EVALUATION OF ANTIOXIDANT, ANTIMICROBIAL, AND ANTFUNGAL POTENTIAL OF *CUCURBITA PEPO* VAR. FASTIGATA SEED EXTRACTS

ROSHNI RS SONI*, MANOJ BAI

1Department of Applied Sciences, Quest Group of Institutions, Jhanjeri, IKG-Punjab Technical University, SAS Nagar, Punjab, India.
2Baba Hira Singh Bhattal Institute of Engineering and Technology, Lehragaga, IKG-Punjab Technical University, Sangrur, Punjab, India.

Email: roshmanjot@gmail.com

Received: 20 April 2018, Revised and Accepted: 27 October 2018

ABSTRACT

Objective: The current study aims to study the antioxidant and antimicrobial and antifungal potential of the methanolic extract of *Cucurbita pepo* var. fastigata seeds (MECS).

Methods: Extraction of the seeds has been carried out with solvents of increasing polarity (chloroform, acetone, and methanol) and the phytochemical study of the methanolic extract have been carried out using standard methods. The free radical scavenging activity of all the extracts was evaluated by DPPH and \( \text{H}_2\text{O}_2 \) methods. Standard disk diffusion method was used to evaluate antibacterial and antifungal activities.

Results: Phytochemical evaluation showed the maximum presence of triterpenoids, phenolic compounds, tannins and small amount of Coumarins. Methanolic extract revealed momentous antioxidant activity as compared to chloroform and ethyl acetate extract. Hence, methanolic extract of *C. pepo* seeds (MECS) at a dose level of 100, 200 and 300 \( \mu \text{g/ml} \) was evaluated for antioxidant potential. Maximum free radical scavenging activity of methanolic seed extract of cucurbita pepo var. fastigata has been found at a dose of 300 \( \mu \text{g/ml} \) to be 63±0.16 % by 1,1-diphenyl-2-picryl hydrazyl model and at a value of 78% at 300 \( \mu \text{g/ml} \) with \( \text{H}_2\text{O}_2 \) model. Methanolic extract also showed the presence of antibacterial activity.

Conclusion: Presence of phytochemicals in the methanolic extract is responsible for the antioxidant potential. Extracts were investigated for antibacterial activity using the standard disc diffusion assay method against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* and for the antifungal activities against *Aspergillus niger* and *Candida albicans*. The seed extract showed the presence of antibacterial activity, but the antifungal activity was found to be absent in the extract.

Keywords: Antimicrobial, Antioxidant, *Cucurbita pepo* var. fastigata, Free radical scavenging.

INTRODUCTION

According to the WHO, medicinal plants as therapeutic aids have momentous role in curing and maintaining appropriate health [1-3]. Nature has been practicing combinational chemistry for eons [4] and numerous natural products and synthetically modified natural product have been developed for treatment [5]. The structural analysis and the ability to amalgamate them permitted chemists to amend them to repress or enhance their solubility and efficiency [6]. The ethanolic and aqueous extract of *Cassia fistula* Linn. shows the presence of various antioxidants such as kaempferol, gallic acid, ellagic acid, coumaric acid, rutin, myricetin, and quercetin [7].

