Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2016; 8(12): 1673-1684

ISSN- 0975 1556

Research Article

Antibacterial Activity of Several Plant Extracts Against *Proteus species*

Wurood Alwan Kadhim[1], Mohanad Jawad Kadhim[2], Imad Hadi Hameed[3*]

[1]*College of Science for Women, University of Babylon, Iraq*
[2]*College of Biotechnology, Department of Genetic Engineering, Al-Qasim Green University, Iraq*
[3]*College of Nursing, University of Babylon, Iraq*

Available Online: 25th December, 2016

ABSTRACT

Infectious diseases are one of the major problems in developing as well as developed countries. Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products used in the traditional systems of medicine. Thus, it is a logical approach in drug discovery to screen traditional natural products. Approximately 20% of the plants found in the world have been submitted to pharmaceutical or biological test and a sustainable number of new antibiotics introduced in the market are obtained from natural or semi synthetic resources. Medicinal plants are finding their way into pharmaceuticals, cosmetics, and neutralceuticals. In pharmaceutical field medicinal plants are mostly used for the wide range of substances present in plants which have been used to treat chronic as well as infectious diseases. Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted. Since antiquity, man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies. The drugs already in use to treat infectious disease are of concern because drug safety remains an enormous global issue. Most of the synthetic drugs cause side effects and also most of the microbes developed resistant against the synthetic drugs. To alleviate this problem, antimicrobial compounds from potential plants should be explored. These drugs from plants are less toxic, side effects are scanty and also cost effective. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. Plants are rich source of antibacterial agents because they produce wide array of bioactive molecules, most of which probably evolved as chemical defense against predation or infection. A major part of the total population in developing countries still uses traditional folk medicine obtained from plant resources with an estimation of WHO that as many as 80% of world population living in rural areas rely on herbal traditional medicines as their primary health care, the study on properties and uses of medicinal plants are getting growing interests. In recent years, this interest to evaluate plants possessing antibacterial activity for various diseases is growing. The present antibacterial review of the plant extracts demonstrates that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases.

Keyword: Antibacterial activity, Traditional medicinal plants, *Proteus*, Virulence factors.

INTRODUCTION

Proteus species are among the commonly implicated pathogens in hospital as well as community acquired infections[1,2]. This pathogen has a diverse mode of transmission, and hence can cause infection in different anatomical sites of the body. Some of the incriminating sources of transmission are soil, contaminated water, food, equipments, intravenous solutions, the hands of patients and healthcare personne[3].

The genus *Proteus*, which was described for the first time by Hauser in 1885, belongs to the *Enterobacteriaceae* family. In this family it is placed in the tribe *Proteeae*, together with the genera *Morganella* and *Providencia*[4]. These bacteria are gram negative rod measuring 0.6 to 6.0 µm in length and 0.3 to 1.0 µm in width, motile by peritrichous flagella, facultative anaerobic non spore forming, non-capsulated, most isolates have fimbriae[5]. *Proteus* species are distinguishable from most other genera by their ability to swarm across agar surfaces of solid media.

Epidemiology

Members of the genus *Proteus* are widely distributed in the natural environment, including polluted water, soil, and manure. Due to their proteolytic activity, the ability to hydrolyze urea to ammonia and carbon dioxide, as well as the oxidative deamination of amino acids, these bacteria are involved in the decomposing of the organic matter of the animal origin. They are also present in the

**Author for Correspondence: imad_dna@yahoo.com*

human gastrointestinal tract of humans and animals[6]. *P. mirabilis* was most frequently isolated from dogs, cows and birds[7]. It is by far the most common species identified in clinical specimens. The most common infections caused by *Proteus* spp. are urinary tract infections (UTIs). *Proteus* spp. can be found to colonize the vaginal introitus prior to onset of bacteruria. Therefore, like *Escherichia coli*, *Proteus* spp. causes urinary tract infections by ascending from the rectum to the periurethra and bladder.

P. mirabilis is by far the most common species identified in clinical specimens. *P. mirabilis* is a common cause of both community-acquired and catheter-associated UTI, cystitis, pyelonephritis, prostatitis, wound infections, and burn infections, and occasionally causes respiratory tract infections, chronic suppurative otitis media, eye infections (endophthalmitis), meningitis, and meningoencephalitis[8]. It is a common cause of bacteremia following catheter-associated UTI[9], and in rare cases has been reported to cause cellulitis, endocarditits, mastoiditis, empyema, and osteomyelitis[10]. It has also been suggested that *P. mirabilis* could have a role in the etiology of rheumatoid arthritis[11].

P. vulgaris, previously considered biogroup 2, has been reported to cause UTIs, wound infections, burn infections, bloodstream infections, and respiratory tract infections[12]. There has also been one case study of *P. vulgaris* causing bacteremia and brain abscesses, with the suspected point of entry being the digestive tract[13]. *P. penneri*, previously biogroup 1, generally causes UTIs, wound infections, burn infections, bloodstream infections, and respiratory tract infections.There has been one case study of *P. penneri* Fournier's gangrene in a child with congenital genitourinary anomalies[14]. There has also been one recent report of *P. penneri* causing "red body disease" of the Pacific white shrimp *Penaeus vannamei*[15]. Notably, *P. penneri* may be incorrectly identified as *P. mirabilis* due to being indole-negative, and it cannot be clearly resolved from *P. vulgaris* by 16S sequencing unless using the 16S-23S internal transcribed spacer[15]. Thus, the burden of human infections caused by this organism may be underestimated.

P. myxofaciens was originally isolated from a gypsy moth and has been isolated from UTIs in India[16]. *P. hauseri*, previously considered biogroup 3, has not been associated with infections in humans. *Proteus* rods are opportunistic bacterial pathogens which under favorable conditions cause urinary tract infections (UTIs), commonly associated with complicated urinary tract infections. They generally affect the upper urinary tract (common site of infection), causing infections such as urolithiasis (stone formation in kidney or bladder), cystitis, and acute pyelonephritis. Rare cases of bacteraemia, associated with UTIs, with *Proteus* spp. have also been reported. Other infections include septicaemia and wound infections, meningitis in neonates or infants and rheumatoid arthritis[17,18]. Kalra Janda *et al*. (2006)[19] and Kalra *et al*. (2011)[20] reviewed endocarditis due to *Proteus* species, and Okimoto *et al*. (2010)[21] reported *P. mirabilis* pneumonia. Brain abscesses during *P. vulgaris* bacteremia were described by Bloch *et al*. (2010)[13]. However, it should be stressed that *Proteus* bacteria cause UTIs with higher frequency. This type of infections is classified as uncomplicated or complicated. Uncomplicated infections occur in patients, who are otherwise considered healthy, whereas complicated infections usually take place in patients with a urinary catheter in place or with structural and/or functional abnormalities in the urinary tract, suffering from another illness, immunocompromised, as well as after surgical intervention in the urogenital system. It was found that *Escherichia coli* is a common cause of uncomplicated infections. Complicated UTIs might be polymicrobial and are usually caused by Gram-negative bacteria *Proteus spp*., *Providencia stuartii*, *Morganella morganii*, *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* as well as some Gram-positive bacteria. *Proteus* species can cause hematogenous infections and ascending infections, however, the latter are more common for these microorganisms[14]. An overview of key Proteus mirabilis virulence factors that contribute to catheter colonization and blockage, infection of the bladder (cystitis) and kidneys (pyelonephritis), and to the formation of urinary stones (urolithiasis). ATFs, ambient-temperature fimbriae; GdhA, glutamate dehydrogenase; IgA, immunoglobulin A; MRK, mannose-resistant Klebsiella-like; MRP, mannose-resistant Proteus-like; PMFs, P. mirabilis fimbriae; Pta, Proteus toxin agglutinin; ZapA, serralysin.

Virulence factors of Proteus spp.

Proteus bacilli possess several virulence factors that explain their uropathogenic potential, many of which have been investigated in a murine model of UTI, where they evolved a number of morphological and biochemical features which are considered as virulence factors (Table 1, Figure 1 and Figure 2). These are fimbriae, important for adhesion, flagella, crucial for bacterial ascent to the kidneys through the ureter, as well as enzymes (urease hydrolyzing urea to CO2 and NH3; leading to the formation of struvite stones, antibody-degrading proteases, complement proteins, and tissue matrix proteins; a-keto acidgenerating amino-acid deaminases which function as iron-binding siderophores), toxins, such as hemolysins, that lyse red cells and release iron, a bacterial growth factor and endotoxin (lipopolysaccharide, LPS)[6].

