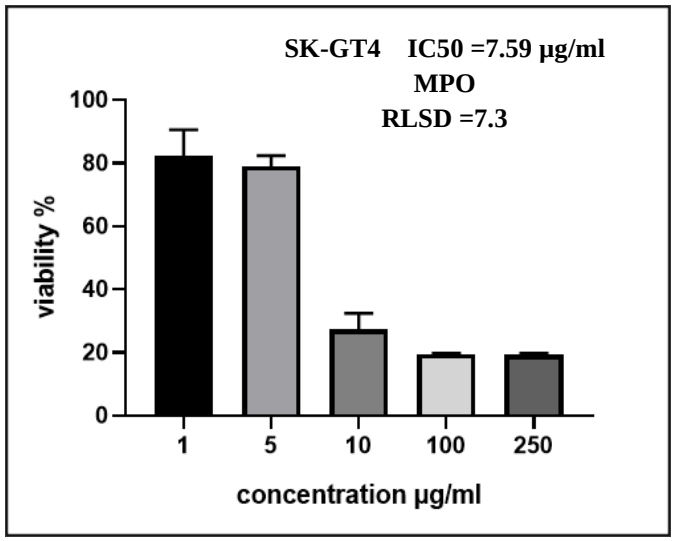

Figure (4.23): Cell viability percentage of (SKGT-4) cell line treated with MPO compound concentrations

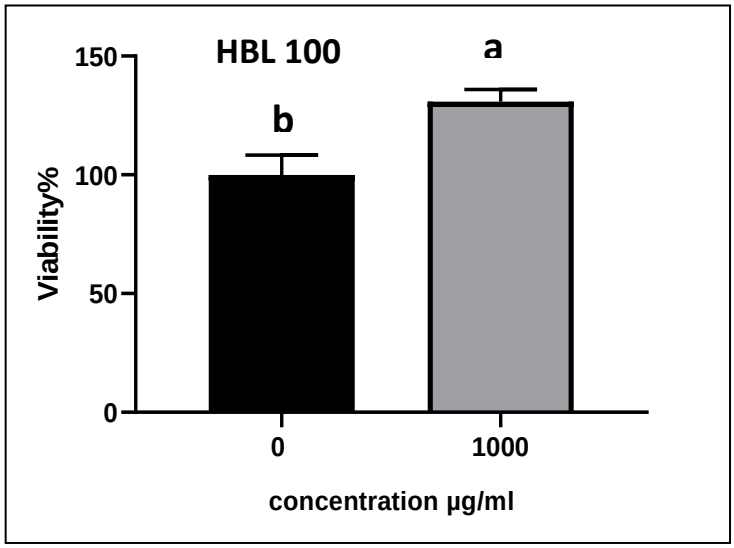

Figure (4.24): Cell viability percentage of MPO compound on normal HBL cell line ;(a) treated with MPO compound (1000µg / ml) ,(b) Negative control (media+DMSO)

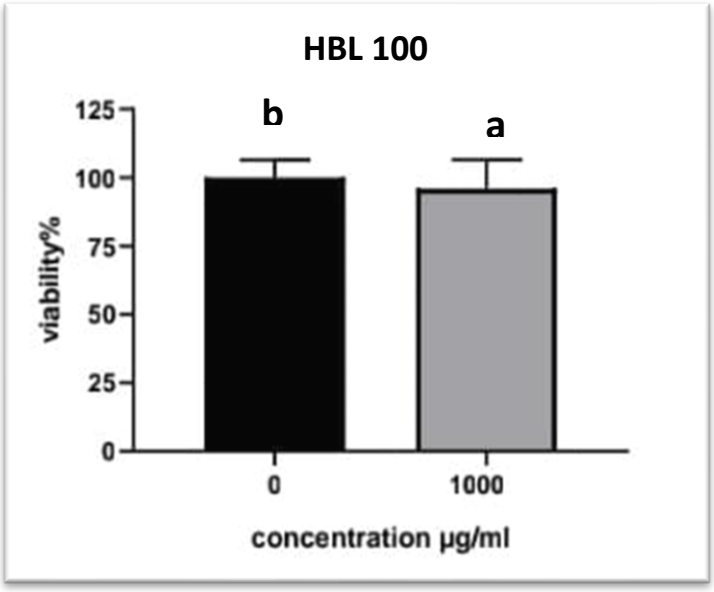

Figure (4.25): Cell viability percentage of Colchicine compound on normal HBL cell line ;(a) treated with Colchicine compound (1000µg / ml) ,(b) Negative control (media+DMSO)

4-8-2 Microscopic View of cell lines

The findings of Microscopic View of cell lines revealed morphological changes following colchicine and MPO compound treatment at various time points, by usng Acridine orange ethidium bromide stain where the viable cells of untreated SK-GT-4 showed green colour, as shown in figure (4-26), while late apoptotic cells after being treated with colchicine and methoxy phenyl oxime alkaloid compound at a concentration of 522 µg/ml and 7.59µg/ml respectively showed reddish or orange fluorescence after 48 hours of incubation as shown in figures (4-27 and 4-28).

