International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2017.481.487

Research Article
Phytochemical Studies, Antioxidant Properties and Antimicrobial Activities of Herbal Medicinal Plants Costus and Cidir Used in Saudi Arabia

[1]Hanan Saeed Alnahdi, [1,2]Enas Nabil Danial, [1]Manal Elsayed Abd Elgaffar Elhalwagy and [1]Najla Othman Ayaz

[1]Department of Biochemistry, Faculty of Science-Al Faisaliah, King Abdulaziz University, P.O. Box 51459, 21453 Jeddah, Saudi Arabia
[2]Department of Chemistry of Natural and Microbial Products, Division of Pharmaceutical Industries, National Research Centre, Cairo, Egypt

Abstract

Background and Objective: Greater use of antibiotics has led to high incidence of resistant bacterial strains infections and associated with increased side effects. The medicinal effect of each of costus and cidir was used as traditional medicine in Saudi Arabia. The objective of this study was to evaluate antioxidant, total phenolic content and enhancement effect of *Saussurea lappa* and *Ziziphus spina-christi* on probiotic bacteria. **Methodology:** Total phenolic content, free radical scavenging, antioxidant as well as antibacterial and antifungal activities of costus (*Saussurea lappa*) and cidir (*Ziziphus spina-christi*) were evaluated in their aqueous extract. Enhancement of probiotic bacteria was investigated by culturing on De Man Rogosa and Shapes Agar Media. **Results:** The aqueous extract of costus (*Saussurea lappa*) is richer with phenolic compounds (80 mg g^{-1} dry weight) than cidir (62 mg g^{-1} dry weight) which induce pronounced antioxidant and anti-scavenging effect. Costus aqueous extract recorded enhancement effect on the growth rate of probiotic bacteria (*Lactobacillus acidophilus*, *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus casei* and *Lactobacillus jensenii*) than cidir (*Ziziphus spina-christi*). It also inhibited the growth of pathogenic Gram +ve bacteria (*Staphylococcus aureus, Sarcina lutea* and *Bacillus subtilis*), Gram -ve bacteria (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*) and fungi (*Aspergillus flavus, Aspergillus niger* and *Candida albicans*). However, cidir (*Ziziphus spina-christi*) recorded remarkable effect against *Aspergillus niger* fungal strains. **Conclusion:** Each of the two extracts has pronounced effect on certain organisms that can be used as precursor for pharmaceutical drug.

Key words: Total phenolic compounds, antioxidants, antimicrobial, antifungal, probiotic bacteria

Received: January 13, 2017 Accepted: May 03, 2017 Published: June 15, 2017

Citation: Hanan Saeed Alnahdi, Enas Nabil Danial, Manal Elsayed Abd Elgaffar Elhalwagy and Najla Othman Ayaz, 2017. Phytochemical studies, antioxidant properties and antimicrobial activities of herbal medicinal plants costus and cidir used in Saudi Arabia. Int. J. Pharmacol., 13: 481-487.

Corresponding Author: Enas Nabil Danial, Department of Chemistry of Natural and Microbial Products, Division of Pharmaceutical Industries, National Research Centre, Cairo, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Saussurea lappa is described as erect, robust, pubescent, perennial herb, roots are stout, dark brown or grey, up to 40 cm long[1]. *Saussurea lappa* Clarke is commonly known as kuth root or costus. The main active compounds are costunolide, dehydrocostus lactone, caustic, palmitic, linoleic acids, β-sitosterol, cyclocostunolide, alantolactone and isoalantolactone[2], which have hypolipidemic, hypoglycemic, immunomodulatory, anti-inflammatory, antimicrobial and antiparasitic medicinal effects[3]. The inhibitory effects of *Saussurea lappa* in ethanol extract on the growth of *Streptococcus mutans* in a dose dependent manner was recorded[3]. *Saussurea lappa* was used in the treatment of dental diseases as it is effective against caries-inducing *Streptococcus mutans*[4]. Ethanolic extract of the roots of *Saussurea lappa* has antioxidant activity through inhibition of nitric oxide production in lipopolysaccharide activated mouse peritoneal macrophages isolated four novel acylated flavonoid glycosides from the roots of *Saussurea lappa*. These compounds have antimicrobial activity against the *Bacillus subtilis*, *Staphylococcus aureus*, Gram-negative bacteria, *Pseudomonas aeruginosa* and *Escherichia coli*. The isolated compounds and their mixture revealed antifungal effect against *Cladosporium cladosporioides*, *Aspergillus ochraceus*, *Aspergillus niger*, *Aspergillus versicolor*, *Penicillium ochrochloron*, *Penicillium funiculosum*, *Aspergillus flavus*, *Trichoderma viride* and *Alternaria alternata*[5]. Also, roots extract of *Saussurea lappa* tested showed excellent antibacterial activity against *B. subtilis*, *E. faecalis*, *S. aureus, P. aeruginosa* and *S. typhi*[6,7].

