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Antimicrobial Activity of Peganum Harmala Fixed Oil

A Dissertation Submitted in Partial Fulfillment of the Requirements of the B.Sc (Hons.) Degree in Chemistry

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1-Introduction

1.1-Natural products

A **natural product** is a chemical compound or substance produced by a living organism—that is, found in nature.[2][3] In the broadest sense, natural products include any substance produced by life.[4][5] Natural products can also be prepared by chemical synthesis (both semi-synthesis and total synthesis) and have played a central role in the development of the field of organic chemistry by providing challenging synthetic targets. The term natural product has also been extended for commercial purposes to refer to cosmetics, dietary supplements, and foods produced from natural sources without added artificial ingredients.[6]Within the field of organic chemistry, the definition of natural products is usually restricted to mean purified organic compounds isolated from natural sources that are produced by the pathways of primary or secondary metabolism.[7] Within the field of medicinal chemistry, the definition is often further restricted to secondary metabolites.[8][9] Secondary metabolites are not essential for survival, but nevertheless provide organisms that produce them an evolutionary advantage.[10] Many secondary metabolites are cytotoxic and have been selected and optimized through evolution for use as

"chemical warfare" agents against prey, predators, and competing organisms.[11]

Natural products sometimes have pharmacological or biological activity that can be of therapeutic benefit in treating diseases. As such, natural products are the active components not only of most traditional medicines but also many modern medicines. Furthermore, because the structural diversity of natural products exceeds that readily achievable by chemical synthesis, and synthetic analogs can be prepared with improved potency and safety, natural products are often used as starting points for drug discovery. In fact, natural products are the inspiration for approximately one half of U.S. Food and Drug Administration-approved drugs.

Several approaches can be employed to extract the plant material. Although water is used as an extractant in many traditional protocols, organic solvents of varying polarities are generally selected in modern methods of extraction to exploit the various solubility's of plant constituents.

Maceration is simple widely used procedure involves leaving the pulverized plant to soak in a suitable solvent in a closed container .simple maceration is performed at room temperature by mixing the ground drug with the solvent (drug solvent ratio : 1:5 or 1:10) and leaving the mixture for several days with occasional shaking or stirring.

The extract is then repeated from the plant particles by straining . The process is repeated for once or twice with fresh solvent .Finally the last residue of extract is pressed out of the plant particles using a mechanical press or a centrifuge.kinetic maceration differe from simple one by continous stirring.

-The method is suitable for both initial and bulk extraction.

-The main disadvantage of maceration is that the process can be quite time-consuming, taking from a few hours up to several weeks

Ultrasound-assisted solvent extraction is a modified maceration method where the extraction is facilitated by the use of ultrasound . The plant powder is placed in a vial. The vial is placed in an ultrasonic bath, and ultrasound is used to induce a mechanical stress on the cells through the production of cavitations in the sample. The cellular breakdown increases the solubilization of metabolites in the solvent and improves extractionyields.

-it is mostly used for the initial extraction of a small amount of material.

In percolation the powdered plant material is soaked initially in a solventin a percolator . Additional solvent is then poured on top of the plantmaterial and allowed to percolate slowly (dropwise) out of the bottom of the percolator. Additional filtration of the extract is not

required because there is a filter at the outlet of the percolator. Percolation is adequate for both initial and large-scale extraction.

The main disadvantages of percolation are **:**fine powders and materials such as resins and plants that swell excessively (e.g., those containing mucilages) can clog the percolator. 2-if the material is not distributed homogenously in the container, the solvent may not reach all areasand the extraction will be incomplete.

Soxhlet extraction is adequate for both initial and bulk extraction . The plant powder is placed in a cellulose thimble in an extraction chamber, which is placed on top of a collecting flask beneath a reflux condenser. A suitable solvent is added to the flask, and the set up is heated under reflux. When a certain level of condensed solvent has accumulated in the thimble, it is siphoned into the flask beneath.

-The main advantage of Soxhlet extraction is that it is a continuous process.

In pressurized solvent extraction the powdered plant material is loaded into an extraction cell, which is placed in an oven. The solvent is then pumped from a reservoir to fill the cell, which is heated and pressurized at programmed levels for a set period of time. The cell is flushed with nitrogen gas, and the extract, which is automatically filtered, is collected in a flask. Fresh solvent is used to rinse the cell and to solubilize the

remaining components. A final purge with nitrogen gas is performed to dry the material.This method offers a more economical and environment-friendly alternative to conventional approaches.

Another method of extraction is done under reflux and steam distillation.

Here the solvent is heated until it reaches its boiling point. As the vapor is condensed, the solventis recycled to the flask.It is commonly applied to the extraction of plant essential oils.The main disadvantage is that thermolabile components risk being degraded.

Supercritical fluids (SCFs) are increasingly replacing organic solvents,e.g., n-hexane, dichloromethane, chloroform, and so on, thatare conventionally used in industrial extraction operations because of regulatory and environmental pressures on hydrocarbon and ozone-depleting emissions. Most of the currently available Solvent Free Extraction systems utilize CO2, which is generally considered as safe for solvent-free extraction processes. The fundamental steps involved in SFE are as follows:

i)Liquid CO2 is forced into supercritical state by regulating its temperature and pressure.

ii) Supercritical CO2 has solvent power and extracts predominantly lipophilic and volatile compounds.

iii) Gaseous CO2 returns to CO2 tank. After a full round, the new extraction starts with circulating CO2.

Countercurrent extraction is a continuous process in which the plant material moves against the solvent. It is suitable procedure for production of large amounts of extracts on an industrial scale. Several types of extractors are available. In the screw extractor the plant material is transported by a screw through a tube and meets the solvent which is pumped in the opposite direction.

1.2- Essential Oil

Humankind has used plants for healing for many thousands of years, and it's from this tradition of that the use of aromatic plant compounds is medicine began. Oils were used in the embalming process, in medicine and in purification rituals. Research has confirmed centuries of practical use of essential oils, and we now know that the 'fragrant pharmacy' contains compounds with an extremely broad range of biochemical effects. There are about three hundred essential oils in general use today by professional practitioners. With the continual bombardment of viral, bacterial, parasitic and fungal contamination in our world, essential oils are a great benefit to help protect our bodies and homes from this onslaught of pathogens. Immune system systems need support and essential oils can give it.