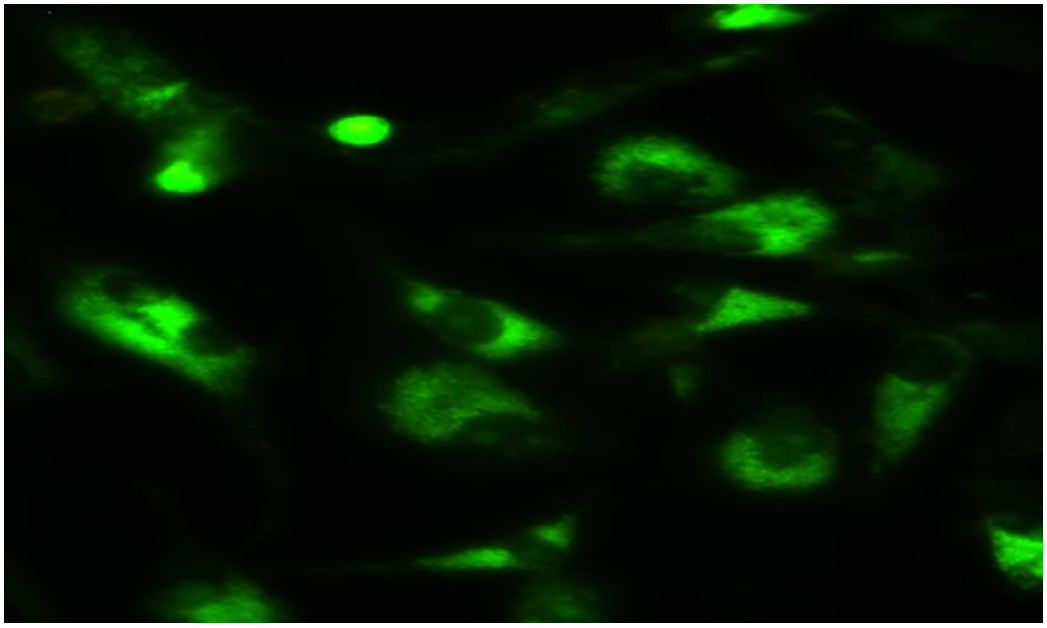

Figure (4-26): Control group (un-treated SK-GT-4 cells)
Acridine orange -Ethidium bromide stain,4000x

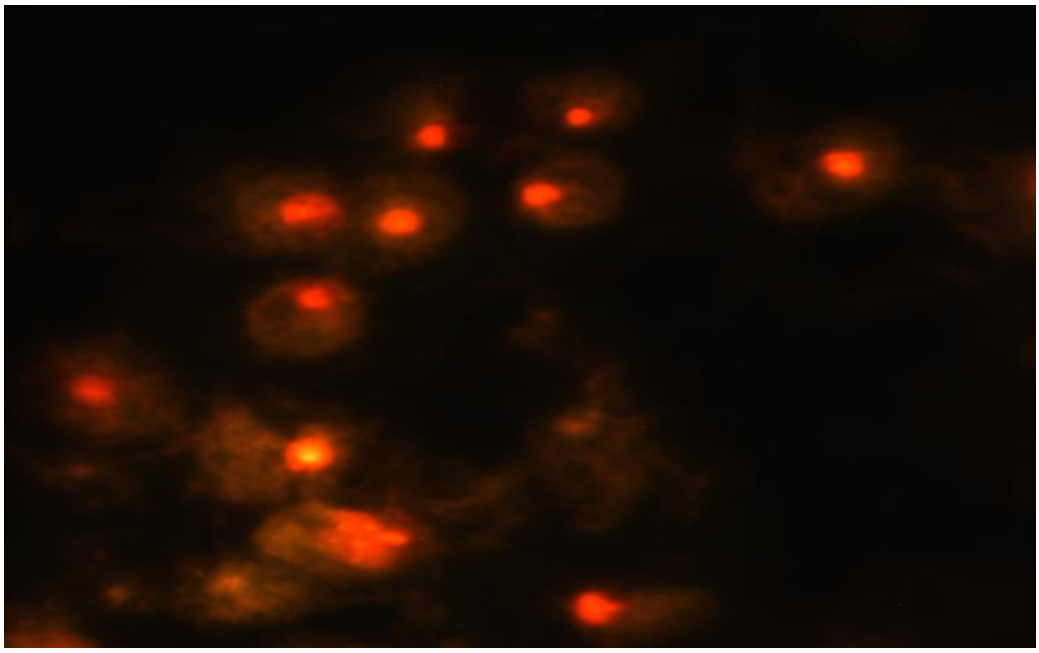

Figure (4-27) : Morphological changes in SK-GT-4 cells after treated(48h) with colchicine compound at IC_{50} =522 µg/Acridine orange -Ethidium bromide stain,4000x

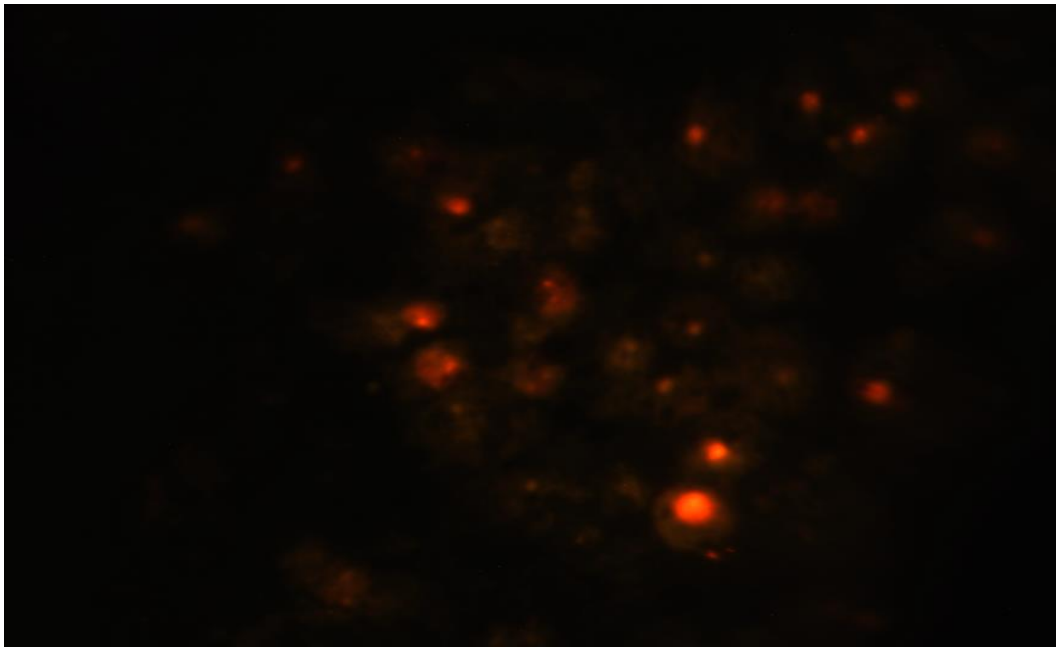

Figure (4-28): Morphological changes in SK-GT-4 cells after treated (48h) with MPO compound at IC50 = 7.59µg/ml. Acridine orange - Ethidium bromide stain,4000x

