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In Vitro Antibacterial, Antifungal and Other Medical Properties of Endangered Medicinal Plant Seeds

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Abstract

The risk created by infectious microorganisms to humans attracted the development of common medicine. To find an alternative source, medicinal plants with diverse metabolites play an important role in curing the diseases and human disorders caused by microbial pathogens. Medicinal plants namely, *Citrullus colocynthis*, *Hyoscyamus muticus*, *Ocimum basilicum*, *Amaranthus lividus*, *Salvia aegyptiaca* and *Ruta chalepensis* are commonly used as a traditional medicine in Gulf countries. The present study aimed to investigate the antibacterial, antifungal and antioxidant potential of the organic crude extracts obtained from the seeds. Besides, the possible antimicrobial mechanisms of the extracts were evaluated by determining the enzyme activities. The antibacterial and antifungal activities of the crude extracts were evaluated by the broth micro dilution method and the effect of the extracts on the pathogens were determined by quantifying the alkaline phosphatase (ALP), lactate dehydrogenase (LDH) enzymes and intracellular protein leakage. Besides, the antioxidant properties were determined using hydroxyl radical scavenging assay, DPPH radical scavenging assay, reducing power assay and superoxide radical scavenging assay. Results indicated that the extracts of *C. colocynthis* showed promising activity against all the tested pathogens, especially the MIC values were ranged from 100 to 150 µg/ml for Gram positive bacteria and 100 to 250 µg/ml for Gram negative bacteria respectively. The MIC values of *H. muticus*, *O. basilicum* and *R. chalepensis* against the fungal pathogens were ranged from 100 to 500 µg/mL respectively. The ALP activity was higher in extract treated *Klebsiella pneumoniae* compared with control, whereas the LDH and protein concentrations for *Escherichia coli* and *Staphylococcus aureus* were comparatively higher. Furthermore, all the studied seed extract showed good antioxidant activities. In conclusion, the studied plant seed extracts documented good antimicrobial and antioxidant activities. Therefore, the medicinal plants would be the excellent source for natural antioxidant and

antibacterial agents for medical and applications.

Keywords

Medicinal Plants, Antimicrobial Activities, Antifungal Activities, Mechanism of Antimicrobial Action, Antioxidant Properties

1. Introduction

Bacteria, fungi, viruses and parasites create infectious disease to human. Among the bacterial pathogens, different species including *Enterococci*, *Salmonella*, *Staphylococcus*, *Bacillus*, and *Pseudomonas* are the major causative agents for the symptom of fever, diarrhea, cough and other human infections [1]. Various infections and disorders caused by these pathogenic strains were cured by the innovative discovery of modern medicine and antibiotics. However, the frequent usage of the common antibiotics and therapeutic compounds for the prevention of disease causing pathogens triggered to the emergence of microbial resistance to the commonly used antibiotics. Also, the production of novel antimicrobial compounds by chemical and pharmaceutical industry has increased tremendous improvement, however, the spreading of the diseases were not comparatively reduced, whereas; creates other side effects such as immune suppression, allergic reactions and hypersensitivity reactions respectively [2]. Therefore, there is an urgent need for the invention of novel molecules with fewer side effects. At present, more than 60% of the world's population is using plant based medicine in the healthcare units [3]. In this regards, novel lead molecules isolated from active beneficial bacteria and traditional rare medicinal plants would be the alternative route for the development good active formulations with side effects. Among the isolated compounds, the novel molecules recovered from plants are shown to produce lesser side effects than the commonly used antibiotics [4]. During the twentieth century, many researchers were interested to identify the useful medicinal plants to unlock the secrets of ancient herbal remedies with various ailments. Indeed, antibacterial, antifungal, antioxidant, antibiofilm, anticancer, antidiabetic, antihpertension, and other cardiovascular protective properties of medicinal plants are reported from different parts of the world [5]. The phytochemicals such as phenolic compounds, flavanoids, anthocyanins, alkaloids, terpernoids, saponins and quinine molecules obtained from traditional medicinal plants are mainly responsible for their potential activity [6] [7] [8] [9]. Due to the presence of wide range of metabolites, medicinal plants from Gulf countries especially kingdom of Saudi Arabia were investigated for various activities [10] [11] [12]. Recently, Al-Juraifani (2011) investigated the antibacterial and antifungal potential of *Thymus vulgaris*, *Salvia officinalis*, *Boswelia carterii* and *Boswelia carterii* against *Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus* sp., *Micrococcus luteus Vibrio tubiashii*, *Cellulosimicrobium cellulans*, *Fusarium oxysporum* and *Aspergillus flavus* respectively [13]. Like other coun-

try Saudi Arabia is also rich in traditional medicinal plants with various biological activities.

Citrullus colocynthis L distributed in the desert area of gulf region belong to *cucurbitaceae* family contains alkaloids such as colocythin and colocynthidin, cucurbitacin, saponin and glycosides were traditionally used in the treatment of jaundice and constipation [14]. Species of *Hyoscyamus* belong to *Solanaceae* family, known for the presence of many phytochemicals such as hyoscine, apohyoscine, belladonines apoatropine, hyoscyamine, skimmianine, tropine caturamine, hyoscypicrin, apoatropine, cuscohygrine, phytin, tropine, hyoscine aphoyoscine choline, alpha and beeta belladonine and hyoscine respectively with various biological activities [15]. *Ocimum basilicum* (Lamiaceae) is another widely studied medicinal plant commonly observed in the warm temperate region is known for its applications towards the treatment of diarrhea, pneumonia, fever respiratory tract infections, ophthalmic, headache, cough, skin disease, and conjunctivitis respectively [16]. *Amaranthus lividus* is also distributed in the warm places and is used in the treatment of various disorders [17]. *Salvia aegyptiaca* is known for the presence of abietane, diterpenoids, triterpenoids and sesquiterpenoids with antimicrobial and anti-leishmania activities [18]. *Ruta chalepensis* is widely used in the treatment of urinary tract infections and the phytochemicals recovered from this plant exhibited comparatively better antimicrobial activity against the clinical pathogens [19]. With these information's, the present study aimed to investigated the antimicrobial and antioxidant properties of the seeds of *C. colocynthis*, *H. muticus*, *O. basilicum*, *A. lividus*, *S. aegyptiaca* and *R. chalepensis*. In addition the possible antimicrobial mechanism of the extract of the seeds also determined.