Because of the enormous amount of raw product used to make wholly natural essential oils, lots of products on the market have been polluted with lower quality, commercial – grade oils or contain other chemical substances to reduce the cost or increase the profit margin – a fact not usually revealed on the label. This is why it is important to study the chemical composition of the volatile fraction once the essential oil is extracted. This fraction is characterized by the complexity in the separation of its components, which belong to various classes of compounds and which are present in a wide range of concentrations. Therefore it is complicated to establish a composition profile of essential oils. The gas chromatographic method (GC) is almost exclusively used for the qualitative analysis of volatiles. The analysis of essential oils was developed in parallel with the technological developments in GC, such as stationary phases, detection devices, etc. However, advances in instrumentation were not the only important factor in the development of analytical methods for essential oils in plants. Sample extraction and concentration were also improved. The most outstanding improvements in the determination of the composition of essential oils came from the introduction of tandem techniques involving prior/further chromatography or spectroscopy.

Essential oils are highly concentrated substances extracted from flowers, leaves, stems, roots, seeds, barks, resins, or fruit rinds. These oils are often used for their flavor and their therapeutic or odoriferous properties,

in a wide selection of products such as foods, medicines, and cosmetics. Extraction of essential oils is one of the most time- and effort-consuming processes. The way in which oils are extracted from plants is important because some processes use solvents that can destroy the therapeutic properties. There are wide number of ways to extract the Essential oil but the quality never remains the same. Here we are using the “Steam Distillation” method for extraction which is the cheapest way for the extraction of Oils from the different parts of the plants. In this process steam is allowed to pass through the extraction chamber which contains plant matter. When steam passes through the herb material under pressure which softens the cells and allows the essential oil to escape in vapor form. The vapor allows passing through condenser and oil is collected in separating funnel and separated. Composition data for Eucalyptus oil were studied and found the Eucalyptus oil can be used as cosolvent which results in depression of cloud point temperature.

Essential oils are extracted from oil 'sacs' in flowers, leaves, stems, roots, seeds, wood and bark. They differ significantly from the well-known vegetable, nut and seed oils which are made up of various fatty acids (essential oils are not). Essential oils are used by the plants in somewhat the same way they are by humans - they fight infection, contain hormone-like compounds, initiate cellular regeneration, and work as chemical defense against fungal, viral, and animal foes. Despite their foliar origins however, essential oils have a similar structure to

some compounds found in blood and tissues, allowing them to be compatible with our own physiology.

The most effective way to use most essential oils is by external application or inhalation, though some can be very beneficial when taken internally. The use of essential oils include body oils, compresses, cosmetic lotions, baths, hair rinses, inhalation by steam, perfumes and room sprays. Essential oils are *very* potent - some will cause skin irritation or have other harmful effects if not used properly. Unless specifically noted, it is best to dilute all essential oils in a carrier of *base* oil like Almond, Jojoba or Apricot Kernel before applying to the skin - appropriate dilution is usually only 1 - 10% essential oil in carrier. For inhalation, a diffuser or oil lamp is effective for releasing essential oils into your environment - a very pleasant way of creating a particular atmosphere.

1.3-Aromatherapy

The treatment of anxiety or minor medical conditions by rubbing pleasant smelling natural oils into the skin or breathing in their smell is known aromatherapy.

It is the use of aromatic essential oils to benefit the body – in emotional and physical health and beauty. Science has discovered that our sense of smell plays a significant role in our overall health.

Many common essential oils have medicinal properties that have been applied in medicine since ancient times and are still widely used today.

For example, many essential oils have antiseptic properties, though some are stronger than the other. In addition, many have an uplifting effect on the mind, though different essential oils have different properties.

The first modern-day distillation of essential oil was performed by the Persian philosopher Avicenna (980-1037 A.D.) who extracted the essence of rose petals through the 'enfleurage' process. His discovery and subsequent use of a wonderful perfume substance eventually lead him to write a book on the healing properties of essential oil of Rose.

Early in the 20th century a French Chemist, Rene-Maurice Gattefosse, began studying what he called " Aromatherapy ." After several burning his arm in a laboratory accident, he thrust the arm into the nearest liquid, which happened to be tub of Lavender Oil. Surprised by the quick healing that followed, Dr. Gattefosse spent the remainder of his life researching the value of Essential Oils. His success made aromatherapy popular, and it became well-known in Europe.

An Essential Oil is inhaled and directly by the olfactory system to the limbic System of the Brain. In true, the brain responds to the particular scent affecting our emotions and chemical balance. Essential Oils also absorbed by the skin and carried throughout the body via the circulatory system to reach all internal organs.

By carefully choosing one or more oils, you can experience beneficial effects promoting overall health - and even specific targets. Benefits

depend upon the unique nature of each person's response to an aromatic stimulus.

1.4-Pharmacological Properties of Essential Oils

-Antiseptics

Essential oils have antiseptic properties and are active against a wide range of bacteria as well as on antibio-resistant strains. Moreover, they are also known to be active against fungi and yeasts (Candida).The most common sources of essential oils used as antiseptics are: Cinnamon, Thyme; Clover; Eucalyptus; Culin savory; Lavender. Citral, geraniol, linalool and thymol are much more potent than phenol**.**

-Expectorants and diuretics

When used externally, essential oils like (L'essence de terebenthine) increase microcirculation and provide a slight local anaesthetic action. Till now, essential oils are used in a number of ointments, cream and gels, whereby they are known to be very effective in relieving sprains and other articular pains. Oral administration of essential oils like eucalyptus or pin oils, stimulate ciliated epithelial cells to secrete mucus. On the renal system, these are known to increase vasodilation and in consequence bring about a diuretic effect.

-Spasmolytic and sedative

Essential oils from the Umbellifereae family, Mentha species and verbena are reputed to decrease or eliminate gastrointestinal spasms.

These essential oils increase secretion of gastric juices. In other cases, they are known to be effective against insomnia.

-Others

Cholagogue; anti-inflammatory; cicatrizing

1.5-Chemical Constituents of Essential Oils

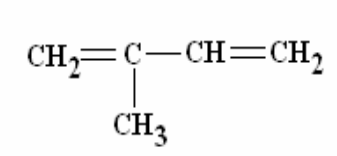
Pure essential oils are mixtures of more than 200 components, normally mixtures of terpenes or phenylpropanic derivatives, in which the chemical and structural differences between compounds are minimal. They can be essentially classified into two groups:

-Volatile fraction: Essential oil constituting of 90–95% of the oil in weight, containing the monoterpene and sesquiterpene hydrocarbons, as well as their oxygenated derivatives along with aliphatic aldehydes, alcohols, and esters.

-Nonvolatile residue: that comprises 1–10% of the oil, containing hydrocarbons, fatty acids, sterols, carotenoids, waxes, and flavonoids.

-Hydrocarbon:

Essential Oils consist of Chemical Compounds that have hydrogen and carbon as their building blocks. Basic Hydrocarbon found in plants are isoprene having the following structure.



(Isoprene)

-Terpenes

Generally have names ending in " **ene**."