Antimicrobial susceptibitlty

Proteus spp. are generally susceptible to broad–spectrum cephalosporins, aminoglycosides, and imipenem. Otherwise, *P. mirabilis* is also susceptible to trimethoprime-sulfamethoxazole, ampicillin, amoxicillin and pipercillin, *P.vulgaris* and *P. penneri* are also susceptible to cefoxitin, cefepime, and aztreonam. *P.mirabilis* is resistant to nitrofurantion. *P.vulgaris* and *P.penneri* are resistant to piperacillin, amoxicillin, ampicillin, cefoperazone, cefuroxime, and cefazolin[5]. *Proteus* can be naturally resistant to antibiotics, such as benzylepenicillin, oxacillin, tetracycline, and macrolides[6]. *Proteus* spp. can acquire resistance to ampicillin through plasmid mediated beta-lactamases, and chromosomal

beta-lactamase expression has now been reported. In the last decade there have also been numerous reports of production of extended-spectrum beta-lactamases (ESBLs) by *Proteus* spp.. The ESBLs can confer resistance to third generation cephalosporins such as cefotaxime, ceftriaxone and ceftazidime, as well as the monobactam, aztreonam[22]. The cephamycins (cefoxitin, cefotetan and cefmetazole) and the carbapenems (imipenem and meropenem) are generally not hydrolyzed by ESBLs[22]. However, resistance to carbapenems is starting to be observed in *Proteus* spp[23-25]. A wide variety of ESBLs have been detected in *P. mirabilis*, and recent reports indicate a rise in ESBL-producing *P. mirabilis*, for instance in Japan. CTX-M-type ESBLs have been detected in *P. mirabilis* isolates from Korea and Taiwan[26]. CTX-M2 is the most common ESBL in Japan[27] and it appears to be spreading rapidly[27]. CTX-M type β-lactamases also appear to be evolving in *P. mirabilis* via recombination[28]. CTX-M has been found on the *P. mirabilis* chromosome as part of an integrative and conjugative element (ICE) in addition to being plasmid-encoded[29]. TEM is another common ESBL in *P. mirabilis*[30], and the most common type of ESBL in *P. mirabilis* isolates from Croatia and Italy[31,32]. A new TEM (TEM-187) has been reported in *P. mirabilis*, which has broad activity against penicillins but lower activity than TEM-1[33,34]. It has been suggested that TEM-187 may represent an evolution of TEM enzymes from penicillinases to ESBLs, leading to underestimation of ESBLs in *P. mirabilis*. Other ESBL types include: VEB-1, an integron borne ESBL that was detected in a *P. mirabilis* isolate from a Vietnamese patient hospitalized in France, a multidrug-resistant isolate from Greece and in Taiwan; PER-1, which was detected in a *P. mirabilis* isolate from Italy; VIM-1, detected in three ESBL *P. mirabilis* isolates from Bulgaria (128); and SHV-type β-lactamases, detected in *P. mirabilis* isolates from Bulgaria[24] and Taiwan[35]. Metallo-beta-lactamases (MBLs) are also being reported in recent *P. mirabilis* isolates. For instance, one study from France identified a *P. mirabilis* isolate with a metallo-beta-lactamases, and a New Dehli metallo-beta-lactamase (NDM-1) has been identified in *P. mirabilis* isolates from New Zealand and India[36]. Interestingly, NDM-1 was present in a genomic island in one isolate of *P. mirabilis* and co-occurred with a VEB-6 ESBL and SGI-1[36], and it has been proposed that the presence of NDM-1 in a genomic island structure may enhance the spread of carbapenemases.

Multidrug resistance in *P. mirabilis* is also becoming more common. SGI-1 (*Salmonella* genomic island 1), an integrative mobilizable element of multidrug-resistant *Salmonella* typhimurim, has recently been detected in a surprisingly high percentage of *P. mirabilis* clinical isolates from France and indicates that *P. mirabilis* is a bacterial species of concern involved in dissemination of this multidrug-resistant element[37]. SGI-1 confers resistance to a wide variety of older drugs that are no longer commonly used to treat human infection, but the multidrug-resistant regions of SGI-1 from *P. mirabilis* isolates had complex mosaic structures and rearrangements capable of facilitating acquisition and/or movement of antibiotic resistance genes that jeopardizes use of third-generation cephalosporins and quinolones. An ESBL-producing *P. mirabilis* isolate has also been identified with both TEM and CTX-M[38]. Interestingly, ESBL production was found to be a risk factor for ciprofloxacin-resistant bacteremia due to *P. mirabilis*, and recent treatment with quinolone antibiotics was a risk factor for carriage of ESBL-producing *P. mirabilis*. A recent study from Tunisia also identified a high prevalence of plasmid-mediated quinolone resistance determinants among ESBL-producing *P. mirabilis* isolates[39-71]. Importantly, ESBL and non-ESBL producing isolates of *P. mirabilis* are frequently susceptible to beta-lactam/beta-lactamase inhibitor combinations. However, there have been some reports of inhibitor resistant TEM mutants (IRT) occurring in *P. mirabilis*[72]. These beta-lactamases are not inhibited by clavulanic acid, sulbactam and tazobactam. It should be noted that these beta-lactamases do not have extended-spectrum activity (that is, they do not hydrolyze third generation cephalosporins).

Another mechanism of beta-lactamase inhibitor resistance in *P. mirabilis* isolates is presence of plasmid-mediated AmpC beta-lactamases. AmpC type beta-lactamases (also termed group 1 or class C beta-lactamases) can either be chromosomally encoded or plasmid encoded in *P. mirabilis*[73]. AmpC has also been found on the chromosome as part of integrative and conjugative elements (ICE). Strains with plasmid-mediated AmpC beta-lactamases are consistently resistant to aminopenicillins (ampicillin or amoxicillin), carboxypenicillins (carbenicillin or ticarcillin) and ureidopenicillins (piperacillin). These enzymes are also resistant to third generation cephalosporins and the 7-α-methoxy group (cefoxitin, cefotetan, cefmetazole, moxalactam). AmpC beta-lactamases generally do not effectively hydrolyze cefepime or the carbapenems. One type of AmpC beta-lactamase is CMY, and clonal spread of CMY-producing *P. mirabilis* has been reported in Europe[74]. CMY is also the predominant AmpC in Taiwan[26], and AmpC has been reported in *P. mirabilis* isolates from Korea and Spain[29]. Carbapenems are generally active against *P. mirabilis*. Meropenem is more potent than imipenem against *P. mirabilis*[75]. Carbapenemases have been found in >*P. mirabilis*[76], albeit rarely. A recent report has documented the presence of the class D carbapenemase, OXA-23, in *P. mirabilis*.

Proteus vulgaris produces a chromosomally encoded beta-lactamase[77], referred to as the cefuroxime-hydrolyzing beta-lactamase (cefuroximase or CumA), which hydrolyzes cephalosporins. The enzyme can be induced by ampicillin, amoxicillin and first generation cephalosporins, weakly induced by carboxypenicillins, ureidopenicillins, cefotaxime and ceftriaxone, and inhibited by clavulanate. Strains of *P. vulgaris* that have a mutation in the regulatory genes of this beta-lactamase produce high levels of the enzyme and are resistant to penicillins, cefuroxime, ceftriaxone and cefotaxime.

However, these isolates will generally be susceptible to ceftazidime, aztreonam, cephamycins, carbapenems and beta-lactam/beta-lactamase inhibitor combinations. Ertapenem and meropenem are substantially more active than imipenem[78]. Quinolones and aminoglycosides are usually active against *P. vulgaris* strains, though *qnr* genes for quinolone resistance have been detected in recent isolates[79]. Tigecycline has lesser activity against *P. vulgaris* than against other *Enterobacteriaceae* (for example, MIC_{50} 4 µg/mL against *P. vulgaris* but 0.25 µg/mL against *E. coli*)[80].

P. penneri: Like *P. vulgaris*, *P. penneri* is naturally resistant to ampicillin, narrow-spectrum cephalosporins and cefuroxime, by virtue of production of a similar beta-lactamase[81]. *P. penneri* is considered to be a nosocomial pathogen with an underestimated potential to cause disease, and a recent case report identified a multidrug-resistant ESBL-producing *P. penneri* isolate[82]. *P. myxofaciens*: One report of *P. myxofaciens* from UTIs in India discussed antibiotic susceptibility, and found this species to be susceptible to imipenem, ciprofloxacin, amikacin, gentamicin, trimethoprim-sulfamethoxazole, aztreonam, ofloxacin and piperacillin and resistant to methicillin and nalidixic acid[16].