2. Materials and Methods

2.1. Collection of Plant Seed Materials

Seeds of six medicinal plants namely *C. colocynthis*, *H. muticus*, *O. basilicum*, *A. lividus*, *S. aegyptiaca* and *R. chalepensis* were collected from the desert region of gulf countries.

2.2. Solvent Extraction of Seeds

Seeds of the medicinal plants were collected and shade dried for three days before solvent extraction. For solvent extraction, the seeds were finely powdered using the blender and the powder was mixed with ethyl acetate. Further, the mixture was thoroughly vortexed and rapped with air-tight cotton plug and tightly covered with aluminum foil. After that the mixtures containing flask was kept in the orbital shaker and mixed in the gradual speed of 100 rpm for three days. Finally, the mixtures was filtered using the whatman No-1 filter paper and the collected supernatant was further centrifuged at 12000 rpm for 15 min for complete removal of the debris. The debris free supernatant was concentrated using vacuum evaporator at 40˚C which was maintained by supplying chilled water under reduced pressure. The collected solvent phase was discarded and the

concentrate containing the photochemical were transferred into the air-tight brown bottle and safely stored in the cold cabinet for further experiments.

2.3. *In-Vitro* Antimicrobial Activity

2.3.1. Pathogenic Microbial Strains

A total of seven Gram positive and Gram negative bacterial strains namely, *Bacillus subtilis* (MTCC 441), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (MTCC 3615), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 15380), *Pseudomonas aeroginosa* (ATCC 27853) and six filamentous fungi namely, *Aspergillus niger* (KACC 40280), *Botrytis cinerea* (KACC 40573), *Candida albicans* (KACC 30003), *Curvalaria lunata* (KACC 40392), *Fusarium oxysporum* (KACC 40051) and *Gibberella moniliformis* (KACC 44022) were evaluated for the antimicrobial activity.

2.3.2. Antimicrobial Activity

Antibacterial activity of the extract was determined by disc diffusion method [6]. Briefly, the bacterial strains were freshly prepared and sub-cultured before performing the experiment. Fifty micro liter of active cell suspension were evenly spread on the nutrient agar plates in aseptic condition. After that the sterile disc impregnated with the extracts were placed on the top of the plates and incubate at 37˚C for 17 h. The antimicrobial activity was determined by measuring the zone of inhibition around the discs. This experiment was performed in triplicates. Whereas for determination of antifungal activity, the fungal spore suspension were mixed with sterile semi-solid potato dextrose agar together with the varying concentrations of the extract. Attention must take care that the fungal spore and the extract should be mixed when the temperature is around 50˚C. Control plate was maintained without addition of the extract. After incubation at 30˚C for two days, antifungal activity was determined by comparing the difference in the fungal biomass growth. This experiment was performed in triplicates for further confirmation.

2.3.3. Minimum Inhibitory Concentration (MIC)

Standard reported method was followed for the determination of minimum inhibitory concentration in 96 well plate [6].

2.4. Determination of Mechanism of the Antimicrobial Activity of the Extract

2.4.1. Alkaline Phosphatase (ALP) Quantification

The content alkaline phosphatase (ALP) was quantified by following the modified method of Arokiyaraj *et al.* 2014 [20].

2.4.2. Lactate Dehydrogenase (LDH) Quantification

Lactate dehydrogenase (LDH) is present in the cytoplasm of the bacteria. LDH level was analyzed to determine the damage caused by the extract to the pathogenic bacteria. The LDH level was quantified by following the method of Arokiyaraj *et al.* (2014) [20].

2.4.3. Intracellular Protein Leakage

The effect of the extract in the intracellular protein level was monitored. For evaluating the extracts influence in the intracellular protein levels, the freshly grown bacteria were cultivated by supplementing the extract. After that the cells were cultivated under micro-aerobic condition in the shaking incubator for 24 h. After incubation the supernatant was collected and level of protein was measured by following the method of Bradford (1976) [21].

2.5. *In-Vitro* Antioxidant Activities

2.5.1. Hydroxyl Radical Scavenging Activity

In vitro hydroxyl scavenging activity of the seed extract was determined by following the method of Sunil *et al.* (2014) [22].

Scavenging activity (%) = [1 − (absorbance of sample-absorbance of blank)/absorbance of control] × 100

2.5.2. DPPH Radical Scavenging Assay

The DPPH scavenging activities of the compounds were determined by following the method of Hanato *et al.* (1988) [23].

Scavenging activity (%) = [1 − (absorbance of sample-absorbance of blank)/absorbance of control] × 100

2.5.3. Reducing Power

Reducing power of the metabolites was determined by following the method of Oyaizu, (1986) [24]. BHT at various concentrations was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power.

2.5.4. Superoxide Radical Scavenging Assay

As described by Sunil *et al.* [22] NBT (tetrazolium reagent) method was followed for the superoxide radical scavenging assay.

Scavenging activity (%) = [1 − (absorbance of sample-absorbance of blank)/absorbance of control] × 100

3. Results

3.1. Antibacterial Activity of the Medicinal Plant Seeds

The antibacterial activity of the selected six medicinal plant seeds ethyl acetate extracts are showed in **Table 1**. The MIC results revealed variable degrees of activity against Gram positive and Gram negative bacterial pathogens, with MICs values ranging from 100 to 250 µg/ml. Among the plants, the extracts of *C. colocynthis* showed promising activity against all the tested pathogens, especially the MIC values were ranged from 100 to 150 µg/ml for Gram positive bacteria, and 100 to 250 µg/ml for Gram negative bacteria respectively. The MIC values of *C. colocynthis* towards *B. subtilis*, *S. epidermidis*, *E. faecalis* and *P. aeroginosa* were 100 µg/ml, *S. aureus* and *E. coli* were 150 µg/ml and towards *K. pneumoniae* 200 µg/ml respectively. The antibacterial activity of *O. basilicum* and *A. lividus* comparatively showed similar profile towards Gram Positive bacteria. The

Table 1. Minimum inhibitory concentration of the extracts against Gram positive and Gram negative bacteria.

