Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2016; 8(11); 1844-1854

ISSN: 0975-4873

Review Article

Proteus species: Characterization and Herbal Antibacterial: A Review

Ghaidaa Jihadi Mohammed[1], Mohanad Jawad Kadhim[2], Imad Hadi Hameed[3*]

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

Available Online: 15th November, 2016

ABSTRACT

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. *Proteus* spp. are part of the human intestinal flora and can cause infection upon leaving this location. They may also be transmitted through contaminated catheters (particularly urinary catheters) or by accidental parenteral inoculation. The specific mode of transmission, however, has not been identified. 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. Many infectious diseases have been known to be treated with herbal remedies based on ethno-botanical 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 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, Bioactive compounds, *Proteus*, Pathogenicity, Motility.

INTRODUCTION

The genus *Proteus* currently consists of five species: *P. mirabilis*, *P. vulgaris*, *P. penneri*, *P. hauseri* and *P. myxofaciens*, as well as three unnamed *Proteus* genomospecies: *Proteus* genomospecies[1]. *Proteus myxofaciens* is the only *Proteus* species without any significance in the pathogenicity of humans, it has been isolated from living and dead larvae of the gypsy moth *Porteria dispar*[2]. However, a recent study indicated that *P. myxofaciens* may represent a separate genus with low similarity to tribe *Proteeae*, and it has been suggested that this organism be renamed *Cosenzaea myxofaciens*[3]. A striking microbiologic characteristic of *Proteus* species is their swarming activity. Swarming appears macroscopically as concentric rings of growth emanating from a single colony or inoculum. On a cellular level, swarming results from bacterial transformation from "swimmer cells" in broth to "swarmer cells" on a surface such as agar, in a process involving cellular elongation and increased flagellin synthesis. The genus name *Proteus* originates from the mythological Greek sea god *Proteus*, who was an attendant to Poseidon. *Proteus* could change his shape at will. This attribute reminded early microbiologists of the morphologic variability of the *Proteus* on subculture, including their ability to swarm.

Synonym or cross reference

Former species of genus *Proteus* now homotypic synonyms with other species: *P. inconstans* with *Providencia alcalifaciens*, *P. morganii* with *Morganella morganii*, and *P. rettgeri* with *Providencia rettgeri*[4,5]. Table 1 shows the common characteristics among three genera. While, Table 2 and Table 3 shows the conventional biochemical tests necessary for the differentiation of *Proteus*, *Providencia*, and *Morganella*[6]. All three genera are positive for phenylalanine deaminase and negative for arginine decarboxylase, malonate utilization, and acid production from dulcitol, d-sorbitol, and l-arabinose.

Sources /Specimens

Samples from urine tract, wounds, and blood samples[7]. Specific sources identified include:

P. mirabilis: Urinary tract, blood, and cerebrospinal fluid.

P. penneri: Urinary tract, blood, wound, feces, eye.

P. vulgaris: Urinary and respiratory tract, wound, and stool.

Primary hazards

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

Use of contaminated catheters (particularly urinary catheters) in medical procedures, and accidental parenteral inoculation and/or ingestion of contaminated material[8-10].

Pathogenicity of Proteus spp.

Proteus rods are opportunistic bacterial pathogens which under favorable conditions cause urinary tract infections (UTIs), commonly associated with complicated urinary tract infections[7]. 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[1,10]. Kalra *et al*. (2011)[2] reviewed endocarditis due to *Proteus* species, and Okimoto *et al*. (2010)[12] 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).

Proteus mirabilis Fimbriae (PMF)

Proteus mirabilis fimbriae (PMF) were identified from a crude fimbrial preparation of *P. mirabilis* strain HI4320. The PMF were isolated and their operon nucleotide sequence was determined by Massad *et al.* (1994)[15]. The genetic organization of the *pmf* operon revealed the presence of five genes that appear to be contained in one operon: *pmfA* (the major structural subunit), *pmfC* (a putative usher), *pmfD* (a putative chaperone), *pmfE* (a putative minor subunit), and *pmfF* (the putative tip adhesin). Such genetic organization resembles that of uropathogenic *E. coli* P fimbriae[16]. The role of PMF in the virulence of *P. mirabilis* was determined by two studies. In the first, an isogenic *pmfA* fimbrial mutant colonized the bladders of transurethrally challenged CBA mice in numbers 83-fold lower than those of the wild-type strain. However, the mutant colonized the kidneys in numbers similar to those of the wild-type strain. The authors suggested that the role of PMF was just in colonization of the bladder but not in the kidney tissue. The second study also evaluated a *pmfA* mutant and the role of PMF in colonization, and virulence in UTI was assessed using a co-challenge ascending UTI model in CD-1 mice. After 7 days of infection, the mutant and the wild-type strains were enumerated on non-swarming Luria–Bertani (LB) agar. The number of viable bacteria counted from bladder and kidneys showed that the mutant strain was significantly out-competed by the wild-type strain in colonizing the bladders and kidneys. This suggests that PMF play a role in localization of uropathogenic *P. mirabilis* to the bladder and kidney[17].

Uroepithelial cell adhesion (UCA)

Uroepithelial cell adhesin (UCA) was initially discovered in an uropathogenic isolate of *P. mirabilis* (strain HU1069) during a screen designed to identify outer membrane proteins that facilitated binding to uroepithelial cells[17]. The identified UCA protein, the major structural subunit later designated *ucaA* was purified to homogeneity for characterization; the purified protein retained the ability to bind to uroepithelial cells and, additionally, organized into long, flexible filaments with a diameter of 4 to 6 nm, consistent with the appearance of fimbriae. N-terminal sequencing was performed on purified UCA. The sequence of the first 25 amino acids of the UCA protein displayed more similarity to the K99 pilus of *E. coli* (which mediates binding to intestinal epithelium) than to adhesins from uropathogenic *E. coli*. Subsequent determination of the sequence of the *ucaA* gene revealed that *ucaA* has the highest similarity to F17 and F111 fimbriae from bovine enterotoxigenic *E. coli*. These findings led to postulation that UCA may function as a primary adhesin for *Proteus* in the intestinal tract, although this hypothesis has not been experimentally tested[18]. UCA fimbriae have since been renamed non-agglutinating fimbriae (NAF) to distinguish them from other *P. mirabilis* fimbriae that contribute to adherence. Tolson *et al*. (1995)[19] confirmed that what they designated the NAF subunit was identical to the previously identified UCA subunit based on N-terminal sequencing. Pre-incubation with monoclonal antibodies specific for NAF significantly reduced binding of *P. mirabilis* to HEp-2 and uroepithelial cells in vitro, providing additional evidence of a role for NAF in adherence to host cells[20].