Resveratrol

Resveratrol is (3,5,4'-trihydroxy-*trans*-stilbene) is a stilbenoid, a type of natural phenol, and a phytoalexin produced naturally by several plants especially the roots of the Japanese Knotweed (*Polygonum cuspidatum*), from which it is extracted commercially when under attack by pathogens such as bacteria or fungi[26]. Phytoalexins are low molecular weight compounds which have been shown to possess biological activity against a wide range of plant and human pathogens. Resveratrol is found in the skin of red grapes and in other fruits. It also has been produced by chemical synthesis and by biotechnological synthesis (metabolic engineered microorganisms.It has a wide range of biological activities and consequently it has many different targets and mechanisms of action. Resveratrol can prevent or slow the progression of several diseases, including cardiovascular disease, carcinogenic disease and neurodegenerative disease. It also prevents many aging processes and increases longevity. Moreover, resveratrol has anti-inflammatory, antioxidant and antimicrobial properties[83]. Recent studies have indicated that resveratrol has growth-inhibitory effects on some bacterial pathogens[84]. In the course of studying the effect of resveratrol on human pathogens, Wang *et al.* (2006)[26] found that resveratrol could inhibit swarming and virulence factor expression in *P. mirabilis*.

p-nitrophenylglycerol (PNPG)

P-nitrophenylglycerol or 1-(4-Nitrophenyl-β-Dglucuronicacid) (PNPG) is a chromogenic β-glucuronidase substrate[85]. The anti-swarming agent PNPG has long been used to aid the isolation of small numbers of many different pathogenic bacteria from specimens contaminated with swarming strains of *Proteus* spp. In addition, PNPG has little effect on the results of a variety of identification tests performed directly on colonies from media containing PNPG[86]. It is relatively cheap, nontoxic and doesn't affect red blood cells; even fastidious pathogens will grow well and with characteristic colony morphology in its presence. Its heat stability and long 'shelf -life' make it convenient to use in the preparation of media[87].

Fatty acids

The antibacterial activity of long-chain unsaturated fatty acids have been well known for many years. Fatty acids function as the key ingredients of antimicrobial food additives which inhibit the growth of unwanted microorganisms. Linoleic and oleic acids are antibacterial components in the herbs (*Helichrysum pedunculatum* and *Schotia brachypetala*). Besides normal fatty acids, fatty acid derivatives showing potent antimicrobial activities exist in nature. These are mainly found in microorganisms, algae, or plants, which may mediate chemical defense against microorganisms. Additionally, long-chain unsaturated fatty acids are bactericidal to important pathogenic microorganisms, including Methicillin-resistant *Staphylococcus aureus*, *Helicobacter pylori* and *Mycobacteria*. These antibacterial actions of fatty acids are usually attributed to long-chain unsaturated fatty acids including oleic acid, linoleic acid, and linolenic acid, while long-chain saturated fatty acids, including palmitic acid and stearic acid, are less active. However, their primary molecular target still remains unknown. Fatty acids are one of the most ubiquitous components of bacterial cell membranes. Interestingly, it has been shown that exogenously added fatty acids modulate various bacterial activities, including motility, virulence, cell growth, and differentiation. Swarming growth is also influenced by fatty acids. Oleic acid stimulates, whereas lauric and myristic acids inhibit this phenomenon, respectively. Straight-chain saturated fatty acids (SCFAs) repress swarming motility and hemolysin production in *Proteus mirabilis* and *Serratia marcescens*[87].

Urea

Urea or carbamide is an organic compound with the chemical formula $CO(NH_2)$[88]. It serves an important role in the metabolism of nitrogen-containing compounds by animals and is the main nitrogen-containing substance in the urine of mammals. It is a colorless, odorless solid, highly soluble in water and practically non-toxic (LD_{50} is 15 g/kg for rat), it is neither acidic nor alkaline. Urea has experimentally been demonstrated to possess anti-swarming properties and recommended for routine laboratory usage[89]. It is commonly used in culture media designed for the identification of pathogens of UTIs including *Proteus* spp. However, reports have been silent on *Proteus* swarming prevention possibilities. Urea is primarily used in selective and composite media to identify urease producing microorganisms. In recent times, the possibilities of exploiting the anti-swarming property of urea to aid isolation and identification of single colonies on solid media are been confirmed[90,91].

Ethanol

Ethanol also called ethyl alcohol, pure alcohol, grain alcohol, is a volatile, flammable, colorless liquid with the structural formula CH_3CH_2OH, often abbreviated as

Table 1: Biochemical characteristics common to the genera *Proteus*, *Morganella* and *Providencia*[7].

Biochemical test	*Proteus*	*Morganella*	*Providencia*
Arginine dihydrolase	–	–	–
Lysine decarboxylase	–	–	–
Ornithine deaminase	+	+	+
Phenylalanine deaminase	+	+	+
Growth on KCN	+	+	+
d-Glucose from acid	+	+	+
Acid from melibiose	–	–	–
Nitrite from nitrate	+	+	+
Oxidase production	-	–	–
ONPG production	-	–	–
Pectate utilization	-	–	–
Tartrate utilization	+	+	+

Symbols and Abbreviations: +, present; −, absent; KCN, potassium cyanide; and ONPG, o-nitrophenyl-β-d-galactopyranoside.

C_2H_5OH or C_2H_6O. It has bactericidal activity and is used often as a topical disinfectant[92,93]. Ethanol at 90% added to the medium at a 5% concentration is also a very effective anti-swarm agent. It allows an easier isolation of gram-postive cocci and members of the families *Enterobacteriaceae* and *Pseudomonaceae.* However, when ethanol is used in blood agar medium, hemolytic reactions cannot be reliably determined. In some cases, the addition of chemical agents such as ethanol can interfere with the growth of other bacteria[94,95].

Sodium Azide

Sodium azide is the inorganic compound with the formula NaN_3. It is used for the preparation of other azide compounds. It is an ionic substance, is highly soluble in water, and is very acutely toxic[96]. It is a potent bacteriostatic that is frequently used to protect a diverse array of stock solutions (e.g., antibodies) and samples (e.g., milk, fecal specimens) from prokaryotic contaminants. NaN_3 binds to heme-iron (cytochrome oxidase, catalase) leading to chemical asphyxiation of affected cells. However, the bacteriostatic effects of NaN_3 appear to be limited to Gram-negative *Bacteria*, whereas Gram-positive *Bacteria* are largely resistant to the compound[97]. The addition of sodium azide to blood agar media was reported to abolish the swarming of *Proteus* without affecting the isolation of clinically important *Staphylococci* and *Streptococci*, but blood agar media containing azide are not widely used in the clinical laboratory because azide turns out to be a poor anti-swarm agent and, it shows growth inhibition of certain *Streptococci*[90].

Antimicrobial activity

Plant based antimicrobials represent a vast untapped source. The use of plant extract for medicinal treatment has become popular when people realized that the effective life span of antibiotic is limited and over prescription and misuse of traditional antibiotics are causing microbial resistance[98]. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties[99]. In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world[100]. At present, nearly 30% or more of the modern pharmacological drugs are derived directly or indirectly from plants and their extracts dominate in homeopathic or ayurvedic medicines[101-115]. Considering the vast potentiality of plants as sources for antimicrobial drugs, this study aimed to detect the antibacterial activities of some natural plant extracts and investigate the effect of some commercial antibiotics against multi-drug resistant human clinical bacterial isolates.

Traditional medicinal plants used

The medicinal properties of those plants were studied by several workers in Tamilnadu. It is very important to document the information about the medicinal plants from traditional healers to protect the knowledge of plant usage, because the younger generation is not interested to carry on the traditional knowledge. Many medicinal plants are given, which are used by traditional healers for their antimicrobial properties. Hereby, the mentioned plants are taken from references which are already included in ethnobotanical surveys[104]. This paper reviews specifically about the plants having antimicrobial properties. The increasing interest on traditional ethnomedicine may lead to discovery of novel therapeutic agent. Since, plant contains potential antimicrobial components that may be useful for evolution of pharmaceutical for the therapy of ailments. Plants with possible antimicrobial activity should be tested against some microbes to confirm the activity. Researchers are increasingly turning their attention to folk medicine looking for new leads to develop better drugs against cancer, as well as viral and microbial infections. The activity of plant extracts on bacteria and fungi has been studied by a very large number of researchers in different place of the world. The specific plants to be used and the methods of application for particular ailments were passed down through oral tradition. Plants with possible antimicrobial activity should be tested against some microbes to confirm the activity.