Microorganism	Minimum Inhibitory Concentration (MIC) ($\mu g \cdot mL^{-1}$)						
	1	2	3	4	5	6	S
Gram positive							
Bacillus subtilis (MTCC 441)	100	>500	250	150	>250	250	2.5
Staphylococcus aureus (ATCC 25923)	150	>250	250	100	250	NA	37.5
Staphylococcus epidermidis (MTCC 3615)	100	250	150	150	100	NA	10
Enterococcus faecalis (ATCC 29212)	100	150	100	250	150	100	25
Gram negative							
Escherichia coli (ATCC 25922)	150	>500	>250	150	NA	500	25
Klebsiella pneumoniae (ATCC 15380)	250	>500	>250	100	NA	>500	25
Pseudomonas aeroginosa (ATCC 27853)	100	500	>250	100	250	>500	50

1, *Citrullus colocynthis*; 2, *Hyoscyamus muticus*; 3, *Ocimum basilicum*; 4, *Amaranthus lividus*; 5, *Salvia aegyptiaca*; 6, *Ruta chalepensis*; S, standard antibiotics. MTCC: microbial type culture collection; ATCC: American type culture collection; MMC: NA: no activity.

MIC values of *O. basilicum* and *A. lividus* were ranged from 100 to 250 µg/ml. However, the extracts of *H. muticus*, *S. aegyptiaca* and *R. chalepensis* exhibited moderate level of activity towards Gram positive and Gram negative bacteria. The MIC of the standard streptomycin were ranged from 2.5 to 50 µg/ml respectively. The extracts of *S. aegyptiaca* and *R. chalepensis* did not expressed activity towards *S. epidermidis*, *S. aureus*, *P. aeroginosa* and *K. pneumoniae*.

3.2. Antifungal Activity of the Medicinal Plant Seeds

The antifungal activity of the selected six medicinal plant seeds extracts are presented in Table 2. Among the seeds, *A. lividus* showed significant activity against the filamentous fungi, *B. cinerea* revealed the lower MIC values (100 µg/mL) and other fungi such as *A. niger*, *C. lunata*, *F. oxysporum*, and *G. moniliformis* showed MIC at 125 µg/mL concentrations. The extracts of *C. colocynthis*, *H. muticus*, *O. basilicum* and *R. chalepensis* exhibited the MIC values ranged from 100 to 500 µg/mL respectively. Among the seeds, *S. aegyptiaca* showed the moderate activity against all the tested fungal pathogens. The extracts of *S. aegyptiaca* did not showed activity towards *A. niger*, *B. cinerea*, *C. albicans* and *C. lunata*, whereas the MIC values of *F. oxysporum* and *G. moniliformis* were above 500 µg/mL respectively. The positive control showed MIC values from 25 - 100 µg/mL for the selected fungal pathogens.

3.3. Antimicrobial Mechanism of the Extract

3.3.1. Quantification of Alkaline Phosphatase (ALP)

The quantification ALP enzyme gives further evidence that the treatment of

Table 2. Minimum inhibitory concentration of the extracts against fungi.

Microorganism	Minimum Inhibitory Concentration (MIC) ($\mu g \cdot mL^{-1}$)						
	1	2	3	4	5	6	S
Aspergillus niger (KACC 40280)	>250	NA	150	125	NA	250	25
Botrytis cinerea (KACC 40573)	500	500	100	100	NA	250	50
Candida albicans (KACC 30003)	125	500	100	100	NA	500	100
Curvalaria lunata (KACC 40392)	250	250	150	125	NA	125	50
Fusarium oxysporum (KACC 40051)	100	>500	>150	125	>500	250	25
Gibberella moniliformis (KACC 44022)	100	500	500	125	>500	250	100

1, *Citrullus colocynthis*; 2, *Hyoscyamus muticus*; 3, *Ocimum basilicum*; 4, *Amaranthus lividus*; 5, *Salvia aegyptiaca*; 6, *Ruta chalepensis*; S, standard antibiotics. KACC: Korean type culture collection; NA: no activity.

extracts to microbial strains strongly inhibited the bacterial cell wall and other physiological nature. Results indicated that the significant increase in the units of the ALP in the cultivation medium. ALP enzyme concentrations were dominant in the *K. pneumoniae* (623, 466, 466 U/L) treated with *C. colocynthis*, *H. muticu* and O. *basilicum* extract, whereas the content of the enzymes were higher in S. typhi (383 and 606 U/L) for *A. lividus* and *S. aegyptiaca* respectively (Figure 1(a)). ALP was noted higher in *P. aeruginosa* in the case of *R. chalepensis* treatment. The increase in the concentration of the ALP enzyme proved that the plant extracts created the unfavorable environment to the bacteria which resulted in the release of the enzymes.

3.3.2. Quantification of Lactate Dehydrogenase (LDH)

The levels of LDH in the extract treated samples were showed in Figure 1(b). All the seeds extract exhibited higher level of enzyme in the culture broth indicated that the extracts directly attach the cell wall of the bacterial and create unfavorable condition for the growth. Particularly, the LDH level of *S. aureus* treated with *C. colocynthis* was 64.7% higher than the control. Also, treatment with *H. muticus* exhibited 61% higher LDH in *K. pneumoniae*. Similarly, the extracts of *O.basilicum*, *A. lividus*, *S. aegyptiaca* and *R. chalepensis* pronounced comparatively higher level of LDH in the spent medium.

3.3.3. Quantification of Intracellular Protein Leakage Level

The results revealed that the treatment of the extract with the microbial strains showed higher release of protein in the supernatant. Among the pathogenic bacteria, the protein concentration *S. aureus* was higher (79%, 76%, 74% and 72%) than the control in *R. chalepensis*, *A. lividus*, *S. aegyptiaca* and *O.basilicum* treated samples, followed by *P. aeruginosa* (70.68%) and *K. pneumoniae* (60.15%) in *C. colocynthis* and *H. muticus* (Figure 1(c)).