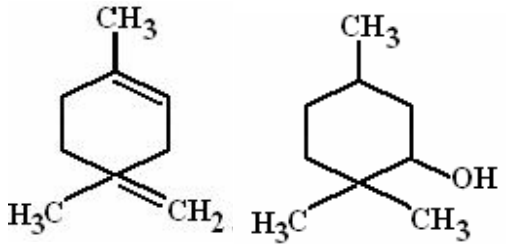
For examples: Limonene, Pinene, Piperene, Camphene, etc. Terpenes are anti-inflammatory, antiseptic, antiviral, and bactericidal. Terpenes can be further categorized in monoterpenes, sesquiterpenes and diterpenes. Referring back to isoprene units under the Hydrocarbon heading, when two of these isoprene

units join head to tail, the result is a monoterpene, when three join, it's a sesquiterpene and four linked isoprene units are diterpenes.

-Monoterpenes [C10H16]

Properties: Analgesic, Bactericidal, Expectorant, and Stimulant.

Monoterpenes are naturally occurring compounds, the majority being unsaturated hydrocarbons (C10).But some of their oxygenated derivatives such as alcohols, Ketones, and carboxylic acids known as monoterpenoids.



(Limonene) (Menthol)

The branched-chain C10 hydrocarbons comprises of two isoprene units and is widely distributed in nature with more than 400 naturally

occurring monoterpenes identified. Moreover, besides being linear derivatives (Geraniol, Citronellol), the monoterpenes can be cyclic molecules (Menthol – Monocyclic; Camphor – bicyclic; Pinenes (α andβ) – Pine genera as well. Thujone (a monoterpene) is the toxic agent found in Artemisia absinthium (wormwood) from which the liqueur, absinthe, is made. Borneol and camphor are two common monoterpenes. Borneol, derived from pine oil, is used as a disinfectant and deodorant. Camphor is used as a counterirritant, anesthetic, expectorant, and antipruritic, among many other uses.

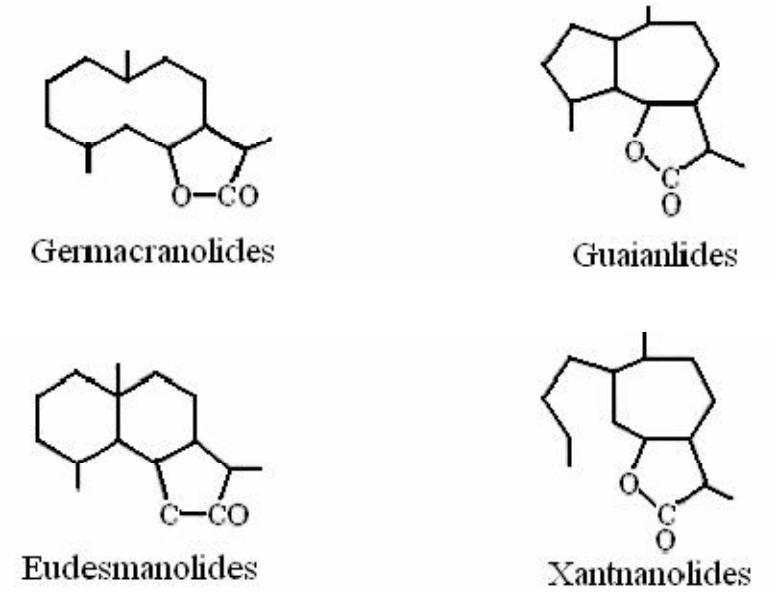
-Sesquiterpenes

Properties of sesquiterpenes include **:** anti-inflammatory, anti-septic, analgesic, anti-allergic.

Sesquiterpenes are biogenetically derived from farensyl pyrophosphate and in structure may be linear, monocyclic or bicyclic. They constitute a very large group of secondary metabolites, some having been shown to be stress compounds formed as a result of disease or injury.

Over 500 compounds of this group are known; they are particularly characteristics of the Compositae but do occur sporadically in other families. Not only have they proved to be of interest from chemical and chemotaxonomic viewpoints, but also possess many antitumor, anti-leukemia, cytotoxic and antimicrobial activities. They can be responsible for skin allergies in humans and they can also act as insect feeding deterrents.

Chemically the compounds can be classified according to their carboxylic skeletons; thus, from the germacranolides can be derived the guaianolides, pseudoguaianolides, eudesmanolides, eremophilanolides, xanthanolides, etc.



A structural feature of all these compounds, which appears to be associated with

much of the biological activity, is the α, β -unsaturated- γ- lactones.

-Diterpenes

Properties of ditrpenes include: anti-fungal, expectorant, hormonal balancers, hypotensive

Diterpenes are made of up four isoprene units. This molecule is too heavy to allow for evaporation with steam in the distillation process, so is rarely found in distilled essential oils. Diterpenes occur in all plant families and consist of compounds having a C20

skeleton. There are about 2500 known diterpenes that belong to 20 major structural types. Plant hormones Gibberellins and phytol occurring as a side chain on chlorophyll are diterpenic derivatives. The biosynthesis occurs in plastids and interestingly mixtures of monoterpenes and diterpenes are the major constituents of plant resins. In a similar manner to monoterpenes, diterpenes arise from metabolism of geranylgeranyl pyrophosphate (GGPP).

Diterpenes have limited therapeutical importance and are used in certain sedatives (coughs) as well as in antispasmodics and antoxiolytics.

-Alcohols

Properties include: anti-septic, anti-viral, bactericidal and germicidal.

Alcohols are the compounds which contains Hydroxyl compounds. Alcohols exist naturally, either as a free compound, or combined with a terpenes or ester. When terpenes are attached to an oxygen atom, and hydrogen atom, the result is an alcohol. When the terpene is monoterpene, the resulting alcohol is called a monoterpenol. Alcohols have a very low or totally absent toxic reaction in the body or on the skin. Therefore, they are considered safe to use.

-Aldehydes:

Properties include **:** anti-fungal, anti-inflammatory, anti-septic, anti-viral, bactericidal, disinfectant, sedative.

Medicinally, essential oils containing aldehydes are effective in treating Candida and other fungal infections.

-Acids

Organic acids in their free state are generally found in very small quantities within Essential oils. Plant acids act as components or buffer systems to control acidity.

-Esters

Esters are formed through the reaction of alcohols with acids. Essential oils containing esters are used for their soothing, balancing effects. Because of the presence of alcohol, they are effective antimicrobial agents. Medicinally, esters are characterized as antifungal and sedative, with a balancing action on the nervous system. They generally are free from precautions with the exception of methyl salicylate found in birch and wintergreen which is toxic within the system.

-Ketones

Properties include **:** anti-catarrhal, cell proliferant, expectorant, vulnery.