Bioactive compounds

The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body and these chemical substances are called phytochemicals. These phytochemicals were used to cure the disease in herbal and homeopathic medicines[117].

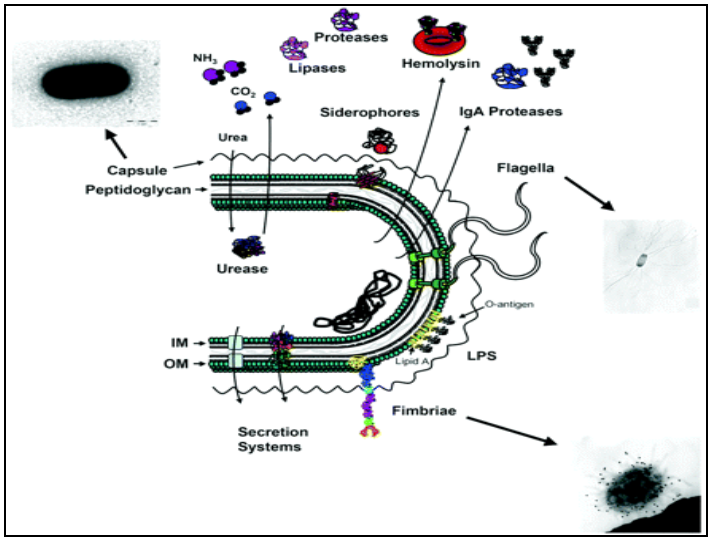

Figure 1: Virulence factors of the gram-negative uropathogens *E. coli* and *P. mirabilis*. IM, inner membrane; OM, outer membrane. (The micrographs are reprinted from references)[14].

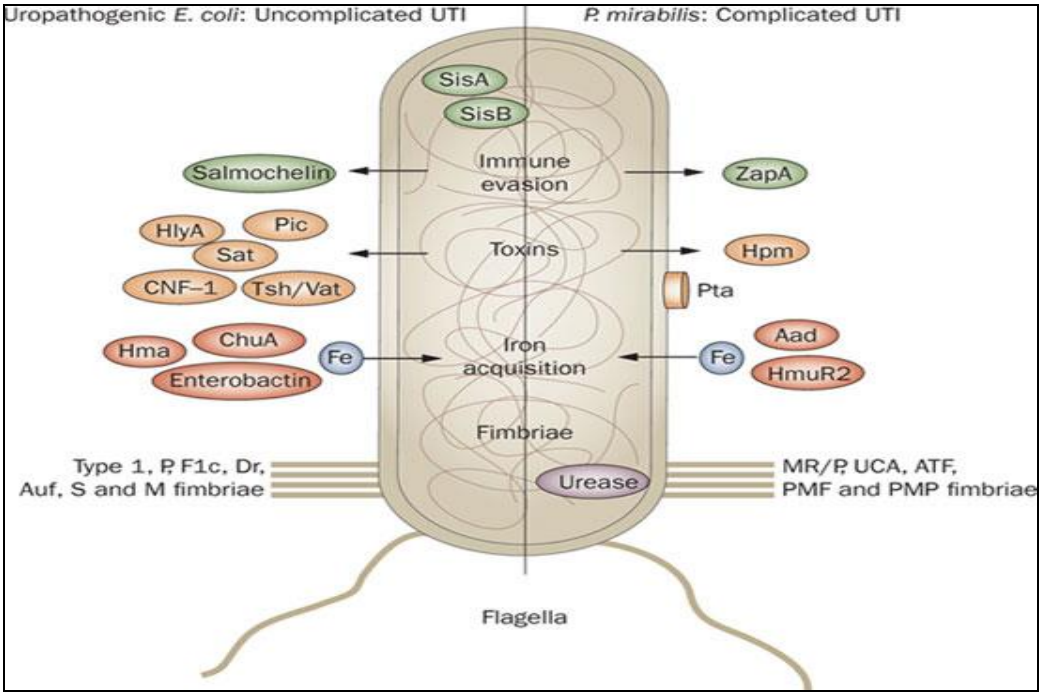

Figure 2: Overview of uropathogen virulence factors. The left hand side of this schematic bacterial cell represents uropathogenic *Escherichia coli*, and the right hand side represents *Proteus mirabilis*. Proteins that contribute to each mechanism of pathogenesis—immune evasion, toxin production, iron acquisition, adherence, and motility—are highlighted for each pathogen. *P. mirabilis* produces urease, which plays a role in the formation of urinary stones.

These are non-nutritive substances, have protective or disease preventive property[106]. There arises a need and therefore to screen medicinal plants for bioactive compounds as a basis for further pharmacological studies. With advances in phytochemical techniques, several active principles of many medicinal plants have been isolated and introduced as valuable drug in modern systems of medicine.

The most important of these bioactive compounds are alkaloids, flavonoids, tannins and phenolic compounds[107]. These are the important raw materials for drug production[118]. Most plants contain several compounds with antimicrobial properties for protection against aggressor agents, especially microorganisms[119]. Antimicrobials of plant origin have enormous therapeutic potential and have been used since time immemorial. They have been proved effective in the treatment of infectious diseases simultaneously mitigating many of the side effects which are often associated with synthetic antibiotics[7]. Many infectious diseases have been known to be treated with herbal remedies based on ethnobotanical knowledge. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the

unmatched availability of chemical diversity. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections. Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections. The antibacterial activity of ethanol extracts was determined by agar well diffusion method. The plant extracts were more active against Gram-positive bacteria than against Gram-negative bacteria among all the pathogens, all Gram-positive bacteria were inhibited by all four plant extract. All Gram-negative bacteria *i.e. Pseudomonas* spp, *Proteus* spp, *Escherichia coli, Shigella dysenteriae, Klebsiella pneumonia* and *Salmonella typhi* were showed zone of inhibition against extract of *Ocimum sanctum*[121].

In vitro microbicidal activity of the methanol extract of *Origanum marjorana* L. was tested against six bacteria (*Bacillus subtilis, B. megaterium, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa* and *Staphylococcus aureus*). The methanol extract of *O. marjorana* can be used as an effective herbal protectant against different pathogenic bacteria[122].

The inhibitory activity was highly significant in the aqueous extracts of *Oxalis corniculata*. Most of the plant extracts showed significant antibacterial activity than bacitracin. MIC of aqueous extract of twelve plants varied between 4-50 µl. Results indicate the potential of these plants for further work on isolation and characterization of the active principle responsible for antibacterial activity and its exploitation as whereas *Oxalis Acacia nilotica* varied between 9-35.5 mm. Whereas *corniculata* was effective against all the tested bacteria in case of *Lawsonia inermis* it varied between 9 to except *Shigella sonnei* and *Proteus mirabilis*[112]. Effectiveness of organic extracts of *Piper nigrum* fruit against pathogenic strains of *Escherichia coli* (MTCC 723), *Staphylococcus aureus* (MTSS 96), S*treptococcus pyogenes* (MTSCC 442), *Proteus mirabilis* (MTCC 1429) by tube dilution method. The study revealed that 70% alcoholic hot extract had higher antibacterial activity as compared to chloroform hot and petroleum ether cold extracts[113]. The aqueous extract was found to be antibacterial and it was studied against various Gram-positive and Gram-negative bacterial strains by using MIC, agar well diffusion method to find zone of inhibition. The MIC results of aqueous extract of *Plectranthus amboinicus* indicated that *Proteus vulgaris*, *Bacillus subtilis* and *Staphylococcus aureus* were least susceptible among the organisms tested and *Escherichia* coli, *Klebsiella pneumonia and Pseudomonas aeruginosa* are not shown any inhibition to aqueous extract of *Plectranthus amboinicus*[122].

CONCLUSION

Many medicinal plants have been found effective in the cure of bacterial diseases. Due to increasing antibiotic resistance in microorganisms and side effects of synthetic antibiotics medicinal plants are now gaining popularity in the treatment of bacterial infections. The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies. Medicinal plants are considered as clinically effective and safer alternatives to the synthetic antibiotics. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Extensive research in the area of isolation and characterization of the active principles of these plants are required so that better, safer and cost effective drugs for treating bacterial infections can be developed.