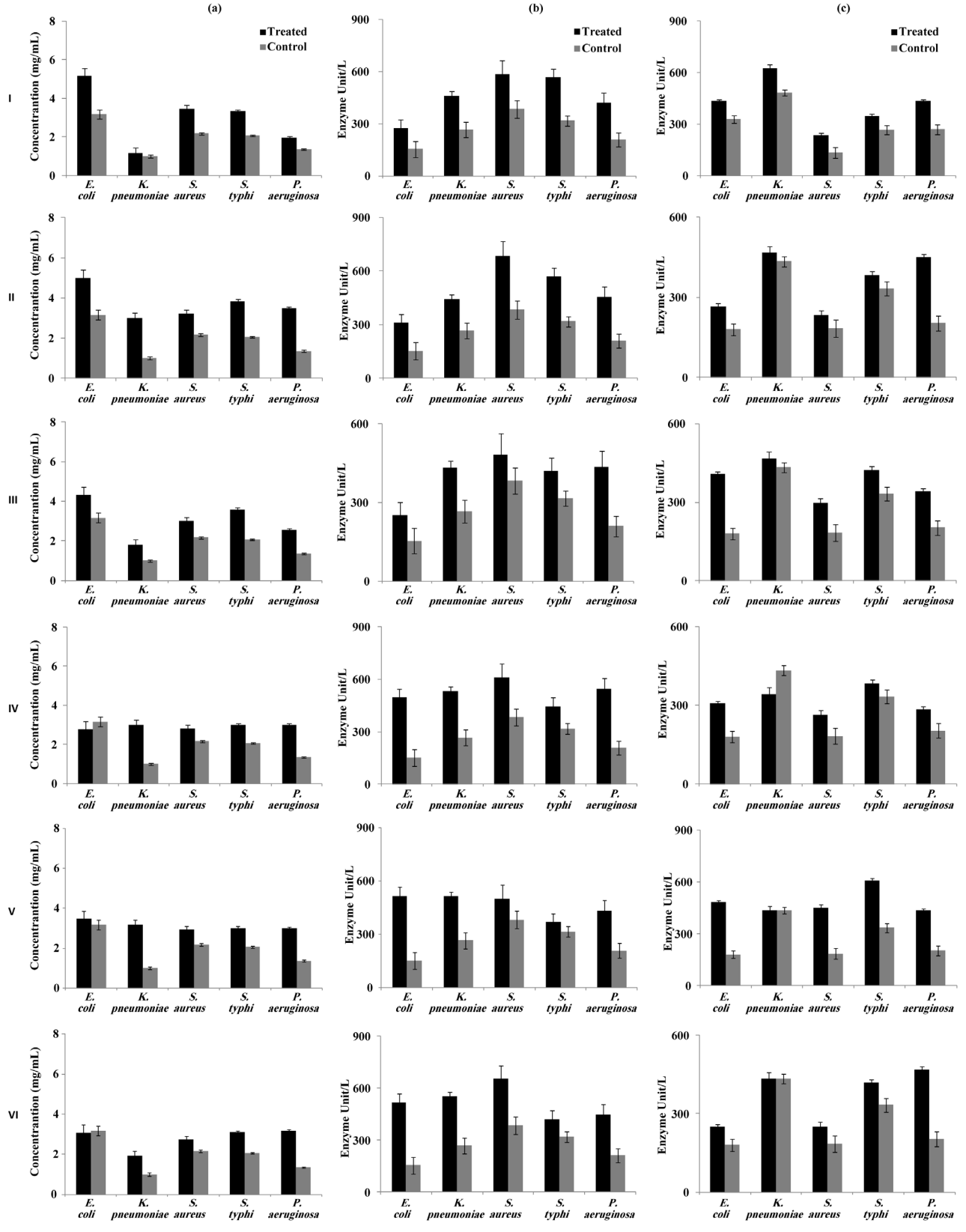

Figure 1. Antimicrobial mechanism of the extract; determined by studying the enzyme activities. I, *Citrullus colocynthis*; II, *Hyoscyamus muticus*; III, *Ocimum basilicum*; IV, *Amaranthus lividus*; V, *Salvia aegyptiaca*; VI, *Ruta chalepensis*; (a), Quantification of Alkaline phosphatase (ALP); (b), Quantification of Lactate dehydrogenase (LDH); (c), Quantification of intracellular protein leakage level.

3.4. Antioxidant Activity of the Medicinal Plant Seeds

3.4.1. Hydroxyl Radical Scavenging Activity

The ability of the seeds extracts to scavenge the hydroxyl radical is displayed in Figure 2(a). All the seeds extracts revealed the scavenging activity in a dose dependent manner. In particular for *C. colocynthis*, the concentrations for 50% inhibition were found to be 163 and 125 μg/mL for the crude ethyl acetate extract and BHT, respectively. The 50% inhibition of other seeds extracts were noted in the figure.

3.4.2. DPPH Radical Scavenging Assay

The ability of the seeds extracts to scavenge DPPH free radicals are shown in Figure 2(b). All the seeds extract revealed varying scavenging effects. However, the DPPH scavenging activities were noted as the dose dependent manner. Fifty