Ketones often are found in plants that are used for upper respiratory complaints. They assist the flow of mucus and ease congestion. Essential oils containing ketones are beneficial for promoting wound healing and encouraging the formation of scar tissue. Ketones are usually (not always) very toxic**.** The most toxic ketone is Thujone found in mugwort, sage, tansy, thuja and wormwood oils. Other toxic ketones found in essential

oils are pulegone in pennyroyal, and pinocamphone in hyssops. Some non-toxic ketones are jasmone in jasmine oil, fenchone in fennel oil, carvone in spearmint and dill oil and menthone in peppermint oil.

-Lactones

Properties include : anti-inflammatory, antiphlogistic, expectorant, febrifuge.

Lactones are known to be particularly effective for their anti-inflammatory action, possibly by their role in the reduction of prostaglandin synthesis and expectorant actions. Lactones have an even stronger expectorant action then ketones.

1.6-The target species-*Peganum harmala*

Peganum harmala L. (Zygophyllaceae) is native to eastern Mediterranean region. Due to resemblance to plants of the rue family, It is also known as Wild Rue or Syrian Rue(Mikaili ,2012). Peganum harmala is a perennial plant which can grow to about 0.8 m tall, but normally it is about 0.3 m tall. It blossoms between June and August in the northern hemisphere . The roots of the plant can reach a depth of up to 6.1 m, if the soil is very dry. The flowers are white and are about 2.5–3.8 cm in diameter. The round seed capsules measure about 1–1.5 cm in diameter(Frison et. al.,2008; El Gendy et.al.,2009; Wanntorp et.al.,2012; Sheahan et.al.,2000).

Pregnan harmala is used traditionally in the treatment of a wide array of human disorders.β-Carboline alkaloids were identified in different parts(seeds,roots,barks) of Pregnan harmala. Various pharmacological surveys demonstrated that harmala alkaloids namely; harmaline, harmine, harmalol and harmol are biologically active compounds.

The plant is employed in ethno-medicine to treat hypertension and cardiac disease(Tahraoui et.al.,2007; Fortunato et.al.,2009). Extracts of seeds exert vasorelaxant effects(Hamsa and Kuttan,2010) and alkaloids of Pregnan harmala were shown to have anti-platelet aggregation effects(Saeed et.al.,1993).Furthermore,these alkaloids were shown to be psychoactive in mammalian body(Airaksinen and Kari , 1981).Various studies demonstrated a wide range of effects produced by Pregnan harmala extracts on the central nervous system including ;analgesic(Airaksinen and Kari , 1981; Monsef and Ghobadi ,2004),hallucination, excitation (Nasehi et.al.,2010) and antidepressant(Fortunato et.al. ,2009; Farzin et.al.,2006) effects.Harmal alkaloids were shown to be involved in pathogensis of Parkinson`s disease(Splettstoesser et.al.,2005; Storch et.al.,2004).

Various studies indicated antiparasidal (Storch et.al.,2008; Akhtar et.al., 2000), antifungal (Akhtar ,2010;Saadabi,2006) ,anti-bacterial (Akhtar ,2010; (Prashanth and John,1999) and insecticidal (Rharrabe et.al., 2007; Jbilou et.al.,2008) effects for Harmal alkaloids.

Significant in vitro and in vivo antileishmanial activity was exhibited by Peganum harmala seeds extract(Rahimi-Moghaddam et.al.,2011) .Also it was reported that methanolic extracts of Peganum harmala reduced the number of living pups and produced a dose- dependent decrease in litter size of model animals(Shapira et.al.,1989). Frequent abortion is observed in animals that digest this plant in dry seasons(Mahmoudian et.al.,2002).

Recently there has been renewed interest in the use of medicinal plants by local communities to treat a broad spectrum of human disorders. In parallel there has been an increased scientific interest in the bioconstituents(steroids,alkaloids,flavonoids….etc) of these plants. A knowledge of bio-active components would evidently site a rationale for traditional uses of medicinal plants and enrich the global database of phytochemicals.The constituents and the antimicrobial potential of Pregnan harmala oil were addressed by many authors. However, such constituents are influenced to some extent by geographical distribution. To the best of our knowledge there are no reports addressing the constituents and antimicrobial potency of the Sudanese material of Prenan harmala fixed oil. So we planned this study to evaluate the antimicrobial activity of fixed oil from Pregnan harmala native to Sudan and to probe the constituents present in this oil.

*Peganu*m harmala

*Peganu*m harmala

Taxanomy:

Harmal
Harmal (Peganumharmala) flower

Scientific classification	
Kingdom:	Plantae
Unranked:	Angiosperms
Unranked:	Eudicots
Unranked:	Rosids
Order:	Sapindales
Family:	Nitrariaceae
Genus:	Peganum
Species:	P. harmala
Binomial name	
Peganum harmala L.[1]	

Aim of this study

This study was designed to:

-Extract oil from *Peganum harmala* seeds

-GC-MS studies of the oil

2-Materials and Methods

2.1-Materials

2.1.1--Plant material

Seeds of *Peganum harmala* were collected from a forest reserve at "Hawata" – eastern Sudan.The plant was identified by direct comparison with reference herbarium sample.

2.1.2-Test organisms

Peganum harmala oil was screened for antibacterial and antifungal activities using the standard microorganisms shown in Table(1).

Table 1: Test organisms.

Ser. No	Micro-organism	Type
1	Bacillus subtilis	G+ve
2	Staphylococcus aureus	G+ve
3	Pseudomonas aeroginosa	G-ve
4	Escherichia coli	G-ve
5	Aspergillus niger	fungi
6	Candida albicans	fungi

2.2-Methods

Extraction of oil from Peganum harmala seeds:-

Powdered shade-dried seeds of Peganum harmala (300g) were exhaustively extracted with n-hexane (soxhlet).The solvent was removed under reduced pressure and the oil was kept in the fridge at 4^{o}C for further manipulation.

2.2.1-Antimicrobial assay

i) Preparation of bacterial suspensions

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours.

The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about 10^{8}-10^{9}colony forming units per ml. The suspension was stored in the refrigerator at 4°C until used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique.

Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dry nutrient agar plates. The plates were allowed to stand for

two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours.

ii)Preparation of fungal suspensions

Fungal cultures were maintained on dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

iii)Testing for antibacterial activity

The cup-plate agar diffusion method was adopted , with some minor modifications, to assess the antibacterial activity. (2ml) of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45°C in a water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes. The agar was left to settle and in each of these plates which were divided into two halves, two cups in each half (10 mm in diameter) were cut using sterile cork borer (No 4), each one of the halves was designed for one of the test solutions. Separate Petri dishes were designed for standard antibacterial chemotherapeutics(ampicillin and gentamycin).

The agar discs were removed, alternate cups were filled with 0.1 ml samples of each test solution using adjustable volume microtiter pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours.