ACKNOWLGMENT

Authors are thankful to Department of Biology, University of Babylon, for providing facilities during preparation of this review article.

REFERENCES

1. Douglas, G., Mandell, G.L., Bennett, J.E. and Dolin, R. Bennett's Principles and Practice of Infectious Diseases. 5th ed. Philadelphia, Pa: Churchill Livingstone. 9,121-126 (2000).
2. Patrick, K.F., Stephen, Y.G., Solomon, N.A., Yaw, A. and Clement O.O. Occurrence, species distribution and antibiotic resistance of *Proteus* isolates: A case study at the Komfo Anokye Teaching Hospital (KATH) in Ghana. *International Journal of Pharma Sciences and Research*, 1(9), 347-352 (2010).
3. Heinzelmann, M., Scott, M. and Lam, T. Factors predisposing to bacterial invasion and infection. *Am J Surg*, 183(2), 179-90 (2002).
4. Rozalski, A. and Staczek, P. *Proteus.* In: Molecular detection of human bacterial pathogens. In: D. Liu (ed.), CRC Press, Taylor and Francis Group. *Boca Raton*, P. 981–996 (2011).
5. Abbott, S.L. *Klebsiella*, *Enterobacter*, *Citrobacter*, *Serratia*, *Plesiomonas*, and other *Enterobacteriaceae*. In P. R. Murray, E. J. Baron, J. H. Jorgensen, M. L. Landry & M. A. Pfaller (Eds.), Manual of Clinical Microbiology (9th ed., pp. 698-711). Washington, USA: ASM Press (2007).
6. Armbruster, C.E. and Mobley, H.L. Merging mythology and morphology: the multifaceted lifestyle of *Proteus mirabilis*. *Nat Rev Micro*. 10, 743-754 (2012).
7. Manos,J. and Belas, R. The Genera Proteus, Providencia, and Morganella. Prokaryotes. Chapter 3.3.12, 6,245–269 (2006).
8. Stock, I. Natural antibiotic susceptibility of *Proteus* spp., with special reference to *P. mirabilis* and *P. penneri* strains. *J Chemother*,15, 12-26 (2003).
9. Melzer, M. and Welch, C. Outcomes in UK patients with hospital-acquired *bacteraemia* and the risk of catheter-associated urinary tract infections. *Postgrad Med J*, 89(1052), 329-334 (2013).

10. Butkevich, O.M. and Vinogradova, T.L. Hospital infectious endocarditis and endocarditis in drug addicts Ter Arkh, 70, 56-8 (1998).
11. Wilson, C., Thakore, A. Isenberg, D. and Ebringer, A. Correlation between anti-*Proteus* antibodies and isolation rates of *Proteus mirabilis* in rheumatoid arthritis. *Rheumatol Int*, 16, 187-189 (1997).
12. Kim, B. N., Kim, N. J., Kim, M. N., Kim, Y. S., Woo, J. H., and Ryu, J. Bacteraemia due to tribe Proteeae: a review of 132 cases during a decade (1991-2000). *Scandinavian Journal of Infectious Diseases*, 35(2), 98-103 (2003).
13. Bloch, J., Lemaire, X., Legout, L., Ferriby, D., Yazdanpanah, Y. and Senneville, E. Brain abscesses during *Proteus vulgaris* bacteremia. Neurol. Sci. 32: 661–663 (2010).
14. Jacobsen, S.M., Stickler, D.J., Mobley, H.L. and Shirtliff, M.E. Complicated catheter-associated urinary tract infections due to *Escherichia coli* and *Proteus mirabilis*. *Clin Microbiol Rev*. 21, 26–59(2008).
15. Cundy, T.P., Boucaut, H.and Kirby, C.P. Fournier's gangrene in a child with congenital genitourinary anomalies. *J Pediatr Surg*, 47, 808-81 (2012).
16. Sharma, I. and Paul, D. Prevalence of community acquired urinary tract infections in silchar medical college, Assam, India and its antimicrobial susceptibility profile. *Indian J Med Sci*. 66(11-12),273 (2012).
17. O'hara, C.M., Brenner, F.W. and Miller, J.M. Classification, identification, and clinical significance of *Proteus, Providencia*, and *Morganella*. *Clin Microbiol Rev*. 13(4), 534-46 (2000).
18. Ronald, A. The etiology of urinary tract infection: traditional and emerging pathogens. *Disease-a-Month: DM*, 49(2), 71-82 (2003).
19. Janda, J.M., Abbott, S.L., Khashe, S. and Probert, W. Biochemical identification and characterization of DNA groups within *Proteus vulgaris* complex. *J Clin Microbiol.* 39 (4), 1231-4 (2006).
20. Kalra, A., Cooley, C. and Tsigrelis, C. Treatment of endocarditis due to *Proteus* species: a literature review. *Int. J. Infect. Dis*. 15, 222–225 (2011).
21. Okimoto, N., Hayashi, T., Ishiga, M., Nanba, F., Kishimoto, M., Yagi, S., Kurihara, T., Asoka, N. and Tamada, S. Clinical features of *Proteus mirabilis* pneumonia. *J. Infect. Chemother*. 16:364–366 (2010).
22. Philippon, A., Labia. R. and Jacoby, G. Extended-spectrum beta-lactamases. *Antimicrob Agents Chemother*, 33, 1131-1136 (2003).
23. Papagiannitsis, C.C., Miriagou, V., Kotsakis, S.D., Tzelepi, E., Vatopoulos, A.C., Petinaki, E. and Tzouvelekis, L.S. Characterization of a Transmissible Plasmid Encoding VEB-1 and VIM-1 in Proteus mirabilis." *Antimicrob Agents Chemother*, 56,4024-4025 (2012).
24. Schneider, I., Markovska, R., Marteva-Proevska, Y., Mitov, I., Markova, B.and Bauernfeind, A. Detection of CMY-99, a Novel Acquired AmpC-Type β-Lactamase, and VIM-1 in *Proteus mirabilis* Isolates in Bulgaria. *Antimicrob Agents Chemother*, 58, 620-621 (2014).
25. Wang, J.T., Chen, P.C., Chang, S.C., Shiau, Y.R., Wang, H.Y., Lai, J.F., Huang, I.W., Tan M.C. and Lauderdale, T.L. TSARHospitals. Antimicrobial susceptibilities of *Proteus mirabilis*: a longitudinal nationwide study from the Taiwan surveillance of antimicrobial resistance (TSAR) program. *BMC Infect Dis*,14, 486 (2014).
26. Kanayama, A., Kobayashi, I. and Shibuya, K. Distribution and antimicrobial susceptibility profile of extended-spectrum β-lactamase-producing *Proteus mirabilis* strains recently isolated in Japan. *Int J Antimicrob Agents*, 45, 113-118 (2015).
27. Fursova, N. and Pryamchuk, S., The Novel CTX-M-116 β-Lactamase Gene Discovered in *Proteus mirabilis* Is Composed of Parts of the CTX-M-22 and CTX-M-23 Genes. *Antimicrob Agents Chemother*, 57, 1552-1555 (2013).
28. Mata, C., Navarro, F., Miró, E., Walsh, T.R., Mirelis, B. and Toleman, M. Prevalence of SXT/R391-like integrative and conjugative elements carrying blaCMY-2 in Proteus mirabilis. *J Antimicrob Chemother*, 66, 2266-2270 (2011).
29. Kaur, M. and Aggarwal, A. Occurrence of the CTX-M, SHV and the TEM Genes Among the Extended Spectrum β-Lactamase Producing Isolates of *Enterobacteriaceae* in a Tertiary Care Hospital of North India. *J Clin Diagn Res*. 7, 642-645 (2013).
30. Sardeli, S., Bedeni, B., Sijak, D., Colinon, C. and Kaleni, S. Emergence of *Proteus mirabilis* Isolates Producing TEM-52 Extended-Spectrum β-Lactamases in Croatia. *Chemotherapy* 56, 208-213 (2010).
31. Siebor, E. and Neuwirth, C. The new variant of *Salmonella* genomic island 1 (SGI1-V) from a *Proteus mirabilis* French clinical isolate harbours blaVEB-6 and qnrA1 in the multiple antibiotic resistance region. *J Antimicrob Chemother*, 66, 2513-2520 (2011).
32. Tonki, M., Mohar, B., Sisko-Kraljevi, K., Mesko-Meglic, K. and Goić-Barisi, I., Novak A., Kovaci A., Punda-Poli V. High prevalence and molecular characterization of extended-spectrum β-lactamase-producing *Proteus mirabilis* strains in southern Croatia. *J Med Microbiol*, 59, 1185-1190 (2010).
33. Corvec, S., Beyrouthy, R., Crémet, L., Aubin, G.G., Robin, F., Bonnet, R. and Reynaud, A. TEM-187, a New Extended-Spectrum β-Lactamase with Weak Activity in a Proteus mirabilis Clinical Strain. *Antimicrob Agents and Chemother*, 57, 2410-2412 (2013).
34. Cremet, L. and Bemer, P. Outbreak caused by *Proteus mirabilis* isolates producing weakly expressed TEM-derived extended-spectrum β-lactamase in spinal cord injury patients with recurrent bacteriuria. *Scand J Infect Dis*, 43, 957-961 (2011).