Motility

There are conflicting results concerning the importance of flagella in the infection. Since flagella mediate motility of bacteria, they seem to be required for virulence of uropathogens, particularly during ascending infection[21]. Indeed, it was shown in vivo that flagella-negative mutant of *P. mirabilis* is less virulent, compared to the wild strain, which suggests an important role of these bacterial surface structures in the pathogenicity[22]. However, it must be stressed, that lack of flagella on P. mirabilis human isolate was found[16]. Flagella play a role of H antigen and are strongly immunogenic. Bacteria can avoid immune response of human organism due to the antigenic variation

process. This phenomenon is based on flagellin genes rearrangement. Flagellar antigenic variations allow bacteria to evade the action of secretory IgA antibodies directed against these organelles[23]. In general, flagella on the surface of bacterial pathogens are thought to assist in host colonization and dissemination, initial attachment, and sensing of the extracellular environment. For *Proteus* species, these surface structures are important in the process known as swarming, a distinct characteristic of these organisms. Swarming may play a role in the migration of *Proteus* strains on catheter materials. Flagella play a role of H antigen and are strongly immunogenic. Bacteria can avoid immune response of human organism due to the antigenic variation process[24]. Swarming cell differentiation is important for the virulence of *Proteus* strains during UTIs since several virulence factors, including flagellin, urease, hemolysin *HmpA*, and IgA metalloprotease *ZapA*, are upregulated in the differentiated swarmer vs. swimmer cells[25]. Swarming is a multicellular differentiation phenomenon that allows a population of bacteria to migrate on a solid surface in a coordinate manner. It is important in movement of *Proteus* species to new locations and most probably helps them in the colonization of macroorganisms. It involves cell to-cell signaling and multicellular interactions and is connected with the possibility of morphological differentiation of bacteria depending on growth media. *Proteus* are dimorphic bacteria, which in liquid media are motile, peritrichously flagellated short rods (1.0 to 2.0 μm in length with 6–10 flagella). These bacteria are called swimmer cells. However, when transferred onto solid media these short rods change into elongated (20–80μm in length), hyperflagellated, multinucleated, nonseptated swarmer cells. The latter migrate out from the inoculation site as long as the population of swarmer cells is reduced on solid surfaces. Then, the consolidation process takes place. In this period of swarming growth, the long rods disintegrate to short bacteria. The processes of differentiation and dedifferentiation of *Proteus* bacteria are cyclic. It results in the formation of characteristic rings of bacterial growth on the agar plate (Figure 1)[26]. Differentiation of swimmer cells into swarmer cells is induced by the contact of bacteria with a solid surface and the inhibition of flagellar rotation. It was found that the addition of thickening agents to liquid media resulted in differentiation of short rods to swarmer cells. A similar effect was obtained when antiflagellar antibodies were added to liquid medium[23]. A number of factors playing a role in swarming regulations have been recognized (Figure 2)[27,28]. The most important gene involved in upregulation of flagellin production is flhDC, class 1 gene of flagellar regulon. This gene encodes FlhD2C2 complex, a heterotetrameric transcriptional regulator, which also regulates the expression of additional genes required for swarmer cells differentiation. The expression of flhDC increases 10-fold during initiation of swarmer cells differentiation and it is influenced by a variety of environmental factors and regulating genes. FlhD2C2 activates class 2 genes coding basal body and hook proteins of flagella, as well as σ28 factor, which activates class 3 genes required for synthesis of flagellin and flagellum assembly. FlhD2C2 activity is regulated by two identified factors DisA (decarboxylase inhibitor of swarming) and Lon protease (ATP-dependent protease) DisA most probably inhibits the assembly of heterotetramer or its binding to DNA[26]. *Proteus* has 16 predicted two-component systems – TCS[29]. TCS consists of sensor kinase, which activates response regulator DNA – a binding protein controlling gene expression. Two of TCS - Rcs and Rpp are involved in swarming regulation. Rcs system contains RcsC sensor kinase, RcsB response regulator, RcsD intermediate transferring phosphate to the RcsB and RcsF which is an outer membrane lipoprotein. Mutation in the genes coding Rcs system in *P. mirabilis* lead to the hyperswarming phenotype, most probably due to an increased expression of the flhDC. *P. mirabilis*. Rcs mutant grows in liquid media as elongated cells. The Rpp system consists of RppA response regulator and RppB histidine sensor kinase like protein. Mutation in the Rpp system in P. mirabilis also results in a hyperswarming phenotype. flhDC expression is also regulated by several proteins including *Umo*, *MrpJ*, *WosA*, *Lrp* and *RsmA*. Umo A-D proteins (umo – upregulated expression of the flhDC master operon) are located in the cell membrane and periplasm. Mutant defective in production of UmoD protein is not able to swarm, however, such effect was not noticed in the case of UmoA and UmoC[30]. *mrpJ* gene is located in the *mrp* operon encoding MR/P fimbriae, which are required for sessile lifestyle of bacteria. It is opposite to the swimming or swarming phenotype of bacteria. The existence of bacteria in one of two forms is regulated by mrpJ gene product – MrpJ protein, a transcriptional regulator. It binds the flhDC promoter, which causes its repression and results in loss of motility of bacteria. wosA gene coding WosA protein, is overexpressed in strains exhibiting hyperswarming phenotype (wos – wild type onset superswarming). WosA overexpression increases the expression of fhlDC and leads to a differentiation of swarmer cells in liquidmedia. The hyperswarming wosA bacteria move quickly and spend less time in the consolidation phase. wosA expression is growth phase dependent, as well as it is partially dependent upon the expression of flaA gene encoding the flagellar filament[31]. Leucine responsive protein (Lrp) is a transcriptional global regulator involved in regulating different processes including amino acids synthesis, peptide transport, and pilin biogenesis[28]. In *P. mirabilis* strains Lrp plays a role in the regulation of the swarming phenomenon, most probably its action leads to the inhibition of hyperflagellation. Mutation in *lrp* results in a decrease in the flhDC, flaA and hpm (hemolysin) expression and, in consequence, a non-swarming phenotype[32]. Thus, initiation of swarming requires the integration of numerous signals and is intimately connected to the metabolic status of the bacterium, membrane integrity, and cell wall changes associated with surface contact. The role of the swarming phenomenon in the pathogenicity of *Proteus* bacteria is till now unclear. It was shown that swarmer cells demonstrate higher production of urease, HpmA hemolysin and IgA metaloprotease ZapA, as well as