The above procedure was repeated for different concentrations of the test solutions and the standard chemotherapeutics. After incubation, the diameters of the resultant growth inhibition zones were measured in triplicates and averaged.

3-Results and Discussion

3.1-Antibacterial activity

In cup plate agar diffusion assay, the oil was evaluated for antimicrobial activity. The averages of the diameters of the growth inhibition zones are shown in Table (5) .The results were interpreted in terms of the commonly used terms ; <9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active) .Tables (6) and (7) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table 5:- Antibacterial activity of *Peganum harmala* oil：M.D.I.Z(,mm)

Drug	Conc.(mg/ml)	Ec	Ps	Sa	Bs	Ca	An
oil	100	15	9	17	17	16	18

Table 6:- Antibacterial activity of standard chemotherapeutic agents :M.D.I.Z (mm)

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Ps.
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 7:- Antifungal activity of standard chemotherapeutic agents against standard fungi.

Drug	Conc. mg/ml	An.	Ca.
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

- Sa.: Staphylococcus aureus
- Ec.: Escherichia coli
- Pa.: Pseudomonas aeruginosa
- An.: Aspergillus niger
- Ca.: Candida albicans
- Bs.: Bacillus subtilis

The oil showed activity against all test organisms,but it was partially active against Pseudomonas aeruginosa. Significant activity was observed against the Gram positive bacteria: Staphylococcus aureus, Bacillus subtilis and and the fungus *Aspergillus niger* .

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50 seeds.[4]

Peganum harmala was first planted in the United States in 1928 in the state of New Mexico by a farmer wanting to manufacture the dye "Turkish Red" from its seeds.[3] Since then it has spread invasively to ArizonaCalifornia, Montana, Nevada, Oregon, Texas and Washington.[6] "Because it is so drought tolerant, African rue can displace the native saltbushes and grasses growing in the salt-desert shrub lands of the

Western U.S."[3]

5mm

Common names:[7]

• African rue

• Esphand (Persian, (دنپس - دنپسا

• Harmal peganum

• Harmal shrub

• Harmel

• Isband

• Ozallaik

• Peganum

• Steppenraute

• Syrian rue

• Yüzerlik, üzerlik (Turkish)

• Üzərlik

• Luotuo-peng (Chinese, 骆驼篷)

Traditional uses

In Turkey *Peganum harmala* is called **yuzerlik** or **uzerlik**. Dried capsules from this plant are strung and hung in homes or vehicles to protect against "the evil eye."

In Afghanistan, Azerbaijan, Iran, Iraq, Turkey, Uzbekistan and Tajikistan, dried capsules (known in Persian as دنپسا *espand* or هناددنفسا *esfand-dāneh* and **испанд** *ispand* or **испандут** *ispandut* by Tajiks and Bukharian Jews of Central Asia) mixed with other ingredients are placed onto red hot charcoal,[8] where they explode with little popping noises, releasing a fragrant smoke that is wafted around the head of those afflicted by or exposed to the gaze of strangers. As this is done, an ancient prayer is recited. This prayer is said by Jews (more specifically, Bukharian Jews) and Muslims as well as by Zoroastrians. This Persian

practice dates to pre-Islamic, Zoroastrian times.

In Iran,this ritual is sometimes performed in traditional restaurants, where customers are exposed to the eyes of strangers.

5mm

Harmal has been used as an entheogen in the Middle East, and in modern Western culture, it is often used as an analogue of *Banisteriopsis caapi* to create an *ad hoc* Ayahuasca, the South American mixture of phytoindoles including DMT with β-carbolines. However, Harmal has distinct aspects from caapi and a unique entheogenic signature. Some scholars identify Harmal with the entheogenic haoma of pre-Zoroastrian Persian religions.[9]

A red dye, "Turkey Red,"[3] from the seeds is often used in Western Asia to dye carpets.[10] It is also used to dye wool.[3] When the seeds are extracted with

water, a yellow fluorescent dye is obtained.[11] If they are extracted with alcohol, a red dye is obtained.[11]
The stems, roots and seeds can be used to make inks, stains and tattoos.[12]

Medicinal uses

Peganum harmala is used as an analgesic and antiinflammatory agent.[13]
In Yemen it was used to treat depression,[14] and it has been established in the laboratory that harmaline, an active ingredient in *Peganum harmala*, is a central nervous system stimulant and a

"reversible inhibitor of MAO-A (RIMA),"[15] a category of antidepressant. Smoke from the seeds kills algae, bacteria, intestinal parasites and molds.[10] *Peganum harmala* has "antibacterial activity,"[16] including antibacterial activity against drug-resistant bacteria.[17]

The "root is applied to kill lice" and when burned, the seeds kill insects.[18] It also inhibits the reproduction of the *Tribolium castaneum* beetle.[19]

It is also used as an anthelmintic (to expel parasitic worms).[18] Reportedly the ancient Greeks used powdered *Peganum harmala* seeds to get rid of tapeworms and to treat recurring fevers (possibly malaria).[20]

Peganum harmala is an abortifacient,[21] and, in large quantities, it can reduce spermatogenesis and male fertility in rats.[22]

Antiprotozoal

It is fairly effective against protozoa including malaria. There is evidence that it may be effective against drug-resistant protozoa.[17] It is given in a decoction for laryngitis.[18]

One of the compounds found in *Peganum harmala*, vasicine (peganine) has been found to be safe and effective against *Leishmania donovani*, a protozoan parasite that can cause potentially "fatal visceral leishmaniasis."[23] "Peganine hydrochloride dihydrate, besides being safe, was found to induce apoptosis in both the stages of L. donovani via loss of mitochondrial transmembrane potential."[24]

Another alkaloid harmine found in *Peganum harmala*,

". . .because of its appreciable efficacy in destroying intracellular parasites as well as non-hepatotoxic and non-nephrotoxic nature, harmine, in the vesicular forms, may be considered for clinical application in humans."[25]

One study using the medicinal plant *Peganum harmala* showed it to have a lifesaving effect on cattle infected with the protozoal East Coast fever,[26] which can be 100% fatal and killed 1.1 million cattle in Africa in 1992.