4-9 In *vitro* antioxidant activity for alkaloid compounds

The findings of DPPH scavenging activity percentage of root crude alkaloid extract CR and two purified alkaloid compounds, MPO and CO, revealed superiority of root crude alkaloid extract CR at all five concentrations (200,100,50,25,12.5) with values reached (96, 90.7,83.3,58.7, 39.4%) respectively to both of MPO and CO alkaloid compounds at significant differences of ($P\leq 0.05$) at values reached (%93.4,%85.3,%73.6,%41.3,%30.6%)and(%39.4,%23.2,%19.5,%13.5,%6.4) respectively.

On the other hand, DPPH scavenging activity percentage of ascorbic acid (Vit C) that was used as a positive control was superior to root crude alkaloid extract CR at significant differences of ($P\leq 0.05$) with values reached (98%,92.3%,86.1%,61.3%,43.1%), as seen in table (4.13).

Besides , significant inhibition percentage on free radical DPPH that was for Vit C at 200 Conc with value reached 98%,in compared to the lowest value which was for CO compound at 12.5 Conc that reached 6.4% at significant differences of ($P\leq 0.05$) as seen in table (4.13). Moreover calculation of IC_{50} for root crude alkaloid extract CR and two purified alkaloid compounds, MPO and CO were performed and the results were reached 17.83,26.3,57.94 (µg\ml**)** respectively as showed in figures (4-29).(4-30).and (4-31). Respectively.

Table 4.13: Free radical scavenging activity percentage by DPPH of (CR) (MPO), Colchicine and Vit. C (positive control).

Alkaloid compounds	**Inhibition%**					**RLSD**
	Concentration(µg\ml)					
	200	**100**	**50**	**25**	**12.5**	Compounds=0.020
Crude alkaloid compound (CR)	96	90.7	83.3	58.7	39.4	
Methoxy-Phenyl Oxime (MPO)	93.4	85.3	73.6	41.3	30.6	
Colchicine (Co)	39.4	23.2	19.5	13.5	6.4	
Vit. C (Ascorbic acid) (Positive control)	98	92.3	86.1	61.3	43.1	
RLSD	Conc.= 0.022 , Intercept = 0.15					

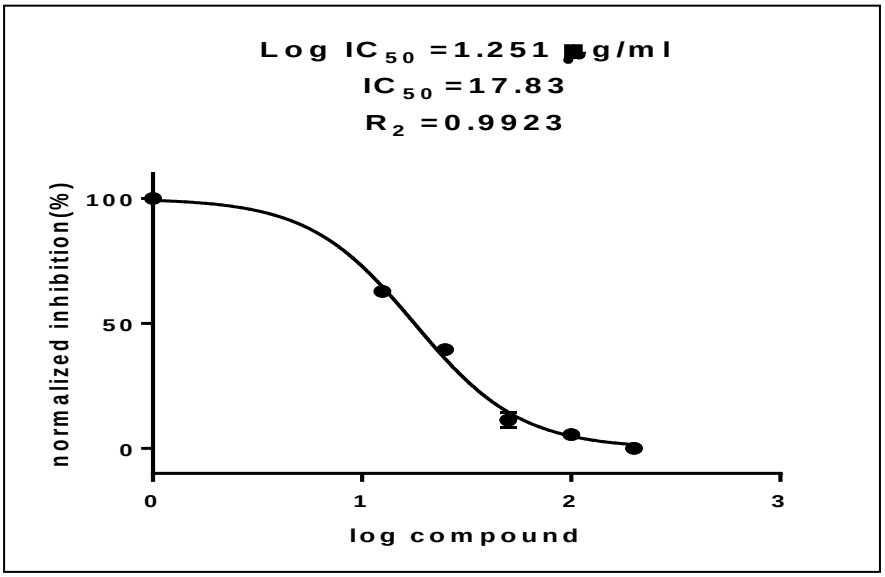

Figure (4-29): Free radical (DPPH) scavenging activity for crude precipitate alkaloid compounds extracted from *C. procera* roots (IC_{50}=17.83).

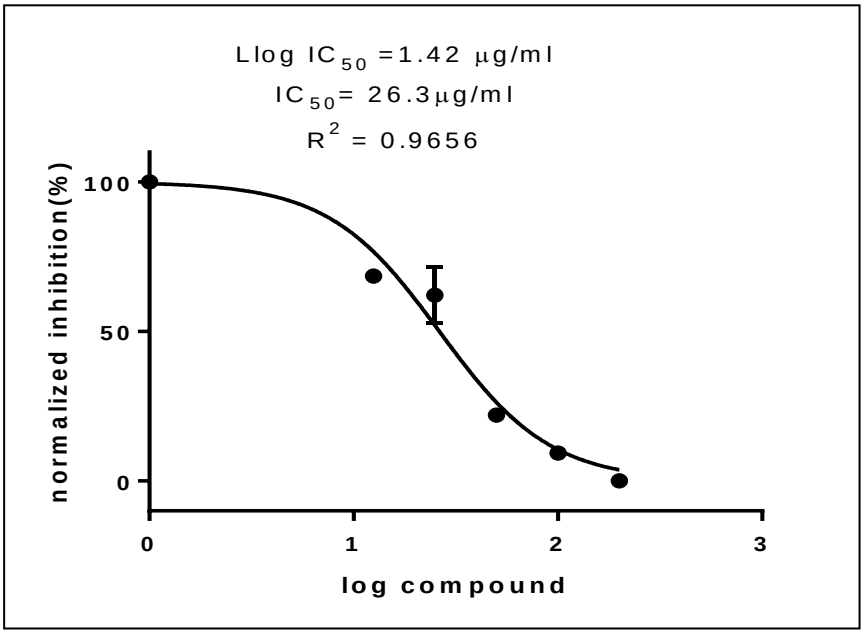

Figure (4-30): Free radical (DPPH) scavenging activity for alkaloid compound MPO purified from *C. lancifolius* leafs (IC_{50}=26.3)