flagellin synthesis, as compared to swimmer cells[28]. There is some controversy concerning invasiveness of swarmer cells. Allison *et al*. (1992)[33] showed in vitro that this morphotype could invade uroepithelial cells, whereas nonflagellated and nonswarming forms were noninvasive. These results were confirmed by Jansen *et al*. (2003)[34] in a mouse model of ascending UTI. These authors have found that the predominant cell type in the urinary tract are short swimmer cells but not elongated swarmer cells. Recently, Fujihara *et al*. (2011)[35] suggested that *P. mirabilis* cells differentiate into hyperflagellated and multinucleated swarmer cells in acidic pH of the host's urine and differentiate back into swimmer forms, when the urinary pH is increased and it is alkaline after urease action. In acidic condition swarmer cells exhibit higher cytotoxicity against T24 line. In alkaline condition *P. mirabilis* showed few elongated cells with high number of flagella and cytotoxic activity. Formation of the Dienes line require direct cell-cell contact by living bacteria, and is thought to involve killing of one strain at the boundary. Interestingly, competitive killing is only observed during swarming as strain sensitive to killing on swarm agar are not outcompeted in broth culture or on agar that is not swarming –permissive[36]. One explanation for the formation of the Dienes line involves the production of proticine capable of killing sensitive strain. Indeed, boundaries form between *P. mirabilis* strains that differ in proticine production and sensitivity[36,37]. However some strains deficient in proticine production still for boundaries, even with other strains lacking proticine production, indicating that another underlying mechanism mediates Dienes line formation[38]. In the research for this mechanism, a transposon mutant was identified that formed a boundary with its parent strain rather than merging and the disrupted locus was named *ids* for identification of self[38]. Further work involving the *ids* locus determined that *idsABCDEF* constitutes an operon, and that *idsD* and *ids*E appear to encode strain-specific factors essential for self-recoginiation while *idsB*, *idsC* and *idsF* encode factors essential for self-recognition that can be complemented by *ids* gene from other strain[38]. As two swarm fronts merge, only a subset of cells in the advancing swarm express the *ids* gene and can traverse the boundary of the approaching swarm, and this subset is sufficient to somehow propagate the signal of self-versus non-self[38]. Furthermore, *ids* expression decreases as an advising swarm approach another swarm of the same strain. The *ids* system alone, however, does not fully explain the differences in strain interactions that occur within this locus allow for boundary formation but without the formation of rounded cells or any apparent competitive killing[38]. As the *ids* locus encodes putative type six secretion system (T6SS) effector proteins Hcp and VgrG, it was hypothesized that T6SS may be involved in Dienes line formation. In agreement with this hypothesis, Mobely's laboratory has identified additional loci encoding T6SS effector and structural proteins involved in Dienes line formation. The T6SS of *Proteus mirabilis* along with proticine and the *ids* gene may therefore mediate a combination of inter bacterial killing during swarming and Dienes line formation, competition on the catheter surface, or even some form of interaction with host during UTI.

Anti–swarmer agents

Many methods and addition of many chemical substances to the agar medium are recommended in order to inhibit the swarming. For example, the use of very dry plates, MacConkey medium containing bile salts, addition of urea and ethanol[39], resveratrol[40], *p*-nitrophenyl glycerin[41], sodium azide, barbiton, activated charcoal and sulfonamide[41-43].

AHL mediated quorum sensing in Gram-negative bacteria

The best-studied quorum sensing systems in Gram-negative bacteria use LuxI-type enzymes, which produce AHLs as small diffusible signal molecules that get bind and activate members of the LuxR transcriptional activator protein family[44,45]. AHL based quorum sensing system functions through three key components: i) AHL signal molecules, ii) AHL synthase protein for synthesis of AHL signals, and iii) a regulatory protein which responds to surrounding concentration of AHL signal[46]. This process initiated with the synthesis and release of AHL signals into the surrounding environment which accumulates in a cell-population-density-dependent manner. When the concentration of AHL signals reaches at higher level; the quorum sensing cells starts responding allowing them to regulate the production of secondary metabolites and control the expression of quorum sensing genes. A majority of Gram-negative bacteria regulates various phenotypes through the secretion and detection of such signaling molecules. However, the efficacy of expression of quorum sensing phenotypes depends upon the presence or absence of surrounding cells. Using quorum sensing bacteria can act to express a specific set of genes responsible for variety of physiological behaviors including bioluminescence, antibiotic production, extracellular polymer production, biosurfactant synthesis, sporulation, release of virulence factors and biofilm formation[47-52]. Bacterial virulence factors are regulated by quorum-sensing molecules. There is no evidence that quorum sensing receptors and AI-1 signal molecules are associated with swarming motility in *Proteus*[23]. Whereas, Daniels *et al*. (2004)[27] showed that the QS signal acylhomoserine lactone enhances swarming motility in *Serratia liquefaciens.*

Culture

Clinical specimens should be cultured on MacConkey agar/ Teepol lactose agar, 6% blood agar and in case of urine on CLED (Cysteine lactose electrolyte deficient agar). Culture media are incubated at 37ºC for 18-24 hours. non fermenting pale colonies, around 2-3 mm in size are formed. On non-inhibtory solid media such as blood and nutrient agar[53]. *Proteus mirabilis* and *P. vulgaris* show characteristic swarming growth in the form of a uniform film, which spreads over the whole surface of the plate. In young swarming cultures, many of the bacteria are long, curved and filamentous, sometimes reaching up to 80 µm in length. When two different strains of swarming *Proteus mirabilis* encounter one another on an agar plate,swarming ceases and a visible line of demarcation forms. This is known as the Dienes phenomenon. Swarming inhibitory

methods: Swarming of Proteus can be prevented by (Matsuyama *et al*.,2000)[54]:

-Increasing the concentration of agar from 1-2% to 6%.
-Incorporation of sodium azide, boric acid, or chloral hydrate.
-Introducing growth inhibitors like sulphonamides.
-On Teepol Lactose agar by Teepol (surface active agent)
-On MacConkey agar or DCA by presence of bile salts.
-On CLED agar by the absence of electrolytes.

