Peganum harmala: a rich source of antimicrobial agents

Uzma Qaisar[1*]

[1]*School of Biological Sciences, University of the Punjab, Lahore*
Quaide Azam Campus, University of the Punjab, Lahore, Pakistan.

Abstract *— Infection causing bacteria are developing resistance against common antibiotics. To treat these antibiotic resistant bacteria, novel effective antimicrobial compounds are synthesized or isolated from various sources. Conventionally, a lot of medicinal plants are used to treat infections caused by pathogens. Peptides isolated from medicinal peptides are gaining a lot of interest owing to their potential to serve as natural antibacterial agents. Peganum harmala is a medicinal plant which contains various natural metabolites, compounds and proteins of pharmaceutical importance. A review of antibacterial agents of P. harmala plants are discussed here.*

Keywords *— antimicrobial peptides, medicinal plants, alkaloids*

I. INTRODUCTION

The use of medicinal plants as a natural source in the cure of diseases and infections has been widely accepted worldwide [1]. As a traditional medicinal approach, plant sources are used by almost 80% of the world population of Africa and Asia and are known to have minimal side effects [2]. A lot of studies have indicated some naturally existing compounds such as aldehydes, alkaloids, essential oils and peptides as antibacterial compounds [3]. These plants are therapeutically very important against many human microbes such as fungi, bacteria and viruses [4]. A lot of research has been carried out to identify the antimicrobial and industrial potential of these plants and their compounds [5, 6].

Various ethanolic, methanolic and aqueous extracts of the stems, leaves and barks has been tested for their pharmaceutical potential [7]. Essential oils, alkaloids, terpenes, sesquiterpenes, naphthoquinones, lactones, flavonoids and some other secondary metabolites were also identified for the medical benefits [8]. Acacia nilotica (Gum Arabic tree) barks are known for the cure of urino-genital diseases. Malvaceae family roots are used in the treatment of nervous and cardiac disorders. *Withania somnifera* is known for the treatment of cough, hiccups, dropsy and also as a sedative in gynecological disorders [9]. Certain spices e.g. thyme, black cumin, rose marry, cinnamon, bay leaves and mustard etc. are used as cure of bacterial infections. Garlic and clove are known to be the most important antimicrobial medicinal plants as several resistant bacterial strains are susceptible to their extracts [10].

Antimicrobial activities of various extracts (petroleum ether, methanol: dichloromethane, diethyl ether and methanol) isolated from medicinal plants i.e. *Hieracium pilosella* (mouse ear hawkweed), *Nepeta cataria* (catnip), *Plantago lanceolata* (ribwort plantain), *Lonicera caprifolium* (Italian honey suckle), *Phytolacca dodecandra* (Pokeweed) and *Helichrysum italicum* (curry plant) were studied. Screening of extracts against various gram positive and negative bacteria were found to possess antimicrobial potential. Diethyl ether extract contained the most efficient antimicrobial substances. Antimicrobial activity was found to be maximum in gram positive bacteria while gram negative were less susceptible to these extracts. It was indicated that maximum antimicrobial activity was observed due to the presence of terpenes and flavonoids [11]. Antimicrobial activity of rosemary and clove was reported and also of their combination. The gram positive bacteria were tested for their susceptibility against these oils. Both these oils showed excellent antimicrobial effects on all of the bacterial strains. The combination of both the oils showed synergistic, antagonistic and additive effects [12].

II. ANTIMICROBIAL POTENTIAL OF *PEGANUM HARMALA*

Peganum harmala is known as ‘Wild Rue’, ‘Syrian Rue’ and ‘harmal’. It belongs to family Zygophyllaceae and is widely distributed in Pakistan, India, Turkey and Iran [13]. According to traditional Iranian medicine and modern phytotherapy, seeds of *P. harmala* are known to be the most important medicinal part of this plant. The well-known active pharmacologic compounds of ‘harmal’ are alkaloids, β-carboline (harmine, harmalol, harman, harmaline etc.) and quinazolone derivatives [14] responsible for antimicrobial, anticancer, cardiovascular, gastrointestinal, neurologic and anti-diabetic effects [15]. Traditional medicinal reports have shown some pharmacologic properties of harmal such as it was used as folk medicine to cure epilepsy, numbness, paralysis, memory loss, dropsy, vision performance, jaundice and some purgative properties as well [16]. These therapeutic observations according to traditional medicine needed experimental evidence

by research and use of modern therapeutics. Some of the documented pharmacologic properties are also confirmed through different techniques in modern phytotherapy. These properties include analgesics for the treatment of toothache, joint pain and sciatica. Some disinfecting properties, anthelminthic, antispasmodic, anti- inflammatory and antibacterial properties are also observed [17].

Harmal possess antifungal potential and is known to cure several fungal diseases. Antifungal proteins isolated from plants can be divided into different types i.e. chitinase like, cyclophilin like, defensins, ribosome inactivating and lipid transfer proteins. Lipid transfer proteins of harmal which are small molecular weight basic proteins that help in lipid exchange between microbodies and mitochondria. These lipid transfer antifungal proteins are known to be involved in various metabolic processes which are important in the secretion of lipids to the cell walls [18]. Antimicrobial potential of *P. harmala* has been observed in the following components.