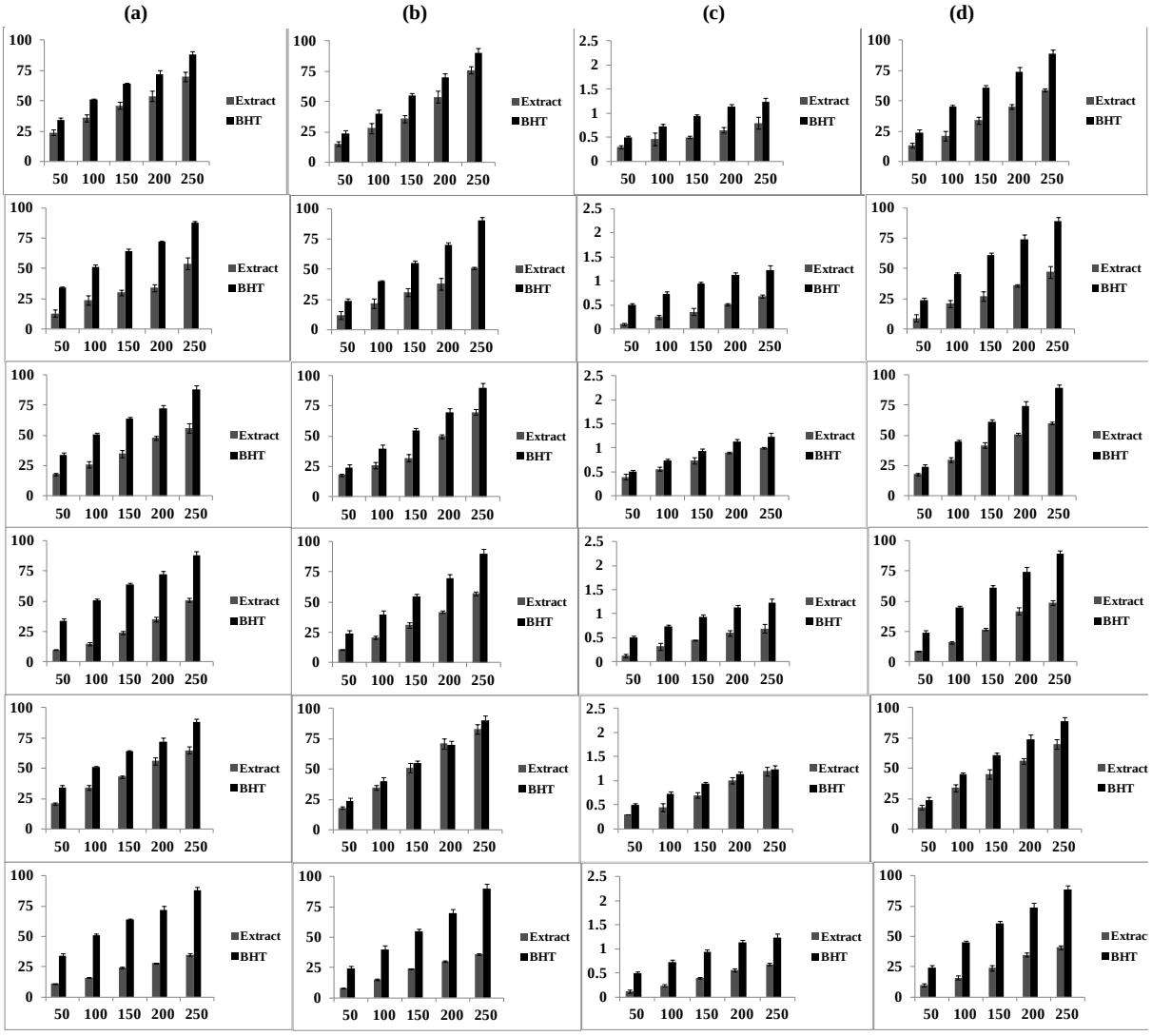

Figure 2. Antioxidant activity of the extract. I, *Citrullus colocynthis*; II, *Hyoscyamus muticus*; III, *Ocimum basilicum*; IV, *Amaranthus lividus*; V, *Salvia aegyptiaca*; VI, *Ruta chalepensis*; (a), Hydroxyl Radical Scavenging Activity; (b), DPPH radical scavenging assay; (c), Reducing power; (d), Superoxide radical scavenging assay.

percentage scavenging ability of the extracts were were found to be 208, 242, 233, 242, 147 and 312 μg/mL for *C. colocynthis*, *H. muticus*, *O. basilicum*, *A. lividus*, *S. aegyptiaca* and *R. chalepensis* respectively. All the extracts revealed the highest scavenging rates at 76%, 51%, 70%, 57% 83% and 36% at 250 μg/mL concentration whereas standard BHT showed 90% at 2500 μg/mL concentration.

3.4.3. Reducing Power

The reducing power ability of the extracts was compared to the standard BHT (**Figure 2(c)**). Results indicated that all the six extracts exhibited differences in their reducing power reactions. In general, all the extracts comparatively showed good reducing power with respect to the concentration of the extract.

3.4.4. Superoxide Radical Scavenging Assay

Figure 2(d) displayed the superoxide radical scavenging activity of the seed extract. Comparatively, all the extracts showed the similar superoxide radical scavenging activity and the activity was directly proportional to the concentration of the crude extract. Fifty percentage of superoxide anion radical generation was scavenged at 221, 277, 179, 278, 167 and 313 μg/mL concentration for *C. colocynthis*, *H. muticus*, *O. basilicum*, *A. lividus*, *S. aegyptiaca* and *R. chalepensis* respectively.

4. Discussion

Novel active lead molecules recovered from different medicinal plants throughout the world create the foundation for the development of new antibiotic or the therapeutic products for the treatment of infectious disease caused by pathogenic microbial strains including bacteria and fungi because of its safe use than the modern synthetic drugs with number of side effects [7]. It is estimated that 80% of the world population attracted the consumption of natural products from various traditional medicinal plants especially herbal medicinal plants contain secondary metabolites such as flavanoids, alkaloids, terpenoids, anthocyanins and saponins with anticancer, antioxidants, antihypertention, anti-inflammatories, anticoagulant, antidiabetic, and other cardiovascular diseases. Also, the novel plant derived molecules involved in the enhancement of the immune system further prolonging the life style [25]. In the recent years, many studies have been done to evaluate the antimicrobial properties of medicinal plants in many countries. Similarly, medicinal plants such as *Datura stramonium*, *Zygophyllum coccineum*, *Lasiurus scindicus* and *Heliotropium digynum* from Saudi Arabia has also explored for the antimicrobial potential against various microbial pathogens [26]. However, it is worthy for the identification of potential medicinal plants from Saudi Arabia with various antimicrobial properties [27]. Especially, plants such as *C. colocynthis*, *H. muticus*, *O. basilicum*, *A. lividus*, *S. aegyptiaca* and *R. chalepensis* have wide level of biological applications attract the researchers to investigate their antimicrobial properties. Therefore, the seeds of *C. colocynthis*, *H. muticus*, *O. basilicum*, *A. lividus*, *S. aegyptiaca* and *R. chalepensis* were collected from the desert region of Saudi Arabia and investigated its antimicrobial

and antioxidant properties. Preliminary screening of the crude extract obtained from the seeds documented comparatively significant antimicrobial activities. There antimicrobial mechanism of the extracts was determined by evaluating the enzyme concentration in the spent medium. Further, the *in vitro* antioxidant properties also evaluated.

From the results, The MIC values of the selected plants extracts against Gram positive and Gram negative microbial pathogens were ranged from 100 to 500 µg/mL. The results indicated that the lowest MIC value (100 µg/ml) of the extracts of *A. lividus* was against *S. aureus*, *K. pneumoniae* and *P. aeroginosa*. MIC value was comparatively higher (500 µg/ml) against uropathogenic bacteria *E. faecalis*. The activity of the extracts against the Gram negative bacteria was interesting, especially, the extracts of *C. colocynthis* documented the lowest MIC (100 µg/mL) was recorded against *P. aeruginosa* and the highest MIC (250 µg/mL) was recorded against *K. pneumoniae*. *E. coli* showed moderate level of MIC values (150 µg/mL). Against all the tested microbial pathogens, we noted that the *A. lividus* extract produced much better antibacterial activities. These results were coincides with the report of Marzouk *et al.* (2010) and Padalia *et al.* (2014), where the extract of *C. colocynthis* and O. *basilicum* showed promising antimicrobial activity against Gram-positive (*S. aureus* and *E. faecalis*) and Gram-negative (*P. aeruginosa* and *E. coli*) respectively [28] [29]. The present study confirmed that the Gram positive bacteria are more susceptible to all the tested extracts as they have a susceptible cell wall layer [30]. The urinary infection causing *K. pneumoniae*, *E.coli* and *P. aeroginosa* also revealed moderate activity against all the six extracts, even though the bacteria contains thick cell wall membrane which rarely permit the molecules inside the cells [30].