In liquid medium (peptone water, nutrient broth), Proteus produces uniform turbidity with a slight powdery deposit and an ammonical odour. Identification is done by standard biochemical reactions mentioned below.

Biochemical Reactions

Like all other members of the family Enterobacteriaceae, all the species of genus Proteus are catalase positive, oxidase negative, reduce nitrates to nitrites and show fermentative reaction on Hugh Leifson's of media. All members of the tribe Proteeae are PPA positive and, hydrolyse urea to ammonia which differentiates them from other Enterobacteriaeceae. *Proteus* spp. are identified by the following biochemical characteristics: positive methyl-red reaction, negative Voges-Proskauer reaction, phenylalanine deaminase production, growth on KCN and urease production. *P. mirabilis* and *P. penneri* are indole-negative, while other *Proteus* species are indole-positive. The *Proteus* genomospecies (4, 5, and 6) can be distinguished from other *Proteus* species based on five biochemical characteristics: esculin hydrolysis, salicin fermentation, L-rhamnose fermentation, and elaboration of DNase and lipase[24].

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[55]. These are non-nutritive substances, have protective or disease preventive property[56]. 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[57]. These are the important raw materials for drug production[58]. Most plants contain several compounds with antimicrobial properties for protection against aggressor agents, especially microorganisms[59]. Medicinal and aromatic plants are used on a large scale in medicine against drug-resistant bacteria, which are considered one of the most important reasons for the lack of success of treatment in infectious diseases. Medicinal plants are the major sources of new medicines and may constitute an alternative to the usual drugs[60]. Aromatic oils are used in many industries, including food preservation, pharmacy, and medicine[61,62]. They are expected to form new sources of antimicrobial drugs, especially against bacteria[63]. The antibacterial effectiveness of aromatic oils has been divided into a good, medium, or bad[64,65]. These oils can also produce some defense products against several natural enemies. In addition, and in order to continue their natural growth and development, aromatic oils may produce some secondary metabolites in response to some external stress[66]. 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*[67]. *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[68-100]. 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

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

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

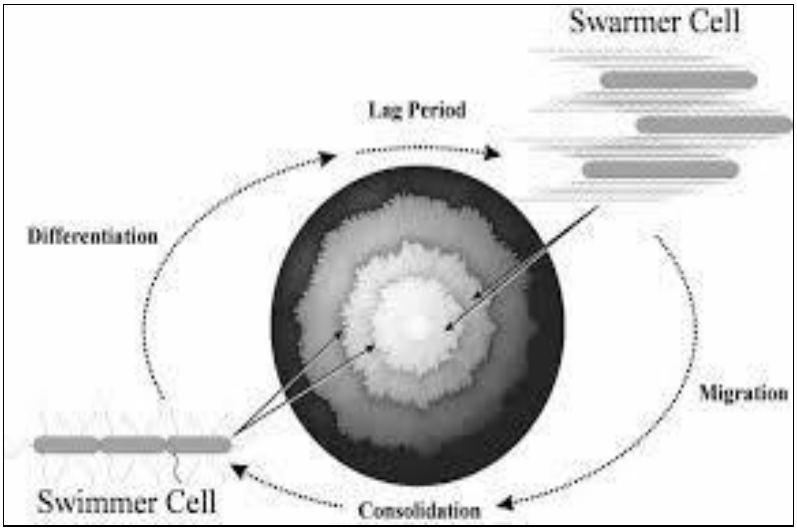

Figure 1: Spatial and temporal cycling of P. mirabilis swarming behaviour and colony formation. Moving clockwise from the lower left, vegetative swimmer cells are induced to differentiate into swarmer cell morphology (elongated, hyperflagellated, polynucleoid cells) upon contact with a surface such as L agar. The microcolony of swarmer cells grows through cell division, but swarming motility is not expressed. This lag period is typically about 3 h on L agar at 37 °C. At this point, an unknown event triggers swarmer cell migration, which is manifested as a seemingly spontaneous uniform movement of swarmer cells outwards from the colony periphery. Migration continues for about 3 h and is followed by a short (30–45 min) consolidation phase where the cells dedifferentiate to swimmer cell morphology and cease to swarm. These events are cyclical, such that on agar media, swarming colony biofilm development is observed as a series of everexpanding concentric rings that are composed of morphologically and physiologically distinct cell types (arrows indicate areas populated by either swimmer or swarmer cells). This cycle leads to the bull's-eye pattern typical of *P. mirabilis* colonies.