The *Cucurbita pepo* var. fastigata belongs to the Cucurbitaceae family which consists of 130 genera and 800 species. Some of the important plants are *Momordica charantia*, *C. pepo*, *Cucurbita andreana*, *Cucurbita ficifolia*, *Cucumis sativus*, *Cucumis melo*, *Citrullus colocynthis*, *Luffa echinata*, *Trichosanthes kirilowii*, *Lagenaria siceraria*, and *Benincasa hispida* [8]. Literature reveals the traditional use of the various parts of the plant by Ayurvedic & Chinese systems as anti-inflammatory, analgesic, anti-diabetic, anti-tumor, and antioxidant. The fruit is used to cure fatigue & thirst, acts as a blood purifier; treats cold. The seeds are diuretic, helpful in headaches neuralgia, bronchitis, fever, Gastritis, burns, febrile diseases, irritable bladder & prostatic complaints. The seeds are also beneficial to spleen, lungs and act as a tonic. The leaves are used for the treatment of nausea & helps to boost haemoglobin [9]. Oil obtained from *C. pepo* seeds is recommended in nutritional and medicinal purpose as it acts as a potential drug able to heal wounds in animal [10]. *C. pepo* is chiefly known for its enhancement in prostatic hyperplasia (benign prostatic hyperplasia) [11], urinary dysfunction, and cytotoxic properties and also has also been used widely as a hypoglycemic agent. Many pharmacological studies have established hepatoprotection, restrain benign prostatic hyperplasia, antioxidant, anticancer, anti-inflammatory, anti-diabetic, and anti-tumor activities supporting its customary uses [9,12]. The seeds, in addition to their roles as food additives and supplements, may also be used as an efficient and economical source of antibacterial agents for the treatment of bacterial infections [13]. The methanolic, chloroform, and ethyl acetate extracts of *C. pepo* fruits significantly reduced the paw swelling in mice dose dependently suggesting the immunomodulatory effects of extracts and can, therefore, act as immunonutrient [14,15]. The natural plant components found in pumpkin could recover the liver against alcohol-induced liver toxicity and oxidative stress in rats [16]. The cell growth inhibition of prostate, breast, and colon cancer cells by ethanolic seed extract authenticates the ethnomedical use of pumpkin seeds for a treatment of benign prostate hyperplasia [17].

The adequate ROS & RNS is vital for the efficient immune response as the imbalance between the production of oxidants/RONS and their removal by antioxidants lead to increased accumulation and, hence, the condition of oxidative stress [18]. Oxidative stress has been recognized as the major reason of the development and progression of numerous ailments. Supplements rich in exogenous antioxidants or boosting endogenous antioxidant defenses of the body are a capable way of diminishing the unwanted effects of ROS-induced oxidative damage. Plants have an innate ability to biosynthesize a wide range of non-enzymatic antioxidants capable of attenuating ROS-induced oxidative damage [19]. Antioxidant defense system plays a key role to overcome...
different diseases caused as a result of free radicals by neutralizing the excess of free radicals. An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage. These antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property [20]. However antioxidant, antimicrobial and antifungal studies have not yet been reported for the seeds of Cucurbita pepo var. fastigata extract. So the present study has carried out to evaluate the antifungal, antimicrobial and antioxidant potential of Cucurbita pepo var. fastigata seeds.

**METHODS**

**Chemicals**

1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydrogen peroxide were procured from Hi-Media. Carrageenan and ascorbic were obtained from Jackson Laboratories, Amritsar, Punjab. The solvents such as hexane, chloroform, ethyl acetate, and methanol were of analytical grade and procured from SD Fine Chemical.

**Plant material**

The seeds of C. pepo var. fastigata were bought from Local Market of Kharar (PB)/Roopnagar (PB)/Chandigarh (UT) and Delhi, 2013. The seeds were authenticated by Prof. Satwinderjeet Kaur and the letter vide Kharar (PB)/Roopnagar (PB)/Chandigarh (UT) and Delhi, 2013. The seeds were cleaned, washed, dried for 2 days, and crudely powdered in a grinder at room temperature. The sample was kept in light-protected air-tightened containers.

**Extraction**

The powdered seed material was subjected to defatting i.e., removal of fats using hexane, and then, extraction was carried out using solvents of increasing polarity such as chloroform, acetone, and methanol by cold maceration process for 24 h. The solvents were completely removed by rotary evaporator and crude extracts were obtained and stored in the refrigerator. These extracts were further used for evaluation of their antioxidant, antibacterial, and antifungal activities.

**Phytochemical screening**

Standard procedures for preliminary phytochemical screenings of the extracts were carried out to analyze the presence of various constituents. The extracts obtained were analyzed for flavonoids, tannins, alkaloids, triterpenes, sterols, proteins, carbohydrates, and amino acids. The identification was done on the basis of change for the respective components [21,22]. Further, seclusion and characterization of pure compounds from the extract are in progress.

**Antioxidant activity**

*Qualitative scavenging activity on DPPH radical*

The qualitative assays were accomplished according to simple screening method for antioxidants [23]. Dilution of 2 mg of each extract was done with 1 ml of the suitable solvent, following which a small quantity of each dilution was cautiously laden onto the baseline of the 20 cm by 10 cm TLC plates, and the sample was allowed to dry for some time. Hexane-ethyl acetate in ratio of 7:3 was used as the mobile phase. The dried plates were sprayed with a 0.2% solution of DPPH in ethanol. The extracts having antioxidant constituent displayed a yellow on purple spot due to the discoloration of DPPH [24].