The local arabic name(s) of *Ziziphus spina-christi* is cidir or Nabka[8]. Cidir essential oil contains linalool (11.5%) and alpha-terpineol (16.4%). The major hydrocarbons were n-pentacosane with (81%), also methyl esters were found in leaves in the form of methyl stearate, methyl palmitate and methyl myristate. Maslinic acid, β-sitosterol and oleanolic acid were the main aglycones of the glycosides also present. Leaf sugars reported were lactose, galactose, glucose, arabinose, rhamnose and xylose[9]. Also the plant contains four saponin glycosides[10]. Flavonoid was found in the leaves (0.66%)[11]. Both bacteria and fungi are affected by *Ziziphus spina-christi*[12]. *Zizyphus spina-christi* was also reported to have free radical scavenging activity[13]. Plant leaves were used in medicine as an anti-inflammatory, antiseptic and antifungal agent for diseases of skin[14]. The saponin fraction of the leaves has an antimicrobial activity against *Candida albicans*[15]. Phytochemical analysis of the crude extracts of the ethanol and water of the leaves of the *Ziziphus spina-christi* declared the presence of alkaloids, tannins, saponins, glycosides, flavonoids, steroids and terpenoids[16]. On the other hand, the antibacterial activity of water and ethanol extract of the leaves of *Ziziphus spina-christi* shows inhibition of *S. typhi*, *S. aureus* and *Shigella*, but was inactive on *E. coli* and *P. aeruginosa*. These plants were used extensively in traditional medicine in Saudi Arabia. Therefore, objective of this study was to investigate the antioxidant, total phenolic content and enhancement effect on probiotic bacteria by the two selected plants, *Saussurea lappa* and *Ziziphus spina-christi*. Antimicrobial effect on different kinds of pathogenic microbes was also investigated.

MATERIALS AND METHODS

Plant sources: Two kind of medicinal plants were evaluated for antioxidant, antimicrobial and total phenolic content. Samples were obtained from the local herbal market of Kingdom Saudi Arabia. Voucher specimens from plant materials were deposited at the Herbal Museum, Department of Pharmacology, Faculty of science, King Abdulazziz University of Medical Sciences, for identification. Fifteen grams of the dried plants were grounded well to fine powder in electrical grinder (ANEX AG-694). Samples were stored in bottles for further studies at 10°C until analyzed.

Extraction: Ten grams of each powdered plants was soaked in 100 mL of water in a shaker at 70°C for 1 h stirring. The suspension was subsequently filtered through Whatman No. 1 filter paper. The resulting final solutions obtained were stored in a freezer at -4°C as a stock solution until use for determination the total phenolic content, antioxidant and antimicrobial activities[17].

DPPH radical scavenging assay: The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay was carried out spectrophotometrically. Aliquots (50 µL) of various plant extracts were added to 5 mL of 0.004% ethanol solution of DPPH. After incubating the samples for 30 min at room temperature, the absorbance was read against a blank at 517 nm[18]:

$$I(\%) = \frac{1- AS}{AC} \times 100 \quad (1)$$

where, AC is the absorbance of the control reaction (containing all reagents except the tested compound) and AS is the absorbance of the tested compound. The percentage of

inhibition was determined from a graph plotting percentage inhibition against extract concentration. All experiments were performed in duplicate.

Total phenolic contents: Total phenolic contents of each plant extract were determined using the Folin-Ciocalteu reagent. The reaction mixture contained 1 mL of plant extract, 0.5 mL of the Folin-Ciocalteu reagent, 0.75 mL of 20 g/100 mL sodium carbonate and 3 mL of pure water. The mixture was heated in a water bath at 40°C for 20 min and then cooled. The absorbance at 765 nm was measured with spectrophotometer (Hitachi U-2001, model 121-0032) and used to calculate the phenolic contents using gallic acid as a standard. The total phenolic contents were then expressed as GAE mg g^{-1} dry sample. All experiments were performed in duplicate[19].