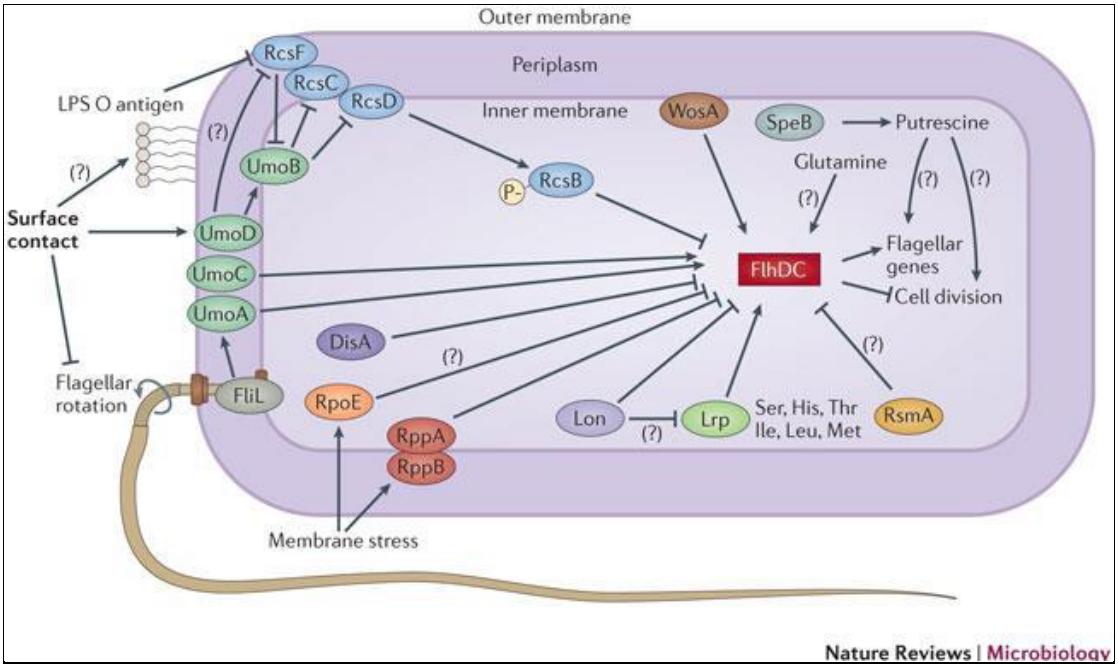

Figure 2: Swarm cell differentiation in *Proteus mirabilis*.

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*[101]. 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[102]. The aqueous extract was found to be antibacterial and it was studied against various Gram-positive and Gram-negative bacterial strains by using MIC,

Table 2: Differentiation among the genera *Proteus*, *Providencia*, and *Morganella*

Biochemical test or property	*Proteus*	*Providencia*	*Morganella*
Citrate utilization	v	+	−
d-Mannose fermentation	−	+	+
Gelatin liquefaction (22°C)	+	−	−
H_2S production (TSI)	+	−	v
myo-Inositol fermentation	−	v	−
Lipase (corn oil)	+	−	−
Ornithine decarboxylase	v	−	(+)
Swarming	+	−	−
Urea hydrolysis	+	v	+

(All data are reactions at 48 h unless otherwise specified): +, 90 to 100% positive; (+), 75 to 89.9% positive; v, 25.1 to 74.9% positive; (−), 10.1 to 25% positive; −, 0 to 10% positive. TSI, triple sugar iron.

Table 3: Biochemical tests used in differentiation within the genus Proteus + 90–100 % positive, - 0 – 9.9 % positive; S – susceptible, R – resistant, V – variable.

Test	*P. mirabilis*	*P. vulgaris*	*P. penneri*	*P. hauseri*	*P. myxofaciens*
Salicin fermentation	-	+	-	-	-
Maltose fermentation	-	+	+	+	+
D-Xylose fermentation	+	+	+	+	-
Esculin hydrolysis	-	+	-	-	-
Ornithine decarboxylase	+	-	-	-	-
Indole production	-	+	-	+	-
Chloramphenicol susceptibility	S	V	R	S	S

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*[103].

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.

ACKNOWLEDGMENTS

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

REFERENCES

1. O'hara CM, Brenner FW, Miller JM. Classification, identification, and clinical significance of Proteus, Providencia, and Morganella. Clin Microbiol Rev. 2000;13(4):534-46.
2. Janda, JM, Abbott SL, Khashe, S, Probert W. Biochemical identification and characterization of DNA groups within *Proteus vulgaris* complex. J Clin Microbiol. 2006;39 (4):1231-4.
3. Giammanco MG, Grimont, P. A. D.; Grimont, F.; Lefevre, M. and Pignato, S. Phylogenetic analysis of the genera *Proteus*, *Morganella* and *Providencia* by comparison of *rpoB* gene sequences of type and clinical strains suggests the reclassification of *Proteus myxofaciens* in a new genus, *Cosenzaea* gen. nov., as *Cosenzaea myxofaciens* comb. nov. International Journal of Systematic and Evolutionary Microbiology. 2011**;** 61(7): 1638 – 1644.
4. Wang Q, Torzewska A, Ruan X, Wang X, Rozalski A, Shao Z, Guo X, Feng L, Wang L. Molecular and genetic analyses of the putative *Proteus* O-antigen gene locus. App. Env. Microbiol. 2010;76: 5471–5480.
5. Farmer J J, III, Davis B R, Hickman-Brenner F W, McWhorter A, Huntley-Carter G P, Asbury M A, Riddle C, Wathen-Grady H G, Elias C, Fanning G R, Steigerwalt A G, O'Hara C M, Morris G K, Smith P B, Brenner D J. Biochemical identification of new species and biogroups of *Enterobacteriaceae* isolated from clinical specimens. J Clin Microbiol. 1985;21: 46–76.
6. 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, 2007; USA: ASM Press.