**Quantitative scavenging activity on DPPH radical**

Antioxidant potential of methanolic seed extract of C. pepo var. fastigata was evaluated by 1, 1-diphenyl-2-picryl hydrazyl radical scavenging activity. The reduction capability of 1, 1-diphenyl-2-picryl hydrazyl radical was determined by the decrease in its absorbance at 517 nm. DPPH radical is scavenged by antioxidants through the donation of a proton, which forms the reduced DPPH and lead to decrease in absorbance at a wavelength of 517 nm [25]. Ascorbic acid was used as standard and blank was used to remove the influence of the color of the samples. A methanolic solution of DPPH was used as negative control.

**Table 1: Phytochemical screening of C. pepo var. fastigata seeds extracts/isolated comp**

| Plant constituent/test | C. pepo var. fastigata |
|------------------------|------------------------|
| Alkaloids              | −                      |
| Carbohydrates          | −                      |
| Phytosterols           | −                      |
| Phenolic compounds and tannins | ++                  |
| Triterpenoids          | +++                    |
| Flavonoids             | +                      |
| Coumarins              |                        |

*C. pepo: Cucurbita pepo*

**Table 2: DPPH scavenging activity by metabolic seed extract of Cucurbita pepo var. fastigata.**

| Concentration (µg/ml) | % age scavenging |
|-----------------------|------------------|
|                       | Methanol extract | Ascorbic acid   |
| 100                   | 45.73±0.27       | 50.23±0.49      |
| 200                   | 58.72±0.45       | 64.74±0.59      |
| 300                   | 76.35±0.52       | 82.88±0.87      |

Values are the average of triplicate experiments and represented as mean±standard error of the mean.

**Table 3: Antioxidant activity of metabolic seed extract of Cucurbita pepo var. fastigata by hydrogen peroxide method**

| Concentration (µg/ml) | Absorbance (nm) | Mean | % age scavenging |
|-----------------------|-----------------|------|------------------|
|                       | Methanol extract | Ascorbic acid |
| 100                   | 0.273           | 0.274| 20.17±0.27       |
|                       | 0.275           |      | 44.43±0.26       |
| 200                   | 0.205           | 0.210| 40.05±0.11       |
|                       | 0.210           |      | 55.03±0.46       |
| 300                   | 0.102           | 0.106| 70.17±0.14       |
|                       | 0.110           |      | 72.17±0.32       |

Values are the average of triplicate experiments and represented as mean±standard error of the mean.

*Fig. 1: Inhibition of bacterial growth of the methanolic extract of Cucurbita pepo. seeds by disc diffusion method*
Table 4: Antibacterial activity of methanolic seed extract of *Cucurbita pepo* var. *fastigata* by disk diffusion method

| S. No | Component          | Concentration (µg/ml) | Zone of inhibition for bacteria |
|-------|-------------------|-----------------------|--------------------------------|
|       |                   |                       | *B. subtilis* | *E. coli* | *S. aureus* | *P. aeruginosa* |
| 1     | *C. pepo* var. *fastigata* | 50        | Resistance | Resistance | Resistance | Resistance |
|       |                   | 75        | Resistance | Resistance | Resistance | Resistance |
|       |                   | 100       | Resistance | Resistance | Resistance | Resistance |
|       |                   | 150       | Resistance | Resistance | Resistance | Resistance |

Table 5: Antifungal activity of methanolic seed extract of *Cucurbita pepo* var. *fastigata* by disk diffusion method

| S. No | Component  | Concentration (µg/ml) | Zone of inhibition for fungus |
|-------|------------|-----------------------|------------------------------|
|       |            |                       | *A. niger* | *C. albicans* |
| 1     | *C. pepo* var. *fastigata* | 50        | Resistance | Resistance |
|       |            | 75        | Resistance | Resistance |
|       |            | 100       | Resistance | Resistance |
|       |            | 150       | Resistance | Resistance |