Antioxidant activity determination in linoleic acid system: Antioxidant activity of extracts was determined by measuring the percentage inhibition of peroxidation in a linoleic acid system[20]. Approximately 1 mL of each extract was added to a solution containing linoleic acid (0.13 mL), 99.8% ethanol (10 mL) and 0.2 M sodium phosphate buffer, pH 7, (10.0 mL). The resulting mixture was then diluted to 25.0 mL with distilled water. Approximately 2.0 mL of the sample solution, was added 1.0 mL of 20 % trichloroacetic acid and 2.0 mL of thiobarbituric acid (TBA) solution. The final sample concentration was 0.02% w/v. The mixture was placed in a boiling water bath for 10 min. After cooling, it was centrifuged at 3000 rpm for 20 min. Absorbance of the supernatant was measured at 532 nm. Antioxidant activity was recorded based on absorbance on the final day. In both methods, antioxidant activity is described by percent inhibition. After stirring (3 min), the absorption was measured at 530 nm. A control was performed with linoleic acid but without extracts. ascorbic acid (200 ppm) was used as a positive control. The maximum peroxidation level observed as 168 h (7 days) in the sample that contained no antioxidant component was used as a test point. Percent inhibition of linoleic acid peroxidation was calculated to express antioxidant with a little modification activity[21]:

$$\text{Percent inhibition of linoleic acid peroxidation} = \frac{\text{Absorbance of control-Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Probiotic bacteria: *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus jensenii* and *lactobacillus casei* were obtained from National Research Centre, Egypt. They were cultured on De Man Rogosa and Shapes agar media (MRS) conditions were adapted using anaerobic Jar and anaerobic gas generating kits from Oxoid, Hampshire, England[22].

Growth of probiotic bacteria in plant extract added media: *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus* and *Lactobacillus casei* were grown for 24 h. Approximately1 mL of plant extract was added at 25% concentration inoculated to 1 mL of each selected probiotic bacterium (10^6 CFU mL^{-1}) comparing to control sample for each bacterium in a media without plant extract. Samples were incubated anaerobically at 37°C for 24 h. The O.D was determined colorimetry using spectrophotometer (Spectro, 23 labomed, Inc.) at 620 nm after 24 h for each selected probiotic bacterium. Anaerobic conditions were kept all over the experiment using anaerobic Jar[22].

Antimicrobial activity

Pathogenic bacteria: The organisms studied were Gram positive bacteria namely *Bacillus subtilis* NRRL B-543, *Staphylococcus aureus*; NRRL B-313 and *Sarcina lutea* ATCC27853, Gram negative bacteria *Escherichia coli*; NRRL B-210, *Pseudomonas aeruginosa* NRRL B23 27853 and *Klebsiella pneumoniae* ATCC 27736, pathogenic yeast *Candida albicans* NRRL Y-477 pathogenic fungi *Aspergillus niger* NRRL-3 and *Aspergillus flavus* ATCC 16883. These microorganisms were obtained from Natural Research centre, Department of Chemistry of Natural and Microbial product Cairo Egypt and were grown and maintained in on nutrient agar media[23].

Well diffusion technique: Screening of antimicrobial activity was performed by well diffusion technique. For pathogenic bacteria the Nutrient Agar ((NA) were used and Potato Dextrose Agar (PDA) for fungi. The plates were seeded with 0.1 mL of the standardized inoculums of each test organism. The inoculums were spread evenly over plates with glass spreader. The seeded plates were allowed to dry in the incubator at 37°C for 20 min[24]. A standard cork borer of 8 mm was used to cut uniform wells on the surface of media and 100 µL of each extract was introduced in the wells. The inoculated plates were incubated at 30-37°C for 24-96 h and zone of inhibition was measured to the nearest millimeter (mm). The zone of inhibition produced by the plant extract was compared with control[25].

RESULTS AND DISCUSSION

DPPH radical scavenging: Figure 1 shows the total phenolic content of *Saussurea lappa* is approximately twice of that in cidir *Ziziphus spina-christi.* These results reflected the increase in antioxidant capacity of costus in compare to cidir. A strong need for effective antioxidants from natural sources is needed as alternatives to synthetic antioxidant to prevent free radicals implicated diseases which cause serious effects on health. A wide range of antioxidant activity in plant material is not common[26]. The main active compounds of *Saussurea lappa* are costunolide, dehydrocostus lactone, caustic, palmitic, linoleic acids, β-sitosterol, cyclocostunolide, alantolactone, cyclocostunolide, isoalantolactone[2]. Isolated bio-active compounds have hypolipidemic, hypoglycemic immunomodulator, anti-inflammatory antimicrobial and antiparasitic medicinal effect due to antioxidant activities[3].