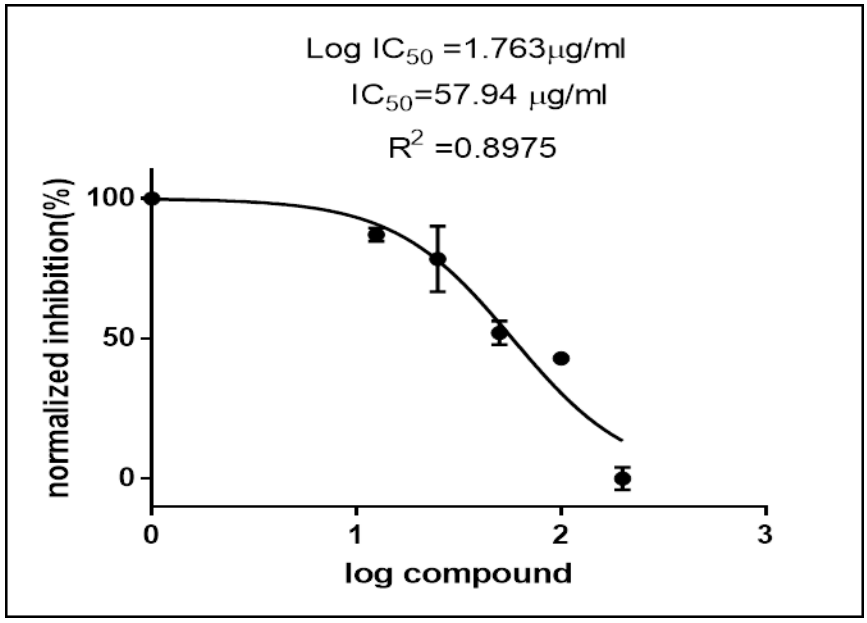

Figure (4-31): Free radical (DPPH) scavenging activity for alkaloid compound CO purified from *C. procera* leafs (IC_{50}=57.94)

Chapter five

Discussion

5-Discussion

Alkaloid compounds are commonly distributed within certain families in the plant kingdom, even though they are mostly founded in small amounts within the plant. but, their characteristic as a bioactive agent made researchers focusing on scanning plants, which may lead to discovering a new alkaloid compound that may cure dangerous diseases.

5-1 Preliminary Biochemical tests for alkaloid compounds detection

As the results showed the presence of alkaloid compounds in leaf only in *C.lancifolius* was in line with a studies by Raheema and Shoker,(2020) and Saadullah *et al*.(2014).wheras the results of phytochemical analysis for *C.procera* that confirmed the presence of Alkaloid compounds in leaf and root of *C.procera* has corresponded with a study of Hassan *et al*.(2006); Oladimeji *et al*.(2006); Naser *et al* .(2019) and Verma *et al*.(2013).

On the other hand, phytochemical analysis for the study of Morsy *et al* .(2016) appeared the presence of alkaloid compounds in *C.procera* in stem and root only and not found in leaf and that because of the synthesis of alkaloid compounds is often affected by environmental conditions such as rainfall, U.V. radiation, atmospheric composition, circadian rhythm, plant age and temperature (Gobbo-Neto and Lopes, 2007).

Also, many previous studies have conducted phytochemical analyses for different parts of *C. procera* (Hanna *et al*.,1999; Hani *et al.*,1999; Hanna *et*

al.,2002; Morsy *et al*.,2010) confirmed the presence of alkaloid compounds in this plant.

5-2 Solvent extraction

The efficiency of methanol in alkaloid extraction in comparison with other two solvents polarity of solvents played a vital role in the extraction process since it would increase the solubility of compounds, methanol extract of *C.lancifolius* contain bioactive compounds twice from that of aqueous extract (Ahmed *et al* .,2016). Methanol's more robust extraction capacity has produced many active constituents responsible for several biological activities (Nattala *et al*., 2019; Abubakar and Haque,2020).

The amount of the bioactive compound is affected by many factors of extraction conditions like temperature, time of extraction, and type and concentration of a used solvent (Ibrahim *et al*., 2013). Aqueous solvent has a better extraction power than pure solvent because mixing non-polar solvent and water may increase the polarity index of solvents and hence may further enhance its extraction efficiency (Razali *et al.*, 2012; Camarena-Tello *et al*.,2018).

Successful extraction of bioactive compounds from plant material largely depends on the type of solvent used (Raman,2012 ; Dhawan and Gupta 2017) .Solvent system selection largely depends on the specific nature of the targeted bioactive compound. Different solvent systems are available to extract the bioactive compound from natural products. Hydrophilic compounds are extracted using polar solvents such as methanol, ethanol or ethyl-acetate. For extraction of more lipophilic compounds,non polar

solvents are suitable like dichloromethane (Cunha *et al*.,2004.; Cosa *et al*.,2006).

The results in the present study showed that hot extraction was more efficient than the cold one in the percentage of alkaloid yield extract in all solvents (methanol, water, dichloromethane) because the amount of a bioactive compound is affected by many factors of extraction conditions like temperature, time of extraction (Altemimi,2017).