Table 5: Antifungal activity of methanolic seed extract of *Cucurbita pepo* var. *fastigata* by disk diffusion method

| S. No | Component  | Concentration (µg/ml) | Zone of inhibition for fungus |
|-------|------------|-----------------------|------------------------------|
|       |            |                       | *A. niger* | *C. albicans* |
| 1     | *C. pepo* var. *fastigata* | 50        | Resistance | Resistance |
|       |            | 75        | Resistance | Resistance |
|       |            | 100       | Resistance | Resistance |
|       |            | 150       | Resistance | Resistance |

Tests were carried out in triplicate. Percentage inhibition was evaluated by using the equation 1.

\[
\text{I(\%)} = \frac{(A_s-A_t)}{A_s} \times 100
\]  

Where, \(A_s\) = values for the absorbance of the negative control. \(A_t\) = The absorbance of the sample [26].

Free radical scavenging activity of metabolic seed extract by hydrogen peroxide method

Hydroxyl radical formation can occur in several ways by far the most important mechanism in vitro is the Fenton reaction where a transition metal involved as a prooxidant in the catalyzed decomposition of superoxide and hydrogen peroxide [27]. Hydroxyl radicals are the most reactive and predominant radical generated endogenously during aerobic metabolism among the ROS which could be formed from superoxide anion and hydrogen peroxide, in the metal ions, such as copper or iron and cause aging of human and some diseases [28,36]. The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells [30]. In the present study, the seed extract was evaluated for their hydroxyl radical scavenging activity.

Antibacterial activity

Extracted dilution was prepared in 50 µL, 75 µL, 100 µL, and 150 µL [31]. Required glassware was washed and dried in a hot air oven. The sterilized agar medium was transferred into the Petri dishes and was allowed to solidify at room temperature. The selected test organism (bacterial) was spread over the solidified agar with the help of a swab stick. Sterile borer was used to make wells of 8 mm diameter. The dilutions of Extracted sample (50 µL, 75 µL, 100 µL, 150 µL) were done in the wells with the help of a sterile syringe needle. The Petri plates were placed in a refrigerator for 5 min to allow diffusion. Later, the Petri plates were incubated in inverted position at 37°C for 24 h in the incubator. After 24 h, the zone of inhibition was observed and diameter in mm was measured and recorded.

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical evaluation revealed that the methanolic extract of *Cucurbita pepo* var. *fastigata* seeds showed maximum presence of triterpenoids, phenolic compounds, tannins and small amount of Coumarins (Table 1), due to which it was further subjected to in vitro antioxidant studies. Polyphenolic compounds, triterpenoid and steroid found in plants, have been reported to have multiple biological effects including antioxidant activity [32-35].

Qualitative DPPH radical scavenging activity

DPPH method was used to estimate the antioxidant activity of the *C. pepo* seed's extracts. The qualitative DPPH radical scavenging activity was demonstrated due to change in the coloration from purple to yellow on the TLC plate by the extract.

Quantitative DPPH radical scavenging activity

In quantitative estimation, DPPH radical was used as a substrate to evaluate the free radical scavenging activity of the methanolic extract of *C. pepo* var. *fastigata* was shown at a dose of 300 µg/ml is 63.0±16% by 1,1-diphenyl-2-picryl hydrazyl model as shown in Table 2.

Free radical scavenging activity of metabolic seed extract by hydrogen peroxide method

Hydroxyl radical formation can occur in several ways by far the most important mechanism in vitro is the Fenton reaction where a transition metal involved as a prooxidant in the catalyzed decomposition of superoxide and hydrogen peroxide [27]. Hydroxyl radicals are the most reactive and predominant radical generated endogenously during aerobic metabolism among the ROS which could be formed from superoxide anion and hydrogen peroxide, in the metal ions, such as copper or iron and cause aging of human and some diseases [28,36]. The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells [30]. In the present study, the seed extract was evaluated for their hydroxyl radical scavenging activity. Maximum free radical scavenging activity of methanolic seed extract of *C. pepo* var. *fastigata* was shown at a dose of 400 µg/ml is 78% by *H. O₂* model as shown in Table 3.