7. Kim, B. N., Kim, N. J., Kim, M. N., Kim, Y. S., Woo, J. H., & Ryu, J. Bacteraemia due to tribe Proteeae: a review of 132 cases during a decade (1991-2000). Scandinavian Journal of Infectious Diseases2003; 35(2), 98-103
8. Coker, C., Poore, C. A., Li, X., & Mobley, H. L. Pathogenesis of Proteus mirabilis urinary tract infection. Microbes and Infection / Institut Pasteur. 2000; 2(12), 1497-1505.
9. Satish S, Raghavendra MP, Raveesha KA. Evaluation of the antibacterial potential of some plants against human pathogenic bacteria. Advances in Biological Research. 2008; 2(3-4): 44-48.
10. Ronald, A. The etiology of urinary tract infection: traditional and emerging pathogens. Disease-a-Month: DM. 2003; 49(2), 71-82.
11. Kalra, A, Cooley C, Tsigrelis C. Treatment of endocarditis due to *Proteus* species: a literature review. Int. J. Infect. Dis. 2011;15: 222–225.
12. Okimoto N, Hayashi T, Ishiga M, Nanba F, Kishimoto M, Yagi S, Kurihara T, Asoka, N, Tamada S. Clinical features of *Proteus mirabilis* pneumonia. J. Infect. Chemother. 2010; 16:364–366.
13. Bloch J, Lemaire X, Legout L, Ferriby, D, Yazdanpanah, Y, Senneville E. Brain abscesses during *Proteus vulgaris* bacteremia. Neurol. Sci. 2010; 32: 661–663.
14. Jacobsen S.M, Stickler, DJ, Mobley HL, Shirtliff ME. Complicated catheter-associated urinary tract infections due to *Escherichia coli* and *Proteus mirabilis*. Clin Microbiol Rev. 2008; 21:26–59.
15. Massad G, Zhao H, Mobley HL. Proteus mirabilis aminoacid deaminase: cloning, nucleotide sequence, and characterization of aad. J. Bacteriol. 1995;177: 5878–5883.
16. Zunino P, V. Sosa G. Allen, A. Preston, G. Schlapp, and D. J. Maskell. *Proteus mirabilis* fimbriae (PMF) are important for both bladder and kidney colonization in mice. Microbiology. 2003; 149: 3231-3237.
17. Rocha SP, WP. Elias, A. M. Cianciarullo, M. A. Menezes, J. M. Nara, R.M. Piazza, M. R. Silva, C. G. Moreira, and J. S. Pelayo. Aggregative adherence of uropathogenic *Proteus mirabilis* to cultured epithelial cells. FEMS Immunol Med Microbiol. 2007;51: 319-326.
18. Cook, S. W., N. Mody, J. Valle, and R. Hull. Molecular cloning of *Proteus mirabilis* uroepithelial cell adherence (*uca*) genes. Infect. Immun. 1995; 63:2082-2086.
19. Tolson, D. L., D. L. Barrigar, R. J. McLean, and E. Altman. Expression of a non-agglutinating fimbria by *Proteus mirabilis*. Infect. Immun. 1995; 63:1127-1129.
20. Latta, R.K.; Schur, M. J.; Tolson, D.L. and Altman, E.. The effect of growth conditions on in vitro adherence, invasion, and NAF expression by *Proteus mirabilis* 7570. Can J Microbiol. 1998;44: 896–904.
21. Shirtliff, M. and Leid, J.G. The Role of Biofilms in Device-Related Infections. 2009; 3:131-132.
22. Mobley HL. Virulence of Proteus mirabilis, p. 245–269. In H. L. T. Mobley and J. W. Warren (ed.), Urinary tract infections. Molecular pathogenesis and clinical management. ASM Press, Washington, D.C. 1996.
23. Belas R, Manos J. Suvanasuthi, R. *Proteus mirabilis* ZapA metalloprotease degrades a broad spectrum of substrates, including antimicrobial peptides. Infect Immun. 2004; 72(9): 5159-67.
24. Rozalski A, Torzewska, A, Moryl M, Kwil, I, Maszewska A, OStrowska K, Drzewiecka D, Zablotni A, Palusak A, Siwinska M, Staczek P. *Proteus sp.* An opportunistic bacterial pathogen classification, swarming growth, clinical significance and virulence factors. Folia Biologica et Oecologica. 2012; 8:1-17.
25. Fraser, GM, L. Claret, R. Furness, S. Gupta, C. Hughes. Swarming-coupled expression of the *Proteus mirabilis hpmBA* haemolysin operon. Microbiology. 2002; 148: 2191-2201.
26. Morgenstein, RM, Szostek, B, Rather PN. Regulation of gene expression during swarmer cell differentiation in *Proteus mirabilis*. FEMS Microbiol. Rev. 2010; 34: 753–763.
27. Daniels R, Vanderleyden, J, Michiles J. Quorum sensing and swarming migration in bacteria. FEMS Microbiol. Rev. 2004; 28: 261–289.
28. Rather PN. Swarmer cell differentiation in Proteus mirabilis. Environm. Microbiol. 2005;7: 1065– 1073.
29. Pearson MM, Mobley HL. Repression motility during fimbrial expression; identification of 14 mrpJ gene paralogues in Proteus mirabilis. Mol. Microbiol. 2008; 69: 548–558.
30. Dufour A, Furness RB, Hughes C. Novel genes that upregulate the Proteus mirabilis flhDC master operon controlling flagellar biogenesis and swarming. Mol. Microbiol. 1998; 29: 741–751.
31. Hatt JK, Rather PN. Characterization of novel gene, wosA, regulating FlhDC expression in *Proteus mirabilis*. J. Bacteriol. 2008; 190: 1946–1955.
32. Hay NA, Tipper D, Gygi, D, Hughes, C. A nonswarming mutant of Proteus mirabilis lacks the Lrp global transcriptional regulator. J. Bacteriol. 1997; 179: 4741–4746.
33. Allison C, L. Emo¨dy, N. Coleman, C. Hughes. The role of swarm cell differentiation and multicellular migration in the uropathogenicity of Proteus mirabilis. J. Infect. Dis. 1994;169: 1155–1158.
34. Jansen, A. M.; V. Lockatell, D. E. Johnson, and H. L. Mobley. Mannose-resistant *Proteus*-like fimbriae are produced by most *Proteus mirabilis* strains infecting the urinary tract, dictate the in vivo localization of bacteria, and contribute to biofilm formation. Infect. Immun. 2004; 72:7294–7305.
35. Fujihara, M., Obara, H., Watanabe, Y., Ono, H.K., Saski J., Goryo, M., Harasawa, R. Acidic environments induce differentiation of Proteus mirabilis into swarmer morphotypes. Microbiol. Immunol. 2011;55: 489–493.