5-3 GC-MS analysis for alkaloid compounds in two studied plants

Using GC-MS analysis as a technique for scanning and identifying alkaloid compounds in two studied plants, where GC-MS is considered the best technique for determining the chemical composition of many compounds such as alkaloids (Selvi and Bhaskar,2012;Narayanan *et al*.,2019).

The results of GC-MS analysis for leaf methanolic crude alkaloids extract of *C. lancifolius* revealed the presence of one type of alkaloid compound, Methoxy phenyl oxime (MPO) where; this is the first study that confirmed the presence of a novel methoxy phenyl oxime compound in this plant that found to be existing in few plants by GC-MS analysis such as methanolic leaves extract for both *Urtica dioica* (Altameme *et al* .,2015) and *Alstonia scholaris* (Swamy *et al*.,2019).

Besides , the study of Chipps *et al* ,(2012) revealed the existence of MPO compound in seeds of *Momordica charantica.* Whereas leaf methanolic alkaloid extract of *C.procera* that appeared the presence of one alkaloid compound Colchicine (CO) has corresponded with a study of (Naser *et*

al.,2019) in Iraq but GC-MS analysis of *C.procera* leaf of other country appeared another type of alkaloid compounds. Also, root methanolic alkaloid extract presence of two alkaloid compounds was in line with a study (Gajar *et al*.,2012).

The differences in chemical composition between the same plant species may refer to many factors that are; different times of collection, different cultivation environments, and even different forms of nutrition plants used thus, secondary metabolites represent a chemical interface between plants and the surrounding environment.

Therefor their synthesis is often affected by environmental conditions such as rainfall, U.V. radiation, atmospheric composition, circadian rhythm, plant age and temperature (Gobbo-Neto and Lopes, 2007).

5-4 Isolation, Purification and Identification of alkaloid compounds by HPLC technique

High-pressure liquid chromatography (HPLC) is a chromatographic technique that can separate a mixture of compounds and is used in phytochemical and analytical chemistry to identify, quantify and purify the individual components of the mix (Sasidharan *et al*.,2011; Piana *et al*.,2013).

HPLC was performed to confirm many bioactive compounds' complete separation and isolation. Therefore, the combination of HPLC and MS facilitates rapid and accurate identification of chemical compounds in medicinal herbs, especially when a pure standard is unavailable (Alwash *et al*.,2020).

HPLC analysis of methanolic leaf alkaloid extract of *C lancifolius* resulted in the separation of one major peak at R.T reached 10.6min at a wavelength of 280 nm that identified as methoxy phenyl oxime by corresponding with R.T for methoxy phenyl oxime compound that separates in a study of (Chipps *et al*.,2012) at an R.T reached (10.4min).

In contrast, HPLC analysis for leaf methanolic alkaloid extract of *C procera* resulted in the identification, separation and purification of colchicine identified by comparison of its actual retention time to the retention time of colchicine standard reference (Sigma Aldrich) at chromatographic conditions, where the two peaks corresponded in their retention time that reached 2.5min.

5-5 Identification and characterization of purified alkaloid compound Methoxy phenyl oxime

5-6 Fourier transform infrared spectroscopy (FT-IR)

FT-IR is an appreciated tool for identifying and characterising compounds or functional groups in an unknown mixture (Mohsen,2021; Kinda *et al*.,2020).

In the present study, the OH stretching vibration was observed at 3351cm-1, which is broad in the FT-IR spectrum. The plane-bending vibration of the OH-group was monitored in the region (1250-1150 cm-1) (Selvaraj *et al*.,2018), and according to the reference, the OH in plane-bending vibrations were assigned at 1246.75 cm^{-1}in the FT-IR spectrum. Stretching vibration of Aromatic- CH_3 embedded with a broad band of OH. Aliphatic C-H bands appeared at 2928. In the present investigation, the asymmetric C=C stretching vibration occurs at 1649.8 cm-1 with strong intensity. CO

and CN absorption in the fingerprint region from 900-1300 cm-1(even if it's not easy to identify).

5-7 The HNMR Spectra

Nuclear magnetic resonance (NMR) spectroscopy is the key technology used for identifying compounds that are particularly convincing due to its simple sample preparation process, high reproducibility, and non-destructive character (Emwas *et al*.,2019;Larive *et al*.,2015). Initially, it was the method of choice for the structure elucidation of pure compounds; however, NMR is increasingly used nowadays to analyse complex mixtures (Wojtowicz *et al*.,2017). This assay was performed to identify MPO alkaloid compounds using DMSO-6 as the solvent, where one signal belongs to this solvent at 2.09 ppm. Aromatic protons appear nearly at (7-8) ppm. (Pavia *et al.*,2016), So signals at 7.27-7.28 ppm belong to aromatic protons. ppm belonging to OH-group (Emwas *et al*.,2019).

5-8 Thin Layer Chromatography (T L C)

This rapid and straightforward separation method helps give an idea about how many different components are present in a mixture and could identify the nature of each component by comparing it with a known standard if they both have the same R*f* (Ciura, *et al*.,2017). As seen in the present study, the Separation of purified MPO *C.lancifolius* by TLC resulted in one clear spot by using Hexane: ethyl acetate : methanol: water (7:3:5:5) solvent system with a value of *hRf* reached 0.71that was identical with *hRf* value in the study of (Chipps *et al*.,2012) were, purified MPO was separated from bitter melon (*Momordica charantia*) seed extracts by TLC that using the same solvent system.