Antibacterial activity

It is evident from the data presented in Table 4 that the sample possesses antibacterial activity. Disk diffusion method showed the resistance of the seed extract to *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* at a concentration of 50 µg/ml and toward *E. coli* and *P. aeruginosa* at a concentration of 75 µg/ml, 100 µg/ml, and 150 µg/ml. It implies that the isolates with a minimum inhibitory concentration at or above or zone diameters at or below the resistant breakpoint are not inhibited by the usually achievable concentration of the agent with normal dosage schedules [36]. As shown in Fig. 1, the largest zones of inhibition were observed in *C. pepo* seed extract against *B. subtilis* 75 µl/ml (14 mm), 100 µl/ml (16 mm), and 150 µg/ml (19 mm) and *S. aureus* 75 µg/ml (13 mm), 100 µg/ml (14 mm), and 150 µg/ml (15 mm). The extract showed high activity against *B. subtilis* and *S. aureus* species.

Then, it is evident from the data presented in Table 5 that the sample does not possess antifungal activity. The disc diffusion method result showed the resistance of the *C. pepo* seed extract toward both *Aspergillus niger* and *Candida albicans* at all the four concentrations.
DISCUSSION

Oxidative stress and microbial infections pose a serious health problem around the world & plants have been an inseparable source of valuable natural products since these are potential source of antioxidants and antimicrobial agents [37]. World Health Organization (WHO) advocates the medicinal plants as the best source of diverse range of drugs and active compounds. Therefore investigations are required in order to explore their properties and understand their safety and efficiency [38]. The present study reports the antioxidant, antibacterial and antifungal activities of methanolic extract of Cucurbita pepo seeds. The evaluation of the antioxidant potential was done using DPPH and ABTS (qualitative and quantitative methods). Hydroxyl radical formation can occur in several ways by far the most important mechanism in vitro is the fenton reaction where a transition metal involved as a prooxidant in the catalyzed decomposition of superoxide and hydrogen peroxide [27]. Hydroxyl radicals are the most reactive and predominant radical generated endogenously during aerobic metabolism among the ROS which could be formed from superoxide anion and hydrogen peroxide, in the metal ions, such as copper or iron and cause ageing of human and some diseases [29,39]. The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells [30]. Both the results thus established the therapeutic potential against oxidative stress. The reason for the antioxidant activity is the presence of phenolic compounds, tannins and triterpenoids. The antimicrobial screening was performed for B. Subtilis, E. Coli, S. aureus & P. aeruginosa at a concentration of 50 µg/ml and towards E. Coli & P. aeruginosa at a concentration of 75 µg/ml, 100 µg/ml &150 µg/ml. Disk diffusion method showed the resistance of the seed extract in all the samples. The Cucurbita pepo var. fastigata seed extract showed high activity against B. subtilis and S. aureus species. The sample do not possess antifungal activity. However no zone of inhibition against antifungal organism was found hence the disc diffusion method results showed the resistance of the Cucurbita pepo seed extract towards both Aniger and Calibicans at all the four concentrations. The insight into the inactivity of the extra against Aniger and Calibicans will require further investigation. The growing bacterial resistant towards antibiotics has become a matter of great concern for researchers’ worldwide [40]. The antibiotic resistant bacteria have been considered as a major problem by intensive care physicians in the treatment of patients [41]. The increase in bacterial resistant has prompted researchers to explore the antimicrobial role of natural herbs against resistant strains [42, 43]. Many infectious diseases caused by resistant microbes can be treated by seed extracts having potential antimicrobial compounds. The results from our study have shown extremely strong activity in the seed extracts of Cucurbita pepo. Traditionally, herbal medicine is used by folklore for the treatment of various infectious diseases. Although most of the cases are not evaluated scientifically but the chemical constituents of even the simplest medicinal preparations are beneficial [44]. Hence the seed extracts offer an ample potential for the development of novel agents effective against infections that are presently difficult to treat.

CONCLUSION

On the basis of the results of the above study, it can be concluded that the methanolic extract of Cucurbita pepo var. fastigata seeds possess noticeable antioxidant and antibacterial activity. The Cucurbita pepo var. fastigata seed extract showed high activity against B. subtilis and S. aureus species, but they have shown no zone of inhibition against antifungal organism which shows that the methanolic extract of seeds does not possess antifungal activity. However, further, investigations are required to comprehend the precise mechanisms of action and isolation of the compound(s) accountable for such activities.