35. Huang, C.W., Chien, J.H., Peng, R.Y., Tsai, D.J., Li, M.H., Lee, H.M., Lin, C.F., Lee, M.C. and Liao, C.T. Molecular epidemiology of CTX-M-type extended-spectrum β-lactamase-producing *Proteus mirabilis* isolates in Taiwan. *Int J Antimicrob Agents* 2015; 45:84-85.
36. Girlich, D., Dortet, L., Poirel, L. and Nordmann, P. Integration of the blaNDM-1 carbapenemase gene into *Proteus* genomic island 1 (PGI1-PmPEL) in a *Proteus mirabilis* clinical isolate. *J Antimicrob Chemother* 2015;70: 98-102.
37. Doublet, B., Poirel, L., Praud, K., Nordmann, P. and Cloeckaert, A. European clinical isolate of Proteus mirabilis harbouring the *Salmonella* 2010;65: 2260-2262.
38. Park, S.D., Uh, Y., Lee, G., Lim, K., Kim, J.B. and Jeong, S.H. Prevalence and resistance patterns of extended-spectrum and AmpC β-lactamase in *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Salmonella* serovar Stanley in a Korean tertiary hospital. *APMIS*, 118: 801-808 (2010).
39. Mahrouki, S., Perilli, M., Bourouis, A., Chihi, H., Ferjani, M., Ben Moussa, M., Amicosante, G. and Belhadj, O. Prevalence of quinolone resistance determinant qnrA6 among broad- and extended-spectrum beta-lactam-resistant *Proteus mirabilis* and *Morganella morganii* clinical isolates with sul1-type class 1 integron association in a Tunisian Hospital. *Scand J Infect Dis*. 45, 600-605 (2013).
40. Kadhim, M.J., Sosa, A.A. and Hameed, I.H. Evaluation of anti-bacterial activity and bioactive chemical analysis of *Ocimum basilicum* using Fourier transform infrared (FT-IR) and gas chromatography-mass spectrometry (GC-MS) techniques. *International Journal of Pharmacognosy and Phytochemical Research*. 8(6), 127-146 (2016).
41. Mohammed, G.J., Kadhim, M.J.and Hussein, H.M. Characterization of bioactive chemical compounds from *Aspergillus terreus* and evaluation of antibacterial and antifungal activity. *International Journal of Pharmacognosy and Phytochemical Research*. 8(6): 889-905 (2016).
42. Hameed, I.H., Altameme, H.J.and Idan, S.A. Artemisia annua: Biochemical products analysis of methanolic aerial parts extract and anti-microbial capacity. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 7(2): 1843- 1868 (2016).
43. Hussein, A.O., Mohammed, G.J., Hadi, M.Y.and Hameed, I.H. Phytochemical screening of methanolic dried galls extract of *Quercus infectoria* using gas chromatography-mass spectrometry (GC-MS) and Fourier transform-infrared (FT-IR). *Journal of Pharmacognosy and Phytotherapy*. 8(3): 49-59 (2016).
44. Sosa, A.A., Bagi, S.H.and Hameed, I.H. Analysis of bioactive chemical compounds of *Euphorbia lathyrus* using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy. *International Journal of Pharmacognosy and Phytochemical Research*. 8(5): 109-126 (2016).
45. Altameme, H. J., Hadi, M.Y. and Hameed, I.H. Phytochemical analysis of *Urtica dioica* leaves by fourier-transform infrared spectroscopy and gas chromatography-mass spectrometry. *Journal of Pharmacognosy and Phytotherapy*. 7(10): 238-252 (2015).
46. Mohammed, G.J., Omran, A.M. and Hussein, H.M. Antibacterial and Phytochemical Analysis of *Piper nigrum* using Gas Chromatography-Mass Spectrum and Fourier-Transform Infrared Spectroscopy. *International Journal of Pharmacognosy and Phytochemical Research*. 8(6): 977-996 (2016).
47. Hamza, L.F., Kamal, S.A. and Hameed, I.H. Determination of metabolites products by *Penicillium expansum* and evaluating antimicobial activity. *Journal of Pharmacognosy and Phytotherapy*. 7(9): 194-220 (2015).
48. Jasim, H., Hussein, A.O., Hameed, I.H. and Kareem, M.A. Characterization of alkaloid constitution and evaluation of antimicrobial activity of *Solanum nigrum* using gas chromatography mass spectrometry (GC-MS). *Journal of Pharmacognosy and Phytotherapy*. 7(4): 56-72 (2015).
49. Hadi, M.Y., Mohammed, G.J. and Hameed, I.H. Analysis of bioactive chemical compounds of *Nigella sativa* using gas chromatography-mass spectrometry. *Journal of Pharmacognosy and Phytotherapy*. 8(2): 8-24 (2016).
50. Hameed, I.H., Ibraheam, I.A. and Kadhim, H.J. Gas chromatography mass spectrum and fourier-transform infrared spectroscopy analysis of methanolic extract of *Rosmarinus oficinalis* leaves. *Journal of Pharmacognosy and Phytotherapy*. 7 (6): 90-106 (2015).
51. Shareef, H.K., Muhammed, H.J., Hussein, H.M. and Hameed, I.H. Antibacterial effect of ginger (*Zingiber officinale*) roscoe and bioactive chemical analysis using gas chromatography mass spectrum. *Oriental Journal of Chemistry*. 32(2): 20-40 (2016).
52. Al-Jassaci, M.J., Mohammed, G.J.and Hameed, I.H. Secondary Metabolites Analysis of *Saccharomyces cerievisiae* and Evaluation of Antibacterial Activity. *International Journal of Pharmaceutical and Clinical Research*. 8(5): 304-315 (2016).
53. Mohammed, G.J., Al-Jassani, M.J. and Hameed, I.H. Anti-bacterial, Antifungal Activity and Chemical analysis of *Punica grantanum* (Pomegranate peel) using GC-MS and FTIR spectroscopy. *International Journal of Pharmacognosy and Phytochemical Research*. 8(3): 480-494(2016).
54. Al-Marzoqi, A.H., Hadi, M.Y. and Hameed, I.H. Determination of metabolites products by *Cassia angustifolia* and evaluate antimicobial activity. *Journal of Pharmacognosy and Phytotherapy*. 8(2): 25-48(2016).
55. Altameme, H.J., Hameed, I.H. and Abu-Serag, N.A. Analysis of bioactive phytochemical compounds of