5-9 Antibacterial activity of alkaloid compounds isolated and purified from two studied plants

The results appeared to be the remarkable antibacterial activity for purified compound MPO against each studied gram-positive bacteria (*Staphylococcus aureus, Bacillus subtillus*) and gram-negative bacteria (*Klebsiella pneumoniae, Escherichia coli*) where methoxy phenyl oxime that has purified from leaves of C. *lancifolius* has the highest average of inhibition zone diameter against four studied bacteria types, that many studies confirmed the antibacterial activity of leaf methanolic extract of *C. lancifolius* against gram-positive and gram-negative bacteria such as study by(Hemalatha *et al.*(2011); Touger *et al* .(2015) and Velmurugan *et al*.,(2012), that proved antibacterial activity of leaf extract of *C. lancifolius* due to the presence of Alkaloids ,that meant most of the antibacterial activity refer to the existence of alkaloid compound methoxy phenyl oxime that purified from leaf extract of C. *lancifolius.*

Methoxy phenyl-oxime compound and all other oximes behave as antibiotics due to the oxime functional group(C=N-OH) responsible for their activity. (Barghouthi *et al*.,2017) . Also, the results of the current study that appeared antibacterial activity of crud precipitates of alkaloid compounds extracted from *C.procera* root that was referring to existing of two different types of alkaloid compounds (1H-Indole,2,3-dihydro-4-methyl (C9H11N)and 1,3, benzene dicarboxylic acid,5-dimethylamino($C_{10}H_{11}NO_4$)) which have many functional groups represented by NH2, CH3, C=Phenyl-ring was in line with the studies of Mainasara *et al*.(2012) and

Oke *et al.* (2004) that confirmed the antibacterial activity of root extract of *C.procera* that containing alkaloid compounds.

Alkaloids remain the focus of much research, their development as antibacterial drugs pursued within academia, industry. (Bogatcheva *et al*., 2011; Hraiech *et al*.,2012; Parhi *et al*.,2012).

Two mechanisms of bacterial inhibition by alkaloids were shown to occur ; one by inhibition of nucleic acid synthesis, or by the troubling of the Z-ring and inhibition of cell division. That carboxyl group –HO function as respiratory inhibitors by reducing oxygen consumption in the treated bacteria (Heeb *et al*., 2011; Tominaga *et al*., 2002). Also alkaloids was showen to have action of disturbing bacterial membrane integrity (Salmi *et al*., 2008).

As shown in this study, most results of antibacterial activity for each MPO and crude alkaloids appeared to have higher activity toward gram-positive bacteria than gram-negative bacteria because most Gram-positive bacteria are surrounded by a coarse peptidoglycan cell wall, so This structure, although mechanically strong, appears to offer little resistance to the diffusion of small molecules such as antibiotics. In contrast, Gram-negative bacteria surround themselves with a second membrane, the outer membrane, which functions as an effective barrier (Nikolaidis *et al*., 2014).

5-10 Hemolysis test for MPO, colchicine and root alkaloid extracts of *C. procera* on human RBCs

Hemolysis is best evaluated using an in vitro method, which can show the effect of increasing concentration and to be sigmoidally related to the logarithm of contact time, as was studied for various surfactants. According to the results shown in the current study, Hemolysis cytotoxicity for purified alkaloid compound MPO on human RBCs showed no Hemolysis because MPO has a low value of erythrocyte disruption effect that percentage value of RBCs hemolysis in the current study for MPO was1.22%. A previous study by Saddeq *et al* .(2019) showed no Hemolysis on human RBCs of aqueous leaf extract of *C.lancifolius*.

Also results for both root crude alkaloid compounds of *C.procera* and colchicine compound showed low percentage hemolysis on RBCs that were 1.54,2.31 respectively in compared with 100% hemolysis for positive control (tap water).

Hemolysis is best evaluated using an *in vitro* method, which can show the effect of increasing concentration and to be sigmoidally related to the logarithm of contact time, as was studied for various surfactants and mechanical stability of the erythrocytic membrane is a good indicator of the effect of various *in vitro* insults levied on it by various compounds for the screening of cytotoxicity and is dependent on their physical and structural properties (Sharma and Sharma, 2001).

5-11 Cytotoxicity study of the colchicine and MPO, alkaloid compounds against MCF-7 and SK-GT-4 cell lines.

Cytotoxicity effect of the two purified alkaloid compounds Colchicine, Methoxy phenyl oxime were tested by studying their ability as anti-proliferative against MCF-7cell line that, characterized by high expression of estrogen and progesterone receptors (Comsa *et al.*,2015; Darwati *et al*.,2021) and SK-GT-4cell line.where, anti-cancer activity done using MTT assay which is a colourimetric assay correlate between cell activity and several viable cells by measuring the absorbance of a specific wavelength to determine the cytotoxic effect of the drugs or substances (Cree,2011).

As confirmed in the present study, colchicine compound has cytotoxic activity against both two cell lines, which was in line with a survey by Krishnasamy *et al*.(2016)and ALQahtani *et al*.(2020) that proved anticancer activity of colchicine compound against another type of cancer cells (Hep-B) and MCF-7 cell lines.

Also, as seen in a studies by Lin *et al*.(2016) and Zhang *et al.*(2019), colchicine has anti-proliferative effects on two human gastric cancer cell lines AGS and NCI-N87 by induced apoptosis. colchicine is very useful as an antimitotic agent in cancer research involving cell culture (Budavari ,1989; Kurek,.2018).

At the same time, (MPO) results in this study showed a selective remarkable cytotoxic activity against the SK-GT-4 cell line only that was identical to results of the cytotoxicity of the MPO compound isolated from Bitter melon seeds, which reported to be caused by its oxime functional group(C=N-OH), which is responsible for its activity in human colon

tumour 116 (HCT116) cells that may lead to an idea of MPO has a selective activity on digestive canal.

Oxime groups have been successfully introduced into a large number of therapeutic leads for the development of kinase inhibitors with anticancer, since some oximes are highly selective. (Schepetkin, *et al*.,2019), while the presence of a terminal oxime group is necessary for the activity of these compounds because of the presence of two H-bond acceptors (N and O atoms) and one donor (OH group).

Additionally, the metabolism of oximes can lead to the release of NO, which may also be therapeutically beneficial.