36. Budding, A.E., C.J. Ingham, W. Bitter,C.M.Vandenbroucke-Grauls, and P.M. Schneeberger. The Dienes phenomenon: competition and territoriality in swarming *Proteus mirabilis*. Journal of Bacteriology. 2009;191(12):3892-900
37. Senior, B. W., L. M. Loomes, and M. A. Kerr. The production and activity in vivo of Proteus mirabilis IgA protease in infections of the urinary tract. J. Med. Microbiol. 1991; 35:203–207.
38. Gibbs, K.A.,L.M.Wenren, and E.P.Greenberg. Identity gene expression in Proteus mirabilis. Journal of Bacteriology. 2008;93(13):3286-92.
39. AL-Kaebi S.J. and Matrood, A. J. The effect of urea and ethanol on swarming phenomenon of local isolate of *Proteus mirabilis.* J. ThiQar. UN. 2011;6(4):41.
40. Euzéby, JP. List of Bacterial Names with Standing in Nomenclature. Int. J. Syst. Bacteriol., 47, 13 July, 2010. Retrieved from http://www.bacterio.cict.fr/m/micrococcus.html
41. 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. 2004;72: 6836–6845.
42. Hernandez, E.; Ramisse, F. and Jean-Didier, C. Abolition of swarming of *Proteus*. J. Clin. Microbiol. 1999;37(10): 3435– 3436.
43. 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. 2004;3 (1):99-104.
44. Juhas M, Eberl L, Tummler B. Quorum sensing: the power of cooperation in the world of Pseudomonas. Environ Microbiol. 2005;7: 459-471.
45. Duan K, Surette MG. Environmental regulation of Pseudomonas aeruginosa PAO1 Las and Rhl quorum-sensing systems. J Bacteriol. 2007;189:4287-4236
46. Shrout JD, Nerenberg R. Monitoring bacterial twitter: does quorum sensing determine the behavior of water and wastewater treatment biofilms?. Environ Sci Technol. 2012; 46:1995-2005.
47. Grossman AD. Genetic networks controlling the initiation of sporulation and the development of genetic competence in Bacillus subtilis. Annu Rev Genet. 1995;29:477-508.
48. Hammer B, Bassler B. Quorum sensing controls biofilm formation in Vibrio cholerae. Mol Microbiol. 2003;50:101-104.
49. Hentzer M, Givskov M, Eberl L. Quorum sensing in biofilms: Gossip in slime city. *O'Toole MGaG, ed.* Microbial Biofilms: ASM press, Washington DC. 2004:118-140.
50. Peter SM. A network of networks: quorum-sensing gene regulation in Pseu-domonas aeruginosa. Int J Med Microbiol. 2006;296:73-81.
51. Nadell CD, Xavier JB, Levin SA. The evolution of quorum sensing in bacterial biofilms. PLoS Biol. 2008; 6(e):14.
52. Parsek MR, Greenberg EP. Sociomicrobiology: the connections between quorum sensing and biofilms. Trends Microbiol. 2005;13:27-33
53. Ryan KJ; Ray CG (editors) (2004). Sherris Medical Microbiology (4th ed.). McGraw Hill. ISBN 0-8385-8529-9.
54. Matsuyama T, Takagi Y, Nakagawa Y, Itoh H, Wakita J, Matsushita M. "Dynamic aspects of the structured cell population in a swarming colony of Proteus mirabilis". J. Bacteriol. 2000;182 (2): 385–93.
55. Chitravadivu C, Manian S, Kalaichelvi K. Antimicrobial studies on selected medicinal plants, Erode region, Tamilnadu, India. Middle-East J Sci Res 2009; 4 (3): 147- 152.
56. Ahmed F, Urooj A. Glucose-lowering, hepatoprotective and hypolipidemic activities of stem bark of Ficus racemosa in streptozotocin induced diabetic rats. J Young Pharm. 2009; 1(2): 160-164.
57. Purkayastha S, 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 2012; 3(1): 64-68.
58. Tullanithi KM, Sharmila B, Gnanendra TS. Preliminary phytochemical analysis and antimicrobial activity of Achyranthes aspera Linn. Int J Bio Tech. 2010: 1(3): 35-38.
59. Silva NCC, Júnior AF. Biological properties of medicinal plants: a review of their antimicrobial activity. J Veno Ani Tox Inclu Trop Dis. 2010; 16(3): 402-431.
60. Tepe B, Daferera D, Sökmen M, Polissiou M, Sökmen A. In vitro antimicrobial and antioxidant activities of the essential oils and various extracts of Thymus eigii M. Zohary et P.H. Davis. J Agric Food Chem. 2004;52: 1132- 7.
61. Elgayyar M, Draughon FA, Golden DA, Mount JR. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. J Food Prot. 2001;64: 1019-24.
62. Singh D, Gupta R, Saraf SA. Herbsare they safe enough? an overview. Crit Rev Food Sci Nutr. 2012; 52:876-98.
63. Raskin I, Ribnicky DM, Komarnytsky S, Ilic N, Poulev A, Borisjuk N, et al. Plants and human health in the twenty-first century. Trends Biotechnol. 2002;20: 522-31.
64. Bankole MA, Shittu LA, Ahmed TA, Bankole MN, Shittu RK, Kpela T, et al. Synergistic antimicrobial activities of phytoestrogens in crude extracts of two sesame species against some common pathogenic microorganisms. Afr J Tradit Complement Altern Med. 2007;4: 427-33.
65. Shan B, Cai YZ, Brooks JD, Corke H. The in vitro antibacterial activity of dietary spice and medicinal herb extracts. Int J Food Microbiol. 2007;117: 112-9.
66. Prabuseenivasan S, Jayakumar M, Ignacimuthu S. In vitro antibacterial activity of some plant essential oils. BMC Complement Altern Med. 2006;6:39.