Anticancer

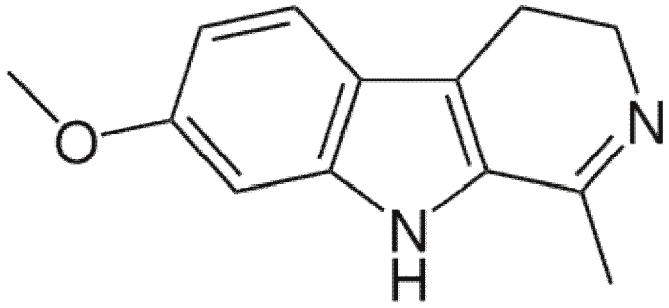
"The beta-carboline alkaloids present in medicinal plants, such as Peganum harmala and *Eurycoma longifolia*, have
recently drawn attention due to their antitumor activities. Further mechanistic studies indicate that beta-carboline
derivatives inhibit DNA topoisomerases and interfere with DNA synthesis."[27]

Peganum harmala has antioxidant and antimutagenic properties.[28]

Peganum harmala as well as harmine exhibit cytotoxicity with regards to HL60 and K562 leukemia cell lines.[29]

Ground *Peganum harmala* seeds have been used occasionally to treat skin cancer and subcutaneous cancers
traditionally in Morocco.[30] Seed extracts also show effectiveness against various tumor cell lines both *in vitro* and
in vivo.[30]

Alkaloids



The active alkaloids of Harmal seeds are the MAOI-A (monoamine oxidase inhibitor A) compounds:

• Harmane, 0.16%[31]

• Harmine, 0.44[32] –1.84%[31] –4.3%[33]

The coatings of the seeds are said to contain large amounts of harmine.[2]

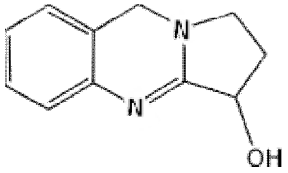
• Harmaline, 0.25%[31] –0.79%[32] –5.6%[33]

• Harmalol, 0.6%[33] –3.90%[31]

• Tetrahydroharmine, 0.1%[33]

Total harmala alkaloids were at least 5.9% per dried weight, in one study.[31]

• Vasicine (peganine),[21] 0.25%[32]



• Vasicinone,[21] 0.0007%[32]

The stems of the plant contain about 0.36% alkaloids, the leaves about 0.52%,[34] and the roots up to 2.5%.[35]

Harmine and harmaline are reversible inhibitors of MAO-A (RIMA).[15]

Toxicity of Peganum harmala

ABSTRACT

Peganum harmala L. is a plant, which grows in semi-arid rangeland. The plant is used traditionally as an emmenagogue and an abortifacient agent

in the Middle East and North Africa. All parts of plant are thought to be toxic and sever intoxication occurs in domestic animals. Digestive and nervous syndromes have been observed in animals that consume sub-lethal amount of the plant. The toxicated animal ap-pears in a narcotic state interrupted by occasional short period of excitement. Abortion is frequent in ani-mals that digest this plant in a dry year. While this plant has traditionally been used in Middle East, it shows toxic effects in human. A case of human overdose with P. harmala seeds is reported in this paper. Symptoms experienced by our patient found to be similar to what has been reported for domestic ani-mals.

Fatty acid constituents of Peganum harmala plant
using Gas Chromatography–Mass Spectroscopy

Abstract :

Fatty acid contents of the Peganum harmala plant as a result of hexane extraction were analyzed using GC–MS. The saturated fatty acid composition of the harmal plant was tetradecanoic, pentadecanoic, tridecanoic, hexadecanoic, heptadecanoic and octadecanoic acids, while the saturated fatty acid derivatives were 12-methyl tetradecanoic, 5,9,13-trimethyl tetradecanoic and 2-methyl octadecanoic acids. The most abundant fatty acid was hexadecanoic with concentration 48.13% followed by octadecanoic with concentration 13.80%. There are four

unsaturated fatty acids called (E)-9-dodecenoic, (Z)-9-hexadecenoic, (Z,Z)-9,12-octadecadienoic and (Z,Z,Z)-9,12, 15-octadecatrienoic. The most abundant unsaturated fatty acid was (Z,Z,Z)-9,12,15-octadecatrienoic with concentration 14.79% followed by (Z,Z)-9,12-octadecadienoic with concentration 10.61%. Also, there are eight non-fatty acid compounds 1-octadecene, 6,10,14-trimethyl-2-pentadecanone, (E)-15-heptadecenal, oxacyclohexadecan-2 one, 1,2,2,6,8-pentamethyl-7-oxabicyclo[4.3.1] dec-8-en-10-one, hexadecane-1,2-diol, n-heneicosane and eicosan-3-ol.

Purification of antioxidant protein isolated from *Peganum harmala* and its protective effect against CCl4 toxicity in rats

Abstract:

The present study was conducted to determine the protective effect of the purified protein from seeds of *Peganum harmala*
against carbon tetrachloride (CCl4)-induced toxicity in male albino rats. The purification steps included ammonium sulfate fractionation
and chromatography on DEAE-cellulose, CM-Sepharose, and Superdex 75 columns. The molecular mass of the purified protein was
132 kDa by gel filtration technique; it consisted of 2 subunits with molecular masses of 30.199 kDa and 38.018 kDa by SDS-PAGE.

Results of the dose-dependent experiment with purified protein prior to CCl4 administration were higher at 4 mg/kg body weight.

The antioxidant activity of the purified protein was determined in vitro by DPPH radical scavenging test. Administration of CCl4 significantly increased the activities of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase in serum.

However, a significant decrease in the level of total serum protein as well as the activities of superoxide dismutase, catalase, and reduced glutathione in liver tissues, and a significant increase in malondialdehyde level, were recorded. Pretreatment with 4 mg/kg body weight of the purified protein significantly altered the deteriorating damage induced by CCl4 toxicity to a near normal range, which was similar to treatment with vitamin C. These results suggest that the purified protein possesses a protective effect against CCl4-induced toxicity and probably acts as an antioxidative defense through free radical scavenging activity.

ANTIBACTERIAL ACTIVITY OF DIFFERENT PARTS OF *PEGANUM HARMALA* L. GROWING IN IRAN AGAINST MULTI-DRUG RESISTANT BACTERIA

Peganum harmala L. (Zygophyllaceae) is one of the most famous medicinal plants used in traditional medicine of Iran. The aim of this

study was to consider antibacterial effects of the methanolic extract of different parts of *P. harmala* including root, stem, leaf, flower and seed against some important human pathogenic bacteria. Antibacterial properties of methanolic extract of mentioned parts were assessed by disc diffusion method. Active extract was fractioned using Thin Layer Chromatography; also their synergism activity in combination with synthetic antibiotic was evaluated. Among the evaluated parts of *P. harmala*, the root and seed extracts presented antibacterial activity against all of tested bacteria even at the lowest concentration. Antibacterial effect of leaf part was moderate while stem and flower extracts showed relatively poor activity. Antibacterial activity of root extract against most of the tested Gram positive bacteria was better than seed extract. Tested against Gram negative bacteria the obtained results were inconsistent. MIC (Minimal Inhibitory Concentration) and MBC (Minimal Bactericidal Concentration) values for both extracts against MRSA (Methicillin Resistant
Staphylococcus aureus) and for seed extract against *E. coli* and *S. typhi* were equal (0.625 mg/ml). TLC (Thin Layer Chromatography) results revealed that seed and root extracts were different in terms of nature and content of their constituents. Furthermore, these two extracts
showed an excellent stability to temperature and pH treatment. Also, the seed and root extracts showed synergism in combination with novobiocin, colistin and carbenicillin. In conclusion,

P. harmala can be assigned as a source of antibacterial compounds for treatment of infections caused by multi-drug resistant (MDR) bacterial pathogens.