Also, the oxime group has a remarkable anticancer activity, as reported by Schepetkin *et al*., (2021) and Surowiak *et al*.,(2020),that suggests that apoptosis may cause the observed cytotoxicity.

Also, recent studies have found cancer-killing properties in oximes extracted from sea sponges, *Cinachyrella alloclada*, *C. apion*, and some oximes isolated from other marine sponges.

The findings of Cui *et al*., (2009) confirmed that the presence of the oxime group enhanced antitumor activity. Our results that found MPO alkaloid compound purified from leaf methanolic extract of *C.lancifolius* can be used as an anticancer drug are supported by all of these investigations using various types of oxime compounds.

5-12 *In vitro* antioxidant activity for alkaloid compounds

As DPPH is a nitrogen-centred, stable free radical that generates deep purple colour when put in methanol solution, antioxidants either donate an electron or hydrogen to neutralize the free radical nature of DPPH, thus balancing the plant's antiradical action (Jebitta and Allwin, 2016). To determine the antioxidants' ability to scavenge free radicals, the DPPH assay uses the stable free radical's ability to decolourize in the presence of antioxidants (Kilic *et al*., 2014). This has been widely utilized for in vitro antioxidant activity assays because it is fast, reliable, and reproducible (Hasan *et al*., 2006). The reducing capacity of compounds could serve as a marker of potential antioxidant activity (Meir *et al*.,1995; Koleva *et al*.,2002; Shah *et al*.,2015; Dalimunthe *et al*.,2016).As the results of antioxidant activity for two purified alkaloid compounds MPO, CO, and crude root alkaloid compounds (CR) showed remarkable scavenging DPPH Activity of CR that was near to activity of positive control (ascorbic acid), that may belong to CR are containing two types of alkaloid compounds1H-Indole,2,3-dihydro-4-methyl($C_9H_{11}N$) and 1,3, benzene dicarboxylic acid,5-dimethylamino ($C_{10}H_{11}NO_4$) which include OH and NH functional groups that could be donating their hydrogen to DPPH which confirmed by Zaher *et al*.(2020) that reported antioxidant activity of methanolic root extracts of *C.procera*. Many researchers reported that alkaloid compounds have major functional O-H, N-H and C-H groups that give them antioxidant activity (Windono *et al*., 2012; Shami,2016). On the other hand, the alkaloid compound Methoxy phenyl oxime purified in the current study from *C.lancifoluis* leaf appeared to have antioxidant activity that occurred as decolourizing DPPH free radical as a reduction process by donating a hydrogen atom. This result was

reported by Surowiak *et al*. (2020) which confirmed the antioxidant activity of all oxime compounds of plant origin.

Conclusions

and

Recommendations

Conclusions:

1. The purified alkaloid compound Methoxy Phenyl Oxime (MPO) is a bioactive compound found at the first time in the leaves of the *C.lancifolius* plant.
2. Hot methanol extraction is the best extraction method for alkaloids

3. The plant *C.procera* has three types of alkaloid compounds represented by colchicine in leaves and two types in the root (1H-Indole, 2,3-dihydro-4-methyl and 1,3, benzene dicarboxylic acid,5-dimethylamino.
4. Purified MPO alkaloid compound has a significant antibacterial activity against all four studied bacteria (equal to broad spectrum Gentamycine antibiotic)
5. A significant cytotoxicity of two Purified MPO,CO, alkaloid compounds on SKG-T4 and MCF-7 cell lines with no cytotoxitciy effect on normal cell made them as an important plant origin sources of anti cancer remedies in the future.
6. The antioxidant activity of root alkaloid compounds and MPO compounds is approximate to standard vitamin C.

Recommendations

1. Further studies on MPO alkaloid compounds are required as an anti-viral, anti-fungal and anti-inflammatory effect.
2. Using more hot extraction methods like (the reflux method) to compare with the soxhlet method in yield alkaloid extract from two studied plants.
3. Studying the cytotoxic effect of MPO and CO alkaloid compounds on more different cancer cell lines.
4. Different antioxidant activity methods are used on root crude alkaloid extracts and MPO compound.
5. Studying the bioactivity of nano particles for MPO, Colchicine and root crude alkaloid compounds.

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الخلاصة

كشفت الدراسة الحالية عن استخلاص, تشخيص وتنقية للمركبات القلويدية من نوعين من النباتات المحلية في محافظة البصرة جنوب العراق وهما نبات الكابرس *Conocarpus lancifolius* Engl. ونبات الديباج *Calotropis procera* (Aiton) R.Br. وتم اظهار فعاليتها ضد البكتيرية لبعض الانواع من البكتريا الموجبة و السالبة لصبغة جرام كما تم اختبار فعاليتها المضادة للأكسدة والمضادة للسرطان ضد نوعين مهمين من الخطوط الخلوية السرطانية.

شملت هذه الدراسة نوعين من النباتات المحلية والتي تم جمعها من مناطق مختلفة من محافظة البصرة وهي: نبات الكابرس *Conocarpus lancifolius* والذي يعود الى عائلة Combretaceae ونبات الديباج *Calotropis procera* و الذي يعود الى عائلة Asclepiadaceae.

شخصت المركبات القلويدية لكل جزء نباتي لكل من النباتين المدروسين وذلك باستخدام نوعين من الكواشف البيوكيميائية والتي تضمنت كاشف ماير وكاشف دراكندورف حيث اظهرت النتائج وجود المركبات القلويدية في الاوراق فقط لنبات الكابرس بينما وجدت في كل من اوراق وجذور نبات الديباج .