two medicinal plants, *Equisetum arvense* and *Alchemila valgaris* seed using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy. Malays. Appl. Biol. 44(4): 47–58 (2015).
56. Hameed, I.H., Hamza, L.F. and Kamal, S.A. Analysis of bioactive chemical compounds of *Aspergillus niger* by using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy. *Journal of Pharmacognosy and Phytotherapy*. 7(8): 132-163 (2015).
57. Hameed, I.H., Hussein, H.J., Kareem, M.A. and Hamad, N.S. Identification of five newly described bioactive chemical compounds in methanolic extract of *Mentha viridis* by using gas chromatography-mass spectrometry (GC-MS). Journal of Pharmacognosy and Phytotherapy. 7 (7): 107-125(2015).
58. Hussein, H.M., Hameed, I.H. and Ibraheem, O.A. Antimicrobial Activity and spectral chemical analysis of methanolic leaves extract of *Adiantum Capillus-Veneris* using GC-MS and FT-IR spectroscopy. *International Journal of Pharmacognosy and Phytochemical Research*. 8(3): 369-385(2016).
59. Hussein, H.J., Hadi, M.Y. and Hameed, I.H. Study of chemical composition of *Foeniculum vulgare* using Fourier transform infrared spectrophotometer and gas chromatography - mass spectrometry. *Journal of Pharmacognosy and Phytotherapy*. 8(3): 60-89 (2016).
60. Kadhim, M.J., Mohammed, G.J. and Hameed, I.H. In *vitro* antibacterial, antifungal and phytochemical analysis of methanolic fruit extract of *Cassia fistula*. *Oriental Journal of Chemistry*. 32(2): 10-309(2016).
61. Altameme, H.J., Hameed, I.H., Idan, S.A. and Hadi, M.Y. Biochemical analysis of *Origanum vulgare* seeds by fourier-transform infrared (FT-IR) spectroscopy and gas chromatography-mass spectrometry (GC-MS). *Journal of Pharmacognosy and Phytotherapy*. 7(9): 221-237 (2015).
62. Hussein, H.M. Determination of phytochemical composition and ten elements content (CD, CA, CR, CO, FE, PB, MG, MN, NI AND ZN) of *CARDARIA DRABA* by GC-MS, FT-IR and AAS technique. *Int. J Pharm Bio Sci.* 7(3): (B) 1009 – 1017(2016).
63. Hussein, H.M. Analysis of trace heavy metals and volatile chemical compounds of *Lepidium sativum* using atomic absorption spectroscopy, gas chromatography-mass spectrometric and fourier-transform infrared spectroscopy. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 7(4): 2529 – 2555(2016).
64. Jaddoa, H.H., Hameed, I.H. and Mohammed, G.J. Analysis of volatile metabolites released by *Staphylococcus aureus* using gas chromatography-Mass spectrometry and determination of its antifungal activity. *Orient J Chem.* 32(4) (2016).
65. Hameed, I.H., Salman, H.D., Mohammed, G.J. Evaluation of antifungal and antibacterial activity and analysis of bioactive phytochemical compounds of *Cinnamomum zeylanicum* (Cinnamon bark) using gas chromatography-mass spectrometry. *Orient J Chem*. 32(4) (2016).
66. Kadhim, M.J., Mohammed, G.J. and Hussein, H.M. Analysis of bioactive metabolites from *Candida albicans* using (GC-MS) and evaluation of antibacterial activity. International Journal of Pharmaceutical and Clinical Research. 8(7): 655-670 (2016).
67. Ubaid, J.M., Hussein, H.M. and Hameed, I.H. Analysis of bioactive compounds of *Tribolium castaneum* and evaluation of anti-bacterial activity. International Journal of Pharmaceutical and Clinical Research. 8(7): 655-670(2016).
68. Hameed, I.H., Jebor, M.A., Ommer, A.J. and Abdulzahra, A.I. Haplotype data of mitochondrial DNA coding region encompassing nucleotide positions 11,719–12,184 and evaluate the importance of these positions for forensic genetic purposes in Iraq. Mitochondrial DNA. 27(2): 1324-1327(2016).
69. Hameed, I.H. A new polymorphic positions discovered in mitochondrial DNA hypervariable region HVIII from central and north-central of Iraq. Mitochondrial DNA. 27(5): 3250-4 (2016).
70. Mohammad, A. and Imad, H. Autosomal STR: From locus information to next generation sequencing technology. Research Journal of Biotechnology (2013).
71. Hameed, I.H., Abdulzahra, A.I., Jebor, M.A., Kqueen, C.Y., Ommer, A.J. Haplotypes and variable position detection in the mitochondrial DNA coding region encompassing nucleotide positions 10,716-11,184. Mitochondrial DNA (2015).
72. Altaee, N., Kadhim, M.J. and Hameed, I.H. Detection of volatile compounds produced by *Pseudomonas aeruginosa* isolated from UTI patients by gas chromatography-mass spectrometry. *International Journal of Current Pharmaceutical Review and Research*. 7(6) (2017).
73. Altaee, N., Kadhim, M.J. and Hameed, I.H. Characterization of metabolites produced by *E. coli* and analysis of its chemical compounds using GC-MS. *International Journal of Current Pharmaceutical Review and Research*. 7(6) (2017).
74. Hussein, J.H., Ubaid, J.M. and Hameed, I.H. Gas chromatography – mass spectrum analysis of volatile components of methanolic leaves extract of *Cordia myxa*. *International Journal of Current Pharmaceutical Review and Research*. 7(6) (2017).
75. Kadhim, M.J. *In Vitro* antifungal potential of *Acinetobacter baumannii* and determination of its chemical composition by gas chromatography-mass spectrometry. *Der Pharma Chemica*, 8(19): 657-665 (2016).
76. Al-Yaseri, A., Kadhim, W.A. and Hameed, I.H. Detection of volatile compounds emitted by *Proteus mirabilis* isolated from UTI patients and its anti-fungal potential. *Der Pharma Chemica*, 8(19): 671-678 (2016).

77. Ubaid, J.M., Kadhim, M.J. and Hameed, I.H. Study of bioactive methanolic extract of *Camponotus fellah* using Gas chromatography – mass spectrum. *International Journal of Current Pharmaceutical Review and Research*. 7(6) (2017).
78. Kadhim, M.J., Kaizal, A.F. and Hameed, I.H. Medicinal plants used for treatment of rheumatoid arthritis: A review. *International Journal of Pharmaceutical and Clinical Research*. 8(11), (2017).
79. Hameed, I.H., Al-Rubaye A.F. and Kadhim, M.J. Antimicrobial Activity of Medicinal Plants and Urinary Tract Infections. *International Journal of Pharmaceutical and Clinical Research*. 8(11), (2017).
80. Mammeri, H., Gilly, L., Laurans, G., Vedel, G., Eb, F. and Paul, G. Catalytic and structural properties of IRT-21 beta-lactamase (TEM-77) from a co-amoxiclav-resistant *Proteus mirabilis* isolate. FEMS Microbiol Lett .205:185-189(2001).
81. Navarro, F., Perez-Trallero, E., Marimon, J.M., Aliaga, R., Gomariz, M. and Mirelis, B. CMY-2-producing Salmonella enterica, *Klebsiella pneumoniae*, Klebsiella oxytoca, *Proteus mirabilis* and *Escherichia coli* strains isolated in Spain (October 1999-December 2000). J Antimicrob Chemother 48:383-389(2001).
82. Garcia-Rodriguez, J.A. and Jones, R.N. MYSTIC Programme Study Group. Antimicrobial resistance in gram-negative isolates from European intensive care units: data from the Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) programme. J Chemother 14:25-32(2002).
83. Sheng, W-H., Badal, R.E. and Hsueh, P.R.SMART Program. Distribution of Extended-Spectrum β-Lactamases, AmpC β-Lactamases, and Carbapenemases among Enterobacteriaceae Isolates Causing Intra-Abdominal Infections in the Asia-Pacific Region: Results of the Study for Monitoring Antimicrobial Resistance Trends (SMART). Antimicrob Agents Chemother 57:2981-2988(2013).
84. Tsai, H.Y., Chen, Y.H., Tang, H.J., Huang, C.C., Liao, C.H., Chu, F.Y., Chuang, Y.C., Sheng, W.H., Ko, W.C.and Hsueh, P.R. Carbapenems and piperacillin/tazobactam for the treatment of bacteremia caused by extended-spectrum β-lactamase–producing Proteus mirabilis. Diagn Microbiol Infect Dis 80:222-226(2014).
85. Bush, K., Jacoby, G.A. and Medeiros, A.A. A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother 39: 1211-33(1995).
86. Livermore, D.M., Carter, M.W., Bagel, S., Wiedemann, B., Baquero, F., Loza, E., Endtz, H.P., Van Den Braak, N., Fernandes, C.J., Fernandes, L., Frimodt-Moller, N., Rasmussen, L.S., Giamarellou, H., Giamarellos-Bourboulis, E., Jarlier, V., Nguyen, J., Nord, C.E., Struelens, M.J., Nonhoff, C., Turnidge, J., Bell, J., Zbinden, R., Pfister, S., Mixson, L.and Shungu, D.L . *In vitro* activities of ertapenem (MK-0826) against recent clinical bacteria collected in Europe and Australia. Antimicrob Agents Chemother 45:1860-7(2001).
87. Guillard T, Grillon A, de Champs C, Cartier C, Madoux J, Berçot B, Lebreil AL, Lozniewski A, Riahi J, Vernet-Garnier V, Cambau E. Mobile Insertion Cassette Elements Found in Small Non-Transmissible Plasmids in Proteeae May Explain qnrD Mobilization. PLoS ONE 2014;9: e87801.
88. Gales, A.C. and Jones, R.N. Antimicrobial activity and spectrum of the new glycylcycline, GAR-936, tested against 1203 recent clinical bacterial isolates. Diagn Microbiol Infect Dis 36:19-36(2000).
89. Liassine, N., Madec, S., Ninet, B., Metral, C., Fouchereau-Peron, M., Labia, R. and Auckenthaler, R. Postneurosurgical meningitis due to *Proteus penneri* with selection of a ceftriaxone-resistant isolate: analysis of chromosomal class A beta-lactamase HugA and its LysR-type regulatory protein HugR. Antimicrob Agents Chemother. 46:216-9(2002).
90. Pandey, A., Verma, H., Asthana, A.K. and Madan, M. Extended spectrum beta lactamase producing *Proteus penneri*: a rare missed pathogen? Indian J Pathol Microbiol .57: 489-491(2014).
91. Docherty, J.J.,McEwen, H.A., Sweet, T.J., Bailey, E. and Booth, T.D. Resveratrol inhibition of *Propionibacterium acnes*. Journal of Antimicrobial Chemotherapy, 59: 1182-1184(2007).
92. Tegos, G., Stermitz, F.R., Lomovskaya, O. and Lewis, K. Multidrug pump inhibitors uncover remarkable activity of plant antimicrobials. Antimicrobial Agents and Chemotherapy, 46: 3133-3141(2006).
93. Sartory, D.P. and J. Watkins. Conventional culture for water quality assessment: is there a future? Journal of Applied Microbiology Symposium Supplement, 05:2258-2338(1999).
94. Jun, R.W., Yu-Tze, H., Hsin-chih, L., Kwen-Tay, L. and Shen-Wu H. Effect of PNPG on cell growth cycle, motility machinery and quorum sensing in *Serratia marcesccens*. J Microbiol Immunol Infect.73:1-7(2004).
95. Liaw, S.J., Lai, H.C. and Wang, W.B. Modulation of swarming and virulence by fatty acids trough the *RsbA* protein in *Proteus mirabilis*. Infect. Immun.72: 6836–6845(2004).
96. Welch, I. "Urea vs UAN". Nitrogen+Syngas, 289:26-27(2007).
97. Fons, J. A., Asten, M. and Gaastra, W. Urea restrains swarming of *Proteus mirabilis. J. Clin. Microbiol.* vol. 37 no. 51652(1999).
98. Iwalokun, B.A., Olukosi, Y.A., Adejoro, A., Olaye, J.A. and Fashade, O. Comparative biochemical and molecular evaluation of swarming of *Proteus* and effects of anti-swarm agents. *African Journal of Biotechnology*, 3 (1), 99-104 (2004).
99. Knoll, Keith, B., West, W., Clark, R., Graves, J., Orban, S., Przesmitzki, and Theiss, T. Effects of