Main Alkaloids of *Peganum harmala* L. and Their Different Effects on Dicot and Monocot Crops

Abstract:

Alkaloids with allelopathic activity are not as well-known as other allelochemicals. Our study revealed that total alkaloids from seeds of the medicinal plant *Peganum harmala* L. possessed significant growth inhibitory effect on four treated plants, with dicot plants (lettuce and amaranth) being more sensitive than the tested monocot plants (wheat and ryegrass). Further investigation led to the isolation of harmaline and harmine as the main active ingredients in the total alkaloids of *P. harmala* seeds.

Harmaline exerted potent inhibitory effects on seedling growth of treated plants, especially dicots, inhibiting root elongation of lettuce and amaranth by 31% and 47% at a very low concentration (5 μg/mL), whereas harmine exhibited much weaker non-selective inhibitory effect on the plants. Considering the high yield and poor utilization of *P. harmala* in China, we anticipate that this plant could be exploited as an alternative weed management tool in the future.

1. Introduction

Peganum species (family Nitrariaceae) are mainly distributed in Africa, the Middle East, central Asia, South America, Mexico, and southern USA [13]. Three species, *i.e.*, *P. harmala* L., *P. nigellastrum*
Bunge and *P. multisectum* (Maxim.) Bobr. are found to grow in northwestern China, generally in arid and semi-arid regions, including Xinjiang Province, where our study site was located [3]. Among them, seeds and whole plant of *P. harmala* have a long history of use as a folk medicine in Turkey,
Iran and China to treat coughs, rheumatism, hypertension, diabetes and asthma [1,4]. Phytochemical studies of *P. harmala* led to the isolation of different types of chemical ingredients such as alkaloids,
steroids, flavonoids, anthraquinones, amino acids, and polysaccharides from its seeds, leaves, flowers, stems and roots [5–7]. Among these compounds, the alkaloids, mostly β-carbolines such as harmine,
harmaline, harmalol, harmol and tetrahydroharmine, were found to be the main substances responsible for the antimicrobial, antidepressant, antinociceptive, analgesic, antitumor and vasorelaxant activities of *P. harmala* [1,8–12]. Like many other medicinal plants, *P. harmala* has been speculated to possess allelopathic
properties, which presumably facilitates its dominance in its habitats and its invasive nature in southern USA [2,13]. It is suggested that allelopathy, which refers to any direct and indirect harmful or beneficial

effect by one plant on another through the production of chemical compounds that are released into the surrounding environment, might influence species distribution and abundance within plant communities, and contribute to the invasion success of many exotic plants [14–17]. However, allelopathy is a notoriously difficult mechanism to demonstrate because it is hard to elucidate how putative allelochemicals might influence the community after being released into the soil or the air [18].

Still, determination of phytotoxic substances in a certain plant is usually a necessary step to evaluate whether allelopathy exists, and the dependence of allelopathic effect occurring upon release of certain compounds into the environment [19]. These compounds are usually biosynthesized in the plants as secondary metabolites. Besides functioning as an ecological factor regulating plant community composition and dynamics, plants with allelopathic traits can also be utilized either directly in weed control, or their active allelochemicals can be developed into environmentally friendly herbicides [20,21]. Previously, *Peganum* species have been reported to exhibit inhibitory effect on neighboring plants' growth. Liu *et al.* [22,23] found that both aqueous and ethanol extracts of *P. multisectum* (Maxim.)

Bobr. greatly affected seedling growth of ryegrass pea, as well as the activities of SOD, CAT and POD. Khan *et al.* [24] found that a methanol extract of *P. harmala* decreased seed germination of radish; Sodaeizadeh

et al. [13,25] reported that aqueous extract and plant residues of *P. harmala* were toxic to treated plants, with the leaves being more toxic than stems and roots, and phenolic acids were proposed as the responsible phytotoxins. In those studies, phenolic acids were identified as the

potential allelochemical candidates mainly because they were the most commonly occurring compounds with allelochemical properties according to the literature [13,26], but it is highly possible that there are other phytotoxins that contribute to the plant growth inhibitory properties of *P. harmala*. In a preliminary experiment, we found that 0.05 g/mL aqueous extract of whole *P. harmala* plant greatly suppressed growth of wheat and lettuce seedling (data not shown), indicating the presence of active phytotoxins in this plant; we thus conducted the following study to evaluate the allelopathic potential of different plant parts (leaves, stems, roots and seeds) of *P. harmala*, and to isolate and identify toxic phytochemical constituents from this plant, which might function as potential allelochemicals and possibly being utilized as cost effective natural herbicides in the future.

2. Results and Discussion

2.1. Phytotoxic Assays of Different Plant Parts of P. harmala

Ethanol extracts of leaf, stem, root and seed all exerted very strong inhibitory activity on seedling

growth of wheat and lettuce (Table 1). Lettuce, a dicot plant, seemed to be more sensitive; its root

length was reduced to 9%, 8%, 4% and 4% of control by 0.05 g/mL ethanol extracts of leaf, stem, root and seed, respectively, whereas root length was 26%, 16%, 17%, and 10% of control for wheat, a monocot plant. Given the fact that seeds possessed the strongest phytotoxic activity, they were chosen for further investigation.

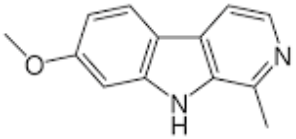
2.2. Isolation and Identification of Two Toxic Alkaloids from Seeds of P. Harmala

Column chromatography and preparative TLC of total alkaloids of seeds of *P. harmala* led to the

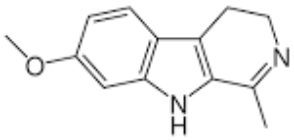
isolation of compound **1** (harmine, 1,150 mg) and compound **2** (harmaline, 140 mg; Figure 1), whose

structures were identified by comparing their spectral data with published literature [4,27].

Figure 1. Chemical structures of harmine and harmaline.