تم اجراء نوعين من طرق الاستخلاص في استخلاص القلويدات وهما الاستخلاص الحار (بالسكسوليت) والاستخلاص البارد (التنقيع) وذلك باستخدام ثلاث انواع من المذيبات (الميثانول ,الماء وثنائي كلوروالميثان) والتي اثبتت تفوق الاستخلاص الحار على الاستخلاص البارد في نسبة صافي مستخلص القلويدات لكل من الميثانول والماء لكلا النباتين. كذلك اظهرت نتائج النسبة المئوية لصافي مستخلص القلويدات للمذيبات الثلاثة تفوق الميثانول بقيمة قاربت 1.677 غم/100غم و بفارق معنوي عن كل من الماء وثنائي كلور والميثان بقيم قاربت 0.619غم\100غم 0.089 غم/100غم على الترتيب .

تم اجراء التحليل الكمي لمستخلصات القلويدات الخام بواسطة تقنية كرماتوغرافي الغاز السائل-مطياف الكتلة (GC-M S)والتي كشفت عن وجود المركب القلويدي Methoxy Phenyl Oxime (MPO), في المستخلص الميثانولي لأوراق نبات الكابرس عند وقت احتجاز بلغ 5.2 ثانية

و بوزن جزيئي بلغ 151.1 غرام\مول وبنسبة مئوية للمساحة بلغت 42.02% بينما كشف عن المركب القلويدي الكولجسين (CO في المستخلص الميثانولي لأوراق نبات الديباج عند وقت احتجاز بلغ 12.8ثانية وبوزن جزيئي بلغ 399غم/مول وبنسبة مساحة بلغت 7.1 % في حين كشف المستخلص القلويدي الخام لجذور نبات الديباج عن وجود نوعين من المركبات القلويدية هما 1H-Indol,2,3-dihydro-4-methyl عند وقت احتجاز 21.9 ثانية وبوزن جزيئي بلغ 133.39 غم/مول ونسبة مساحة بلغت 42.42% والمركب القلويدي 1,3,benzenedicarboxylic acid ,5-dimethylamino عند وقت احتجاز بلغ 23.7 ثانية وبوزن جزيئي بلغ 209.2غم/مول وبنسبة مساحة بلغت 45 %.

تم تشخيص وتنقية المركبات القلويدية بواسطة تقنية كروماتوغرافي السائل عالي الاداء HPLC - التحليلي- مع اجراء بعض التحوير كذلك تم اجراء المزيد من التشخيص للمركبات القلويدية باستخدام كل من تقنيات كروماتوغرافي الطبقة الرقيقة TLC,مطياف الاشعة تحت الحمراء FT-IR و HNMR .

تضمنت دراسة الفعالية البايلوجية للمركبين المنقيين CO,MPO مع المستخلص الميثانولي الخام لجذور نبات الديباج CR دراسة الفعالية ضد البكتيرية و كل من الفعالية ضد السرطانية وضد التأكسدية خارج الجسم الحي حيث اظهرت نتائج الفعالية الضد بكتيرية والتي تم اختبارها ضد اربعة انواع مختلفة من البكتريا الممرضة والتي تضمنت بعض البكترية الموجبة لصبغة غرام *Bacillus subtilis* , *Staphylococcus aureus* والبكتريا السالبة لصبغة غرام *Escherichia coli* ,*Klebsiella pneumoniae* وباستخدام ثلاث تراكيز(200,150,50)ميكروغرام/ملليتر. حيث اظهر المركبMPO اعلى قيمة معنوية ضد بكتريا *B.subtilis* حيث بلغت (22.66mm) بينما كانت اقل قيمة للمركبCO عند التركيز50 مايكوغرام ضد بكتريا *K. pneumonia* بلغت (1.6mm)

اوضحت نتائج الفعالية ضد السرطانية التي اجريت ضد نوعين من الخطوط الخلوية وهي الخط الخلوي لسرطان الثديMC-F-7 والخط الخلو لسرطان المريء SK-GT-4 سمية خلوية كبيرة لمركب الكولجسين ضد الخط الخلوي لسرطان الثدي MC-F-7 وبقيمة لنصف التركيزالقاتل (IC_{50}) بلغت55.33 ميكروغرام/مليلتر بالمقارنة مع كل من المركب MPO و المستخلص الميثانولي الخام لجذور نبات الديباج CR اللذان لم يبديا اي سمية خلوية ضد هذا النوع من الخطوط

الخلوية في حين اظهر المركب MPO سمية خلوية انتقائية معنوية ضد الخط الخلوي لسرطان المريء SK-GT-4 وبقيمة لنصف التركيز القاتل(IC_{50}) بلغت 7.85 ميكروغرام/مليلتر في حين اظهر مركب الكولجسين CO سمية خلوية وبقيمة لنصف التركيز القاتل(IC_{50}) بلغت 522 ميكروغرام/مليلتر ولم يظهر المستخلص الميثانولي الخام لجذور نبات الديباج CR اي سمية خلوية ضد هذا النوع من الخطوط السرطانية ايضا .

اجري النشاط المضادة للأكسدة باستخدام اختبار إزاحة الجذور الحرة المتولدة من مادة DPPH لكل من المركبين MPO,CO و المستخلص الميثانولي الخام لجذور نبات الديباج CR مع مقارنتها ب حامض الاسكوربك (فيتامين- C) الذي يمثل معامل السيطرة الموجب حيث ظهرت النتائج وبفوارق معنوية على الترتيب التالي: فيتامين سي >CR >MPO > CO .

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الفعالية الحيوية لبعض المركبات القلويدية المعزولة من نباتي *Calotropis procera* و *Conocarpus lancifolius*

اطروحة مقدمة الى كلية التربية للعلوم الصرفة كجزء من متطلبات
نيل درجة دكتوراه فلسفة في علوم الحياة – علاجات نباتية

من قبل

منى عبد المطلب يحيى

الحاصلة على شهادة الماجستير في علوم الحياة (2006)

بإشراف

الاستاذ الدكتور
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الاستاذ المساعد الدكتور
ميثم ايوب الحمداني

2022 م

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