Total phenolic contents: The total phenolic contents in the studied *Saussurea lappa* and *Ziziphus spina-christi* L. were illustrated in Fig. 1. The highest total phenolic contents were observed in *Saussurea lappa* (80 mg g^{-1} dry weight) followed by *Ziziphus spina-christi* L. (62 mg g^{-1} dry weight). The total phenolic contents of *Ziziphus spina-christi* L., extract in this study were similar to the result of Zhou and Yu[19] who reported that the total phenols in *Ziziphus spina-christi* L. are in the similar range.

Total antioxidant activity by linoleic acid system: Total antioxidant activity of the plant extracts was assessed by monitoring their ability to inhibit lipid peroxidation (Fig. 1). The level of inhibition of linoleic acid oxidation by *Saussurea lappa and Ziziphus spina-christi* L., water extracts were found to be moderate, 53-72. 4%. It attribute of the presence of higher amount of phenolic compounds in these extracts[27]. These data was parallel with that reported *Saussurea lappa*[25] contain fatty oil 30%, ash 3.5%, volatile oil and sulphuric acid. *Saussurea lappa* also contain phytochemicals such as, costunolide, dehydrocostus lactone, caustic, palmitic, linoleic acids, sitosterol, cyclocostunolide, alantolactone, cyclocostunolide, isoalantolactone. These compounds are antidiabetic, antioxidant, anti-inflammatory and antimicrobial remedy[28].

Regarding to gradual inhibition of linoleic acid demonstrated in Fig. 2 *Saussurea lappa* induced higher inhibition to the linoleic acid peroxidation than that of cider *Ziziphus spina-christi* L., throughout 7 days to be nearly to the

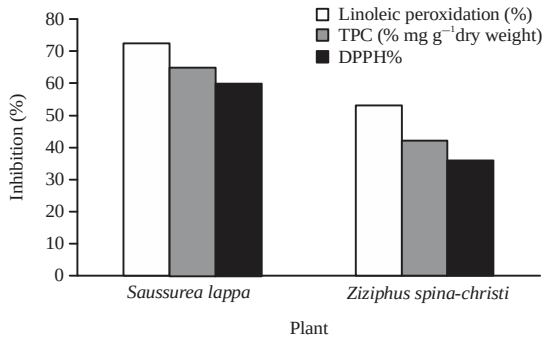


Fig. 1: Total phenolics antioxidant scavenging and percentage inhibition of linoleic acid peroxidation for *Saussurea lappa* and *Ziziphus spina-christi* L.

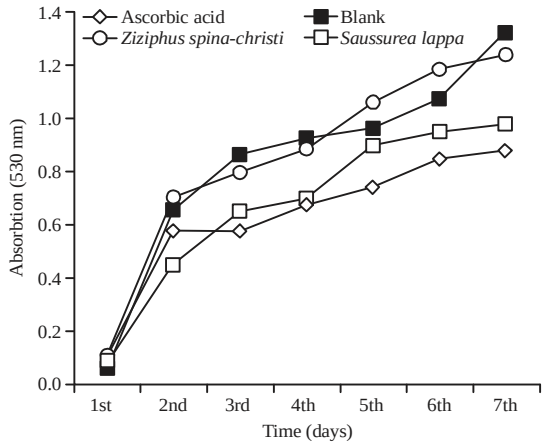
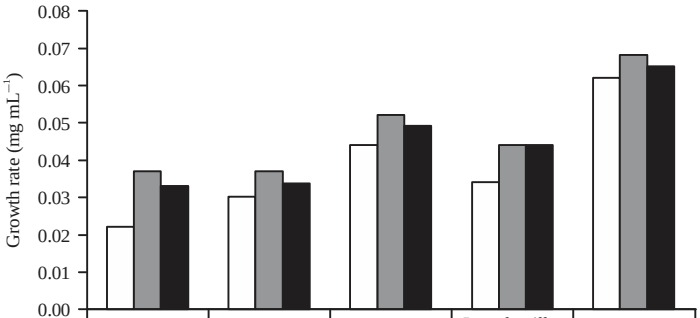


Fig. 2: Antioxidant activity of plant extracts as linoleic acid system

+ve control (ascorbic acid) effect. The antioxidant properties of phenolics arose from their high reactivity as hydrogen or electron donors and from the ability of polyphenol-derived radicals to stabilize and delocalize the unpaired electron or from their ability to chelate transition metal ions[29].