67. Park KM, You JS, Lee HY, Baek NI, Hwang JK. Kuwanon G: an antibacterial agent from the root bark of Morus alba against oral pathogens. Journal of Ethnopharmacology. 2003; 84(2-3): 181-185.
68. Joshi B, Lekhak S, 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. 2009; 5(1): 143-150.
69. Kadhim MJ, Sosa AA, Hameed IH. 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. 2016; 8(6): 127-146.
70. Mohammed GJ, Kadhim MJ, Hussein HM. Characterization of bioactive chemical compounds from *Aspergillus terreus* and evaluation of antibacterial and antifungal activity. International Journal of Pharmacognosy and Phytochemical Research. 2016; 8(6): 889-905.
71. Hameed IH, Altameme HJ, Idan SA. Artemisia annua: Biochemical products analysis of methanolic aerial parts extract and anti-microbial capacity. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2016; 7(2): 1843- 1868.
72. Hussein AO, Mohammed GJ, Hadi MY, Hameed IH. 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. 2016; 8(3): 49-59.
73. Sosa AA, Bagi SH, Hameed IH. 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. 2016; 8(5): 109-126.
74. Altameme H J, Hadi MY, Hameed IH. Phytochemical analysis of *Urtica dioica* leaves by fourier-transform infrared spectroscopy and gas chromatography-mass spectrometry. Journal of Pharmacognosy and Phytotherapy. 2015a; 7(10): 238-252.
75. Mohammed GJ, Omran AM, Hussein HM. Antibacterial and Phytochemical Analysis of *Piper nigrum* using Gas Chromatography-Mass Spectrum and Fourier-Transform Infrared Spectroscopy. International Journal of Pharmacognosy and Phytochemical Research. 2016; 8(6): 977-996.
76. Hamza LF, Kamal SA, Hameed IH. Determination of metabolites products by *Penicillium expansum* and evaluating antimicobial activity. Journal of Pharmacognosy and Phytotherapy. 2015; 7(9): 194-220.
77. Jasim H, Hussein AO, Hameed IH, Kareem MA. Characterization of alkaloid constitution and evaluation of antimicrobial activity of *Solanum nigrum* using gas chromatography mass spectrometry (GC-MS). Journal of Pharmacognosy and Phytotherapy. 2015; 7(4): 56-72.
78. Hadi MY, Mohammed GJ, Hameed IH. Analysis of bioactive chemical compounds of *Nigella sativa* using gas chromatography-mass spectrometry. Journal of Pharmacognosy and Phytotherapy. 2016; 8(2): 8-24.
79. Hameed IH, Ibraheam IA, Kadhim HJ. Gas chromatography mass spectrum and fourier-transform infrared spectroscopy analysis of methanolic extract of *Rosmarinus oficinalis* leaves. Journal of Pharmacognosy and Phytotherapy. 2015; 7 (6): 90-106.
80. Shareef HK, Muhammed HJ, Hussein HM, Hameed IH. Antibacterial effect of ginger (*Zingiber officinale*) roscoe and bioactive chemical analysis using gas chromatography mass spectrum. Oriental Journal of Chemistry. 2016; 32(2): 20-40.
81. Al-Jassaci MJ, Mohammed GJ, Hameed IH. Secondary Metabolites Analysis of *Saccharomyces cerievisiae* and Evaluation of Antibacterial Activity. International Journal of Pharmaceutical and Clinical Research. 2016; 8(5): 304-315.
82. Mohammed GJ, Al-Jassani MJ, Hameed IH. 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. 2016; 8(3): 480-494.
83. Al-Marzoqi AH, Hadi MY, Hameed IH. Determination of metabolites products by *Cassia angustifolia* and evaluate antimicobial activity. Journal of Pharmacognosy and Phytotherapy. 2016; 8(2): 25-48.
84. Altameme HJ, Hameed IH, Abu-Serag NA. 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. 2015b; 44(4): 47–58.
85. Hameed IH, Hamza LF, Kamal SA. Analysis of bioactive chemical compounds of *Aspergillus niger* by using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy. Journal of Pharmacognosy and Phytotherapy. 2015b;7(8): 132-163.
86. Hameed IH, Hussein HJ, Kareem MA, Hamad NS. 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. 2015; 7 (7): 107-125.
87. Hussein HM, Hameed IH, Ibraheem OA. 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. 2016; 8(3): 369-385.
88. Hussein HJ, Hadi MY, Hameed IH. Study of chemical composition of *Foeniculum vulgare* using Fourier

transform infrared spectrophotometer and gas chromatography - mass spectrometry. Journal of Pharmacognosy and Phytotherapy. 2016; 8(3): 60-89.
89. Kadhim MJ, Mohammed GJ, Hameed IH. In *vitro* antibacterial, antifungal and phytochemical analysis of methanolic fruit extract of *Cassia fistula*. Oriental Journal of Chemistry. 2016; 32(2): 10-30.
90. Altameme HJ, Hameed IH, Idan SA, Hadi MY. 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. 2015c; 7(9): 221-237.
91. Hussein HM. 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. 2016;7(3): (B) 1009 – 1017.
92. Hussein HM. 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. 2016;7(4): 2529 – 2555.
93. Jaddoa HH, Hameed IH, Mohammed GJ. Analysis of volatile metabolites released by *Staphylococcus aureus* using gas chromatography-Mass spectrometry and determination of its antifungal activity. Orient J Chem. 2016;32(4).
94. Hameed IH, Salman HD, Mohammed GJ. 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. 2016;32(4).
95. Kadhim MJ, Mohammed GJ, Hussein HM. Analysis of bioactive metabolites from *Candida albicans* using (GC-MS) and evaluation of antibacterial activity. International Journal of Pharmaceutical and Clinical Research. 2016; 8(7): 655-670.
96. Ubaid JM, Hussein HM, Hameed IH. Analysis of bioactive compounds of *Tribolium castaneum* and evaluation of anti-bacterial activity. International Journal of Pharmaceutical and Clinical Research. 2016; 8(7): 655-670.
97. Hameed IH, Jebor MA, Ommer AJ, Abdulzahra AI. 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. 2016; 27(2): 1324-1327.
98. Hameed IH. A new polymorphic positions discovered in mitochondrial DNA hypervariable region HVIII from central and north-central of Iraq. Mitochondrial DNA. 2016; 27(5): 3250-4.
99. Mohammad A, Imad H. Autosomal STR: From locus information to next generation sequencing technology. Research Journal of Biotechnology. 2013.
100. 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.
101. Leeja L, Thoppil JE. Antimicrobial activity of methanol extract of Origanum marjorana L. (Sweet marjoram). Journal of Environmental Biology. 2007; 28(1): 145-146.
102. Patil MK, et al. Antibacterial efficacy of Piper nigrum fruit. Journal of Veterinary Pharmacology and Toxicology. 2007; 2(1-2): 69-71.
103. Kumar VS, Ahmed SM, Badami S, Anil TM and David B. Antibacterial activity of aqueous extract of Coleus amboinicus. Pharmacology online. 2008; 3: 224-226.