Harmine (**1**)



Harmaline (**2**)

2.3. High-Performance Liquid Chromotography (HPLC) Analysis of Harmine and Harmaline

HPLC analysis indicated that the amount of harmine and harmaline varied greatly in different plant parts of the *P. harmala* samples collected from our study site. Previous studies showed that seeds and roots of *P. harmala* contained the highest levels of alkaloids, with low levels in stems and leaves, and absence in flowers [9]. Our results revealed that harmaline was only abundant in seeds (2.87%), and harmine was abundant in both seeds (2.02%) and roots (0.69%). Stems contain low amounts of harmine (0.017%) but no harmaline, and leaves had very low amounts of harmaline (0.0006%) and harmine (0.0008%). The high amount of alkaloids in seeds and roots might explain the significant inhibitory activities of their ethanol extracts; however, total contents of both alkaloids in stems and leaves of *P. harmala* were very low, indicating that the toxicity of their ethanol extracts could be attributable to other chemicals too, for instance, phenolic acids [13].

2.4. Phytotoxic Effects of Total Alkaloids, Harmine and Harmaline

Among the total alkaloids, harmine and harmaline, harmaline exhibited the most potent inhibitory effect on seedling growth of the tested plants, whereas total alkaloids showed relatively moderate

activity, and harmine had the least inhibitory effect, all in a dose-dependent manner. Lettuce and amaranth were more sensitive to total alkaloids and harmaline, compared with the monocots wheat and ryegrass; on the other hand, the phytotoxicity of harmine did not distinguish between dicots and

monocots. In comparison with the herbicide glyphosate, total alkaloids and harmine exerted weaker toxicity on receiving plants, whereas harmaline showed stronger growth inhibitory effect on the dicot plants (lettuce and amaranth), and comparatively toxic effect on wheat and ryegrass, two monocot plants Root elongation of lettuce and amaranth was significantly inhibited by 30% and 43% when treated with 20 μg/mL total alkaloids; in comparison, root growth of wheat and ryegrass was not significantly affected by total alkaloids at such concentrations. When the concentration of total alkaloids increased to 100 μg/mL, root growth of two dicot plants, *i.e.*, lettuce and amaranth, was reduced by 76% and 86%, and only by 40% and 46% for wheat and ryegrass. At the highest concentration (500 μg/mL), root length of wheat, ryegrass, lettuce and amaranth was suppressed by 70%, 82%, 89% and 93%, respectively, with two dicots consistently being more sensitive than the monocots. Compared to total alkaloids, harmaline exhibited much stronger inhibitory effect on four treated plants, especially on dicot plants. At a very low concentration (5 μg/mL), root elongation of lettuce and amaranth was significantly affected by 31% and 47%, respectively. When the concentration reached 20 μg/mL, roots of lettuce and amaranth seedlings turned brownish, indicating the occurrence of tissue damage, which caused seedling death a few days later. In comparison, tissue damage of roots was triggered by 100 μg/mL harmaline for ryegrass, and 500 μg/mL for wheat. Seedling growth of

wheat was least influenced by harmaline, which only caused 62% reduction on root growth at 100 μg/mL, but 87%, 89% and 89% root reduction on ryegrass, lettuce and amaranth, respectively. At the highest concentration (500 μg/mL), harmaline nearly completely killed seedlings of ryegrass, lettuce and amaranth, and root length of wheat was still 17% of control. On the other hand, harmine showed weaker phytotoxicity on tested plants compared to total alkaloids and harmaline. Root elongation of all receiver plants was inhibited by less than 15% when treated with harmine at 20 μg/mL; even when harmine was applied at the highest concentration (500 μg/mL), root length of wheat, ryegrass and lettuce was still 42% , 56%, and 47% of control.

Amaranth, whose root elongation was only 5% of control when cultivated in 500 μg/mL harmine solution, was the most sensitive plant. Under *in vitro* bioassay conditions, both aqueous and ethanol extracts of *P. harmala* exhibited significant inhibitory effects on treated plants. It is evident that this medicinal plant can produce phytochemical compounds with plant growth inhibitory activities. Total alkaloids from seeds of *P. harmala* were found to possess strong plant growth inhibitory activity, and harmaline and harmine were identified as the major responsible compounds. Compared to other isolated allelochemicals, the toxicity of harmaline is relatively strong, and harmine is rather weak [16,19,28–30]. It is believed that almost all plant species can produce chemicals that are toxic to one species or another; therefore, it is insufficient to declare the

occurance of allelopathy of a certain plant species simply because of the presence of phytotoxins [31]. In fact, phytotoxins will not function as active allelochemicals unless they can be released into the environment, and persist in toxic forms in the medium (soil/air/water) at allelopathic levels for a certain period of time [32–34]. Therefore, it needs to be demonstrated that these alkaloids can be released into the soil matrix, possibly via leaching, litter decomposition and root exudation; meanwhile, like other allelochemicals, the fate of these alkaloids depends greatly on the environment. Once they enter the soil, these chemicals are exposed to various physicochemical and biological processes, which might trigger degradations or chemical reactions that lead to the production of novel compounds with different biological activities [35]. On the other hand,

besides allelopathy, *P. harmala* also possess other biological characteristics that might contribute to its dominance and invasiveness. For example, *P. harmala* is extremely drought tolerant; its deep taproot is characterized with 2 or 3 rings of anomalous vascular bundles surrounding the central cylinder, which is considered to be an important adaption to dry conditions [36]; it is unpalatable to animals [37]; *etc.* Taken together, we believe that the ecological success of *P. harmala* is attributable to the combination

of various biological properties, possibly including its inherent allelopathic traits, thus, further investigation is needed to demonstrate the possible involvement of allelopathy.

Synthetic herbicides are widely used in weed management, however they are usually toxic and may cause environmental problems. Moreover, overuse of certain chemical herbicides has created the problem of development of herbicide-resistant weeds. By 1998, 216 herbicide-resistant weed biotypes had been recorded in 45 countries, and the number of new herbicide-resistant weeds continues to increase, at an average of nine new cases per year worldwide [38]. Under such circumstances, developing environment-friendly new herbicides by utilizing natural products as lead compounds seems to be one approach to help solve these problems. There have been successful examples of using natural products, including allelochemicals, as sources to develop commercial herbicides [39]; for instance, mesotrione, a synthesized analogue of leptospermone that is produced by the "bottle brush" plant *Callistemon citrinus* [40], and cinmethylin, a derivative of 1,4-cineole that is a natural phytotoxin found in the essential oils of a number of plants [41]. More importantly, natural phytotoxins were found to act on a large number of unexploited herbicide target sites, which can be used to deal with the rapid evolving resistance to synthetic herbicides [42]. Besides utilizing allelochemicals as herbicides, plants

with allelopathic properties can also be applied in integrated weed management, for instance,

they can be used as cover crops, intercrops, green manure, and so on [21,43]. In a greenhouse experiment conducted by Sozaeizadeh *et al.* [25], plant residues of *P. harmala* were found to significantly suppress seedling growth of two wild weeds, indicating the possibility of utilizing this

plant in weed control. Considering the high yield and poor utilization of *P. harmala*, we anticipate that this plant could be exploited as an alternative weed management tool in the future.