AUTHORS’ CONTRIBUTION

Roshni R.S. Soni - Conceived idea of the study, participated in its design, performed laboratory work and coordinated and helped to draft the manuscript, and also performed statistical analysis. Manoj Bali - Participated in the sequence alignment and drafted the manuscript & Supervised the study from conceiving of idea to drafting of manuscript.

CONFLICTS OF INTEREST STATEMENT

We declare that we have no conflicts of interest.

REFERENCES

1. Vinale F, Sivashithamparam K, Ghisalberti EL, Woo SL, Nigro M, Marra R, et al. Trichoderma secondary metabolites active on plants and fungal pathogens. Open Mycol J 2014;8:127-39.
2. Sofowora A. African Medicinal Plants. Ile-Ife (Nigeria): University of Ife Press; 1984.
3. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. Ibadan (Nigeria): Spectrum Books Ltd.; 1993.
4. Chesney JD, Venkataraman SK, Henri JT. Plant natural products: Back to the future or into extinction? Phytochemistry 2007;68:2015-22.
5. Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 years. J Nat Prod 2007;70:461-77.
6. Newman DJ. Natural products as leads to potential drugs: An old process or the new hope for drug discovery? J Med Chem 2008;51:2589-99.
7. Abid R, Mahmood R, Rajesh KP, Swamy BE. Potential in vitro antioxidant and protective effect of cassis fistula linn: Fruit extracts against induced oxidative damage in human erythrocytes. Int J Pharm Res Pharm Sci 2014;6:497-505.
8. Dhiman K, Gupta A, Sharma DK, Gill NS, Goyal A. A review on the medicinally important plants of the family Cucurbitaceae. Asian J Clin Nutr 2012;4:16-26.
9. Martha R, Gutierrez P. Review of Cucurbita pepo (Pumpkin) its phytochemistry and pharmacology. Med Chem 2016;6:12-21.
10. Bardaa S, Halima N, Alou F, Ben Mansour R, Jabeur H, Bouaziz M, et al. Oil from pumpkin (Cucurbita pepo L) Seeds: Evaluation of its functional properties on wound healing in rats. Lipids Health Dis 2016;15:73.
11. Abdel-Rahman MK. Effect of pumpkin seed (Cucurbita pepo L.) Diets on Benign Hyperplasia Hyperplasia (BPH ): Chemical and morphometric evaluation in rats. World J Chem 2006;1:13-40.
12. Ratnam N. A review on Cucurbita pepo. Int J Pharmacogn Phytocchem Res 2017;9:1190-94.
13. Obi RK, Nwanedu FC, Nduhuisi UU, Orji NM. Antibacterial qualities and physicochemical screening of the oils of Cucurbita pepo and Brassica nigra. J Med Plants Res 2009;3:429-32.
14. Jafarian A, Zolfaghari B, Parnianfar M. The effects of methanolic, chloroform, and ethylacetate extracts of the Cucurbita pepo. On the delay type hypersensitivity and antibody production. Res Pharm Sci 2012;7:217-24.
15. Immaculata M, Issanu M, Anne C, Dass S. Development of immunonutrient from pumpkin (Cucurbita moschata Duchense Ex. Lamk.) Seed. Procedia Chem 2014;13:105-11.
16. Sayed H, Seif A. Ameliorative effect of pumpkin oil (Cucurbita pepo L) against alcohol-induced hepatotoxicity and oxidative stress in albino rats. Beni Suef Univ J Basic Appl Sci 2014;3:178-85.
17. Medjakovic S, Hohger S, Ardidom-Maekert K, Bucar F, Jungbauer A. Pumpkin seed extract: Cell growth inhibition of hyperplastic and cancer cells, independent of steroid hormone receptors. Fitoterapia 2016;110:150-6.
18. Salman KS, Ashraf S. Reactive oxygen species: A link between chronic inflammation and cancer. Asia Pac J Mol Biol Biotechnol 2013;21:41-9.
19. Kasote DM, Katryar SS, Hegde MV, Bae H. Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int J Sci Biotech 2015;11:982-91.
20. Halliwell B. How to characterize an antioxidant: An update. Biochem Soc Symp 1995;61:73-101.
21. Edeoga HO, Okwu DE, Mboebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol 2005;4:685-88.
22. Harborne JB. Phytochemical Methods a Guide to Modern Techniques of Plant Analysis. 3rd ed. Netherlands: Springer; 1998.
23. Takao T, Kritiani F, Watanabe N, Yagi A, Sakata K.A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish. Biosci Biotechnol Biochem 1994;58:1780-3.
24. Motlhanka DM. Free radical scavenging activity of selected medicinal plants of Eastern Botswana. Pak J Biol Sci 2008;11:805-8.
25. Chang MJ, Ho YL, Huang GJ, Lin IH. Study on the antioxidant activities of crude extracts from the roots of *Arnebia euchroma* and *Lithospermum erythrorhizon*. Mid Taiwan J Med 2008;13:113-21.