The increasing rate of fungal infections such as aspergillosis and candidiasis leads to severe immune-suppression diseases [31]. In the present study all the extracts exhibited comparatively moderate activity against all the tested fungi and the MIC values ranged within 100 - 500 µg/mL respectively. The extract of *C. colocynthis*, documented MIC value >250 µg/mL for *A. niger*, 500 µg/mL (*B. cinerea*), 125 µg/mL (*C. albicans*), 250 µg/mL (*C. lunata*), 100 µg/mL (*F. oxysporum*) and 100 µg/mL (*G. moniliformis*). Similarly, Eidi *et al.* (2015), claimed that the crude extract of *C. colocynthis* documented the MIC of 1.56 to 12.5 mg/ml against *Aspergillus fumigates*, *A. niger*, *Candida guilliermondii* and *Candida kreusei* respectively [32]. The inhibitory activity of the extract might be due to the presence of active compounds including glucosides and resins which are water soluble and inhibit enzymatic activity in the cytoplasmic membrane [33]. Cota *et al.* (2013), reported that the phytochemicals present in the medicinal plants actively diffuse through the cytoplasmic membrane and compete for the active sites of certain enzymes inside the cell thereby arresting the strains to grow [34]. In addition many studies evidenced the antifungal activity of *C. colocynthis* [35] [36] [37].

The antimicrobial mechanisms of the plant derived metabolites were elucidated by several researchers. However, the exact mechanisms of action were not

completely reported. Researchers claimed that the active molecules attach the cell wall of the pathogenic microorganisms and create the unfavorable environment to the outer cellular membranes leading to the alteration of cellular contents and leakage of inner membrane contents [38]. Relatively, constituents of plants such as phenolics, flavonoids, quinines, tannins, coumarins, alkaloids, terpenoids, lectins and polypeptides inhibit the ATPase syntheses which directly stimulate the alteration in the physiology of the bacterial cells and leads to cell death [39] [40] [41]. The phenolic compounds derived are known for the lysis of cell membranes and cause cell death [39] [42]. Similarly, the present report claimed that the crude extracts of the six medicinal plants documented variation in the cellular components especially the contents of total protein and the units of enzymes such as ALP and LDH. Therefore, it is attributed that the combination of the phyto constituents present in the seed extracts of the medicinal plants create cell damage, causing leakage of cellular materials and resulted in the suppression of cell growth.

Molecules prevent or stop the oxidation of cellular components are known as antioxidants [43]. In general, most of the identified medicinal plants functional compounds such as alkaloids (terpenoid indole alkaloids, tropane alkaloids and purine alkaloids), terpenoids (monoterpenes, sesquiterpenes and diterpenes), carotenoids (beta-carotene), phenolics (phenolic acids, flavonoids, lignans, stilbenes and tannins) have the promising antioxidant potentials [44] [45]. Among the different functional metabolite, phenolic compounds protect the human body from various major causative agents for various life threatening diseases, including neurodegenerative and cardiovascular disease respectively [46]. Similar to the report, in the present study, the plant materials exhibited comparatively good antioxidant activity. Yoshikawa *et al.* (1997) and Nirmala and Ramanathan, (2011) reported that the presence of phenolic compounds in the medicinal plant materials enhance the activity of antioxidant enzymes such as glutathione peroxidase, superoxide dismutase and catalases respectively [47]. The extracts obtained from the medicinal plants could be useful for the protection of oxidative stress related diseases.

5. Conclusion

In conclusion, the antibacterial, antifungal and antioxidant properties of six medicinal plants seed collected from Saudi Arabia were investigated. All the extracts revealed good antibacterial activity against the Gram positive and Gram negative pathogens, especially the MIC of the extracts against the fungal pathogens were ranged from 100 to 500 μg/mL respectively. All the studied extracts showed promising activity against the filamentous fungal pathogens. The mechanisms of antimicrobial potential of the extracts were confirmed by evaluating the contents of ALP, LDH and extracellular protein contents. The elevated levels of the enzyme concentration in the extract treated microbial pathogens were its advantage. Additionally, all the extract showed promising antioxidant activity. Future studies would plan to isolate the novel active metabolites from the crude

extract and investigate its application in treating infectious diseases and oxidative stress-related diseases. Also, accurate *in vitro* cultivation methods would be developed for the propagation of the medicinal plants for bulk cultivation.

Conflict of Interest

Declare no conflict with the manuscript.

References

[1] Bibi, Y., Nisa, S., Chaudhary, F.M. and Zia, M. (2011) Antibacterial Activity of Some Selected Medicinal Plants of Pakistan. *BMC Complementary and Alternative Medicine*, **11**, 52. https://doi.org/10.1186/1472-6882-11-52

[2] Adwan, G. and Mhanna, M. (2008) Synergistic Effects of Plant Extracts and Antibiotics on *Staphylococcus aureus* Strains Isolated from Clinical Specimens. *Middle-East Journal of Scientific Research*, **3**, 134-139.

[3] Awouafack, M.D., McGaw, L.J., Gottfried, S., Mbouangouere R., Tane, P., Spiteller, M. and Eloff, J.N. (2013) Antimicrobial Activity and Cytotoxicity of the Ethanol Extract, Fractions and Eight Compounds Isolated from *Eriosema robustum* (Fabaceae). *BMC Complementary and Alternative Medicine*, **13**, 289. https://doi.org/10.1186/1472-6882-13-289

[4] Walsh, F.M. and Amyes, S.G. (2011) Microbiology and Drug Resistance Mechanisms of Fully Resistant Pathogens. *Current Opinion in Microbiology*, **7**, 439-444.