Intermediate Ethanol Blends on Legacy Vehicles and Small Non-Road Engines, Report. National Renewable Energy Laboratory, Golden, Colorado, pp. 3-3(2009).
100. Hernandez, E., Ramisse, F. and Jean-Didier, C. Abolition of swarming of *Proteus*. *J. Clin. Microbiol*. 37(10):3435– 3436 (1999).
101. Difco and BBL. Manual of Microbiological Culture Media, 2nd Ed. P. 434(2009).
102. Betterton, E.A. "Environmental fate of sodium azide derived from automobile airbags". *Critical Reviews in Environmental Science and Technology*. 33 (4), 423–458 (2003).
103. Kerros, M-E., Winter, C. and Weinbauer, M.G. Effects of Sodium Azide on the abundance of prokaryotes and viruses in marine samples. *PLoS ONE* 7(5), e37597 (2012).
104. Alam, M.T., Karim, M.M., and Khan, S.N. Antibacterial activity of different organic extracts of *Achyranthes aspera* and *Cassia alata*. Journal of Scientific Research, 1, 393-398 (2009).
105. Chopra, R.N., Nayer, S.L., and Chopra, I.C. Glossary of Indian Medicinal Plants, 3rd edn. Council of Scientific and Industrial Research, New Delhi, pp. 7–246(1992).
106. Sohn, K.M., Kang, C.I., Joo, E.J., Ha, Y.E., Chung, D.R., Peck, K.R., Lee, N.Y. and Song, J.H. Epidemiology of Ciprofloxacin Resistance and Its Relationship to Extended-Spectrum β-Lactamase Production in Proteus mirabilis Bacteremia. *Korean J Intern Med*. 26, 89-93 (2011).
107. Williamson, D.A., Sidjabat, H.E., Freeman, J.T., Roberts, S.A., Silvey, A., Woodhouse, R., Mowat, E., Dyet, K., Paterson, D.L., Blackmore, T., Burns, A. and Heffernan, H. Identification and molecular characterisation of New Delhi metallo-β-lactamase-1 (NDM-1)- and NDM-6-producing Enterobacteriaceae from New Zealand hospitals. *Inter J Antimicrob Agents*. 39, 529-533 (2012).
108. Nimri, L.F., Meqdam, M.M., and Alkofahi, A. Antibacterial activity of Jordanian medicinal plants. *Pharmaceutical Biology*, 37 (3), 196–201 (1999).
109. Murugesan, S., Pannerselvam, A., Tangavelou, A.C. Phytochemical screening and antimicrobial activity of the leaves of *Memecylon umbellatum* burm. F. *Journal of Applied Pharmaceutical Science*, 1, 42-45 (2011).
110. Jabeen, R., Ashraf, M., and Ahmad, I. Evaluating the effects of cold water diffusates against Xanthomonas oryzae Pv. Oryzae causing bacterial leaf blight in rice. Archives of Phytopathology and Plant Protection. 40, 1-9 (2007).
111. Banso, A. Phytochemical and antibacterial investigation of bark extracts of *Acacia nilotica*. *Journal of Medicinal Plants Research*. 3, 82-85 (2009).
112. Bhaskar, A., Samant, L.R. Traditional medication of Pachamalai Hills, Tamilnadu, India. *Global J Pharmacol*. 6 (1), 47-51 (2012).
113. Chitravadivu, C., Manian, S. and Kalaichelvi, K. Antimicrobial studies on selected medicinal plants, Erode region, Tamilnadu, India. Middle-East J Sci Res. 4 (3), 147- 152 (2009).
114. Ahmed, F. and Urooj, A. Glucose-lowering, hepatoprotective and hypolipidemic activities of stem bark of Ficus racemosa in streptozotocin induced diabetic rats. J Young Pharm. 1(2), 160-164 (2009).
115. Purkayastha, S. and Dahiya P. Phytochemical analysis and antibacterial efficacy of babchi oil (Psoralea corylifolia) against multi-drug resistant clinical isolates. International Conference on Bioscience, Biochemistry and Bioinformatics. IPCBEE 3(1): 64-68(2012).
116. Tullanithi, K.M., Sharmila, B. and Gnanendra, T.S. Preliminary phytochemical analysis and antimicrobial activity of Achyranthes aspera Linn. Int J Bio Tech. 1(3): 35-38(2010).
117. Silva, N.C. and Júnior, A.F. Biological properties of medicinal plants: a review of their antimicrobial activity. J Veno Ani Tox Inclu Trop Dis 16(3): 402-431(2010).
118. Park, K.M., You, J.S., Lee, H.Y., Baek, N.I., Hwang, J.K. and Kuwanon, G. An antibacterial agent from the root bark of Morus alba against oral pathogens. Journal of Ethnopharmacology. 84(2-3), 181-185 (2003).
119. Joshi, B., Lekhak, S. and Sharma, A. Antibacterial property of different medicinal plants: Ocimum sanctum, Cinnamomum zeylanicum, Xanthoxylum armatum and Origanum marjorana. Kathmandu university journal of science, engineering and technology. 5(1), 143-150 (2009).
120. Leeja, L. and Thoppil, J.E. Antimicrobial activity of methanol extract of Origanum marjorana L. (Sweet marjoram). Journal of Environmental Biology. 28(1): 145-146(2007).
121. Satish, S., Raghavendra, M.P. and Raveesha, K.A. Evaluation of the antibacterial potential of some plants against human pathogenic bacteria. Advances in Biological Research. 2(3-4), 44-48 (2008).
122. Patil, M.K., et al. Antibacterial efficacy of Piper nigrum fruit. Journal of Veterinary Pharmacology and Toxicology. 2(1-2), 69-71 (2007).