III. ANTIBACTERIAL EXTRACTS OF P. HARMALA

A lot of research work has been reported on different extracts of harmal plant. In-vitro antibacterial activities of chloroform, methanolic and petroleum ether extracts of *P. harmala* against bacterial strains of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Proteus vulgaris* was observed. Comparison of activities of these three extracts with the standard antibiotic chloramphenicol showed that methanolic extract was the best to show positive antibacterial results, followed by chloroform extract and the petroleum ether showed an average effect. Minimum inhibitory concentration (MIC) for methanolic extraction was 0.39 mg/ml for *P. vulgaris* and 1.56 mg/ml for *P. aeruginosa* [19]. It has been very common to explore different plant parts and their different extracts in the development of antimicrobial compounds. Different parts of *P. harmala* plant possessed antibacterial activity against some notorious multi drug resistant bacterial strains important in human infections. Methanolic extracts of flower, leaves, roots and stems were used to check out the bacterial susceptibility against these extracts by using disc diffusion method. By thin layer chromatography (TLC), active fractions of these extracts were identified. It was observed that the seed and root extracts were the most effective among all other as they possessed remarkable antibacterial activity even at extremely low concentrations. Stem and flower extracts showed minimum activity and were unable to inhibit majority of these pathogenic strains. Leaves showed an average effect among all other extracts. Inhibitory effect of these extracts against methicillin resistant *S. aureus*, *Salmonella typhi* and *E. coli* were found. When TLC analysis was done, root and seed extracts were found to have different constituents and chemical composition indicating that they both were different in nature. Seed and root extracts were also stable at different pH and temperature fluctuations. So, it was concluded that the extracts of *P. harmala* were excellent antibacterial compounds and can be used in the cure of infections caused by pathogenic clinical multidrug resistant bacterial strains [20].

Smoke extracts of harmal plant had been used as a disinfectant for several years and is very well known in traditional medicine. Smoke extract is prepared by collection of smoke from smoldering seeds in an evaporating chamber using dichloromethane and n-hexane as solvent. Clinical bacterial strains used in a study were *E. coli*, *Bacillus subtilis*, *K. pneumoniae*, *S. epidermidis*, *S. typhi*, *S. aureus*, *P. aeruginosa*, *S. dysenteriae* and *Serratia marcescens.* Smoke preparation was analyzed by gas chromatography and mass spectrometry and different chemical composition was found out. Dichloromethane extract and harmine was active against bacterial strains tested and exhibit remarkable antimicrobial activity. In n-hexane extract, harmine was not detected and this extract did not show antimicrobial activity against many of the strains tested [21].

IV. P. HARMALA ANTIMICROBIAL ALKALOIDS

Alkaloids of different plants are important to possess antibacterial and antifungal properties. Harmal plant also possess several alkaloids which were isolated and showed antimicrobial properties. β – carboline alkaloids, harmaline, harmalol, harmine and other alkaloids are important active constituents of harmal plant and possess different important bioactivities [22]. Antimicrobial activities of harmine against *B. subtilis*, *P. vulgaris* and *Candida albicans* was reported. Total alkaloid extract was shown to have maximum synergistic antimicrobial effect against these microbes [23]. Thus alkaloids of harmal act as strong antimicrobial compound. Modern phytotherapy has revealed several pharmacological effects of *P. harmala* and presence of some basic alkaloids. Roots, barks, stems and leaves of this plant had been used in folk medicine for centuries. These alkaloids are really very important in the treatment of infectious diseases and recent research have elaborated very wide applications of the active compounds of this plant.

V. P. HARMALA ANTIMICROBIAL PEPTIDES

Purification of a large number of antimicrobial peptides from kernels of *Zea maize* which were acid soluble and basic in nature were purified as well as characterized. MBP-1 was found to have antimicrobial activity against several pathogenic

fungi i.e. *Fusarium graminearum*, *F. moniliforme*, *Aspergillus flavus* and *Alternaria longipes* which inhibited spore formation or hyphal growth. Bacterial strains found to be susceptible to this antimicrobial peptide was *E. coli* and *Clavibacter michiganense* [24]. Some antimicrobial peptides are being synthesized to check their potential to inhibit bacterial and fungal strains.

Crude protein extracts of harmal have been known to contain antifungal and anticancer compounds. These protein extracts were extracted and purified by using 80% ammonium sulphate and then used to check growth inhibition. HeLa cells were found to be inhibited and apoptosis was induced by these crude harmal proteins [25]. In another study, antifungal harmal protein of 16 kDa was isolated, purified and characterized by using cation exchange chromatography and gel filtration columns. This protein was seen to have lipid binding properties and inhibit mycelial growth of various fungal species i.e. *Penicillium digitatum*, *Magnaporthe grisea*, *A. alternate* and *Rhizopus stuolonifer*. This novel antifungal protein did not show any antibacterial and hemagglutinating properties [26].

VI. MECHANISM OF ANTIMICROBIAL ACTIVITY

There are three models proposed for the action of antimicrobial peptides. First one is 'Barrel-Stave' model which explains that transmembrane pore formation in bacterial lipid core is made by direct insertion of peptides secondary structures [27]. These peptides bind in the form of monomer leading to the recruitment of more monomers causing increase in pore size. Cytoplasmic contents are leaked and bacterial cell death occurs [28]. Second model is 'Toroidal Model' in which peptide residues are inserted into bacterial membrane in the form of bundles and lipid molecules tend to bend along with them [29]. This lead to the formation of toroidal pore. Examples include Magainin, Melittin and Protegrins [30]. In the 'Carpet Model', antimicrobial peptides disrupts bacterial cell membrane by electrical attractive forces which cover the surface disturbing complete architecture. The formation of pores and disruption of membrane lead to cell lysis, depend on concentration of peptides [31]. All these three models suggest that the antimicrobial peptides disrupt bacterial cell membrane leading to cytoplasm leaking and cell death.

VII. CONCLUSION

P. harmala is a rich source of antibacterial agents such as alkaloids, secondary metabolites and peptides. In the present era where microbes are developing resistance against most of the antibiotics, *P. harmala* should be used as a source of novel natural antibiotics. Antibiotic agents should be purified and tested through clinical trials for use against multi- resistant bacteria and other pathogens.

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