26. Dutra RC, Leite MN, Barbosa NR. Quantification of phenolic constituents and antioxidant activity of *Pterodon emarginatus* Vogel seeds. Int J Mol Sci 2008;9:606-14.

27. Stols SJ, Bagchi D. Oxidative mechanisms in the toxicity of metal ions. Free Radic Biol Med 1995;18:321-36.

28. Walling C. Fenton’s reagent revisited. Acc Chem Res 1975;8:125-31.

29. Siddhuraju P, Manian S. The antioxidant activity and free radical-scavenging capacity of dietary phenolic extracts from horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) Seeds. Food Chem 2007;105:950-8.

30. Hochstein P, Atallah AS. The nature of oxidants and antioxidant systems in the inhibition of mutation and cancer. Mutat Res 1988;202:363-75.

31. Bauer AW, Kirby WM, Sherris JC, Turek M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966;45:493-6.

32. Shad AA, Ahmad S, Ullah R, AbdEl-Salam NM, Fouad H, Rehman NU, et al. Phytochemical and biological activities of four wild medicinal plants. Sci World J 2014;2014:857363.

33. Nyakudya E, Jeong JH, Lee NK, Jeong YS. Platycosides from the roots of *Platycodon grandiflorum* and their health benefits. Prev Nutr Food Sci 2014;19:59-68.

34. Gil MI, Ferreres F, Tomás-Barberán FA. Effect of postharvest storage and processing on the antioxidant constituents (Flavonoids and Vitamin C) of fresh-cut spinach. J Agric Food Chem 1999;47:2213-7.

35. Esmaeili A, Mousavi Z, Shokrollahi M, Shafaghat A. Antioxidant activity and isolation of luteoline from *Centaurea behen* grown in Iran. J Chem 2013;2013:1-5.

36. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. CLSI Supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.

37. Singh AR, Bajaj VK, Sekhawat PS, Singh K. Phytochemical estimation and antimicrobial activity of aqueous and methanolic extract of *Ocimum sanctum* L. J Nat Prod Plant Resour 2013;3:51-8.

38. Motiana RA, Gruenert R, Bednarski P, Lindequist U. Evaluation of the *in vitro* anticancer, antimicrobial and antioxidant activities of some Yemeni plants used in folk medicine. Pharmazie 2009;64:260-8.

39. Siddhuraju P, Becker K. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata*) Seed extracts. Food Chem 2007;101:10-9.

40. Gardam MA. Is methicillin-resistant *Staphylococcus aureus* an emerging community pathogen? A review of the literature. Can J Infect Dis 2000;11:202-11.

41. Lepape A, Monnet DL, participating members, European Society of Intensive Care Medicine. Experience of European intensive care physicians with infections due to antibiotic-resistant bacteria, 2009. Euro Surveill 2009;14:19393.

42. Alviano DS, Alviano CS. Plant extracts: Search for new alternatives to treat microbial diseases. Curr Pharm Biotechnol 2009;10:106-21.

43. Hemaiswarya S, Kruhiventi AK, Doble M. Synergism between natural products and antibiotics against infectious diseases. Phytotherapy 2008;15:639-52.

44. Barnes J, Anderson LA, Phillipson JD. Herbal Medicine. 3rd ed. London: Pharmaceutical Press; 2007.