[5] Bhalodia. N.R. and Shukla, V.J. (2011) Antibacterial and Antifungal Activities from Leaf Extracts of *Cassia fistula* l: An Ethnomedicinal Plant. *Journal of Advanced Pharmaceutical Technology*, **2**, 104-109. https://doi.org/10.4103/2231-4040.82956

[6] Rejiniemon, T.S., Arasu, M.V., Duraipandiyan V., *et al.* (2014) *In Vitro* Antimicrobial, Antibiofilm, Cytotoxic, Antifeedant and Larvicidal Properties of Novel Quinone Isolated from *Aegle marmelos* (Linn.) *Correa*. *Annals of Clinical Microbiology and Antimicrobials*, **13**, 48. https://doi.org/10.1186/s12941-014-0048-y

[7] Antonisamy, P., Duraipandiyan, V., Ignacimuthu, S. and Kim, J.-H. (2015) Anti-Diarrhoeal Activity of Friedelin Isolated from *Azima tetracantha lam*. in Wistar Rats. *South Indian Journal of Biological Sciences*, **1**, 34-37. https://doi.org/10.22205/sijbs/2015/v1/i1/100440

[8] Balamurugan, R. (2015) *Smilax chinensis* Linn. (*Liliaceae*) Root Attenuates Insulin Resistance and Ameliorate Obesity in High Diet Induced Obese Rat. *South Indian Journal of Biological Sciences*, **1**, 47-51. https://doi.org/10.22205/sijbs/2015/v1/i1/100443

[9] Abou-zeid, A.M., Altalhi, A.D. and El-Fattah, R.I. (2008) Fungal Control of Pathogenic Fungi Isolated from Some Wild Plants in Taif Governorate, Saudi Arabia. *Malaysian Journal of Microbiology*, **4**, 30-39.

[10] Fardos, M.B. (2009) Antifungal Activity of Some Medicinal Plants Used in Jeddah, Saudi Arabia. *Mycophathology*, **7**, 51-57.

[11] Nehal, S.E. and Rokayah, S.A. (2009) Inhibitory Effects of Powdered Caraway and Peppermint Extracts on Pea Root Rot under Greenhouse Conditions. *Saudi Journal of Plant Protection Research*, **49**, 93-96.

[12] Al-Juraifani, A.B. (2011) Antimicrobial Activity of Some Medicinal Plants Used in Saudi Arabia. *Canadian Journal of Pure and Applied Sciences*, **5**, 1509-1512.

[13] Alkamel, M.L. (2005) Antimicrobial Activity of Aqueous Extract of *Citrullus colocynthis* L. Fruit. *Tikrit Journal of Pharmaceutical Sciences*, **1**, 9-15.

[14] Dulger, B., Ceyhan, M., Alitsaous, M. and Ugurlu, E. (1999) *Artemisia absinthium* L. (Pelin)'un Antimikrobiyal Aktivitesi. *Turkish Journal of Biology*, **23**, 377-384.

[15] Keita, S.M., Vincent, C., Schmidt, J.P. and Arnason, J.T. (2000) Insecticidal Effects of *Thuja occidentalis* (*Cupressaceae*) Essential Oil on *Callosobruchus maculatus* (*Coleoptera*: *Bruchidae*). *Canadian Journal of Plant Science*, **81**, 173-177. https://doi.org/10.4141/P00-059

[16] He, H.P., Cai, Y., Sun, M. and Corke, H. (2002) Extraction and Purification of Squalene from Amaranthus Grain. *Journal of Agricultural Food Chemistry*, **50**, 368-372. https://doi.org/10.1021/jf010918p

[17] Mehmood, S., Riaz, N., Ahmad, Z., Afza, N. and Malik, A. (2008) Lipoxygenase Inhibitory Lignans from *Salvia santolinifolia*. *Polish Journal of Chemistry*, **82**, 571-575.

[18] Rathi, M.A., Meenakshi, P. and Gopalakrishnan, V.K. (2015) Hepatoprotective Activity of Ethanolic Extract of *Alysicarpus vaginalis* against Nitrobenzene-Induced Hepatic Damage in Rats. *South Indian Journal of Biological Sciences*, **1**, 60-65. https://doi.org/10.22205/sijbs/2015/v1/i2/100420

[19] Arokiyaraj, S., Choi, S.H., Lee, Y., Bharanidharan, R., Hairul-Islam, V.I., Vijayakumar, B., Oh, Y.K., Dinesh-Kumar, V., Vincent, S. and Kim, K.H. (2015) Characterization of Ambrette Seed Oil and Its Mode of Action in Bacteria. *Molecules*, **20**, 384-395. https://doi.org/10.3390/molecules20010384

[20] Bradford, M.M. (1976) A Rapid and Sensitive Method for the Quantification of Microgram Quantities of Protein Utilizing the Principle of Protein Dye Binding. *Analytical Biochemistry*, **72**, 248-254.

[21] Santhosh, S.K., Venugopal, A. and Radhakrishnan, M.C. (2016) Study on the Phytochemical, Antibacterial and Antioxidant Activities of *Simarouba glauca*. *South Indian Journal of Biological Sciences*, **2**, 119-124. https://doi.org/10.22205/sijbs/2016/v2/i1/100358

[22] Hanato, T., Kagawa, H., Yasuhara, T. and Okuda, T. (1988) Two New Flavonoids and Other Constituents in Licorice Root: Their Relative Astringency and Radical Scavenging Effects. *Chemical and Pharmaceutical Bulletin*, **36**, 2090-2097. https://doi.org/10.1248/cpb.36.2090

[23] Oyaizu, M. (1986) Studies on Product of Browning Reaction Prepared from Glucoseamine. *Japanese Journal of Nutrition*, **44**, 307-315. https://doi.org/10.5264/eiyogakuzashi.44.307

[24] Madhuri, S. and Pandey, G. (2009) Some Anticancer Medicinal Plants of Foreign Origin. *Current Science*, **96**, 779-783.

[25] Ara, I., Bukhari, N.A., Solaiman, D. and Bakir, M.A. (2012) Antimicrobial Effect of Local Medicinal Plant Extracts in the Kingdom of Saudi Arabia and Search for Their Metabolites by Gas Chromatography-Mass Spectrometric (GC-MS) Analysis. *Journal of Medicinal Plants Research*, **6**, 5688-5694.

[26] Al-Daihan, S., Al-Faham, M., Al-shawi, N., Almayman, R., Brnawi, A., *et al.* (2012) Antibacterial Activity and Phytochemical Screening of Some Medicinal Plants Commonly Used in Saudi Arabia against Selected Pathogenic Microorganisms. *Journal of King Saud University-Science*, **25**, 115-120.

[27] Marzouk, B., Marzouk, Z., Décor, R., Mhadhebi, L., *et al.* (2010) Antibacterial and Antifungal Activities of Several Populations of Tunisian *Citrullus colocynthis* Schrad. Immature Fruits and Seeds. *Journal de Mycologie Médicale*, **20**, 179-184.

[28] Padalia, R.C., Verma, R.S., Chauhan, A., Goswami, P., *et al.* (2014) Compositional Variability and Antifungal Potentials of *Ocimum basilicum*, *O. tenuiflorum*, *O. gra-*

tissimum and *O. kilimandscharicum* Essential Oils against *Rhizoctonia solani* and *Choanephora cucurbitarum*. *Natural Product Communication*, **9**, 1507-1510.

[29] Balachandran, C., Duraipandiyan, V., Emi, N. and Ignacimuthu, S. (2015) Antimicrobial and Cytotoxic Properties of *Streptomyces* sp. (ERINLG-51) Isolated from Southern Western Ghats. *South Indian Journal of Biological Sciences*, **1**, 7-14. https://doi.org/10.22205/sijbs/2015/v1/i1/100436

[30] Sreeshma, P.S., Raphael, K.R. and Baby, A.A. (2016) Pharmacognostic Studies of Leaves of *Naravelia zeylanica* (Linn) DC. *South Indian Journal of Biological Sciences*, **2**, 179-182. https://doi.org/10.22205/sijbs/2016/v2/i1/100389

[31] Puthur, J.T. (2016) Antioxidants and Cellular Antioxidation Mechanism in Plants. *South Indian Journal of Biological Sciences*, **2**, 14-17. https://doi.org/10.22205/sijbs/2016/v2/i1/100335

[32] Nandhini, V.S. and Stella Bai, G.V. (2015) *In Vitro* Phytopharmacological Effect and Cardio Protective Activity of *Rauvolfia tetraphylla* L. *South Indian Journal of Biological Sciences*, **1**, 97-102. https://doi.org/10.22205/sijbs/2015/v1/i2/100430

[33] Cota, B.B., Bertollo, C.M. and de Oliveira, D.M. (2013) Anti-Allergic Potential of Herbs and Herbal Natural Products—Activities and Patents. *Recent Patents Endocr Metab Immune Drug Discovery*, **7**, 26-56. https://doi.org/10.2174/187221413804660935

[34] Takhi, D., Ouinten, M. and Yousfi, M. (2011) Study of Antimicrobial Activity of Secondary Metabolites Extracted from Spontaneous Plants from the Area of Laghouat, Algeria. *Advanced Environmental Biology*, **5**, 469-476.

[35] Doss, A., Vijayasanthi, M., Anand, S.P., *et al.* (2011) Screening of Antimicrobial Activity of Essential Oil and Methanol Extracts of *Citrullus colocynthis* (L.) Schrad. *South Asian Journal of Biological Science*, **1**, 7-15.

[36] Marzouk, B., Marzouk, Z., Mastouri, M., Fenina N. and Aouni, M. (2011) Comparative Evaluation of the Antimicrobial Activity of *Citrullus colocynthis* Immature Fruit and Seed Organic Extracts. *African Journal of Biotechnology*, **10**, 2130-2134.

[37] Tsuchiya, H. and Iinuma, M. (2000) Reduction of Membrane Fluidity by Antibacterial Sophoraflavanone G Isolated from *Sophora exigua*. *Phytomedicine*, **7**, 161-165.

[38] Negi, P.S. (2012) Plant Extracts for the Control of Bacterial Growth: Efficacy, Stability and Safety Issues for Food Application. *International Journal of Food Microbiology*, **156**, 7-17.

[39] Gill, A.O. and Holley, R.A. (2006) Disruption of *Escherichia coli*, *Listeria monocytogenes* and *Lactobacillus sakei* Cellular Membranes by Plant Oil Aromatics. *International Journal of Food Microbiology*, **108**, 1-9.

[40] Gill, A.O. and Holley, R.A. (2006) Inhibition of Membrane Bound ATPases of *Escherichia coli* and *Listeria monocytogenes* by Plant Oil Aromatics. *International Journal of Food Microbiology*, **111**, 170-174.

[41] Upadhyay, A., Upadhyaya, I., Kollanoor-Johny, A. and Venkitanarayanan, K. (2014) Combating Pathogenic Microorganisms Using Plant-Derived Antimicrobials: A Mini Review of the Mechanistic Basis. *BioMed Research International*, **2014**, 18. https://doi.org/10.1155/2014/761741

[42] Halliwell, B. (2007) Biochemistry of Oxidative Stress. *Biochemical Society Transaction*, **35**, 1147-1150. https://doi.org/10.1042/BST0351147

[43] Myburgh, K.H. (2014) Polyphenol Supplementation: Benefits for Exercise Performance or Oxidative Stress. *Sports Medicine*, **1**, 57-70. https://doi.org/10.1007/s40279-014-0151-4

[44] Blokhina, O., Virolainen, E. and Fagerstedt, K.V. (2003) Antioxidants, Oxidative Damage and Oxygen Deprivation Stress: A Review. *Annals of Botany*, **9**, 179-194. https://doi.org/10.1093/aob/mcf118

[45] Kasote, D.M., Katyare, S.S., Hegde, M.V. and Bae, H. (2015) Significance of Antioxidant Potential of Plants and Its Relevance to Therapeutic Applications. *International Journal of Biological Sciences*, **11**, 982-991. https://doi.org/10.7150/ijbs.12096

[46] Yoshikawa, M., Murakami, T., Komatsu, H., Murakami, N., Yamahara, J. and Matsuda, H. (1997) Medicinal Foodstuffs: IV. Fenugreek Seeds. (1): Structures of Trigoneosides Ia, Ib, IIb, IIIa, and IIIb, New Furostanol Saponins from the Seeds of Indian *Trigonella foenum* graecum L. *Chemical and Pharmacology Bulletin*, **45**, 81-87. https://doi.org/10.1248/cpb.45.81

[47] Nirmala, P. and Ramanathan, M. (2011) Effect of Myricetin on 1,2 dimethylhydrazine Induced Rat Colon Carcinogenesis. *Journal of Experimental Therapeutics and Oncology*, **9**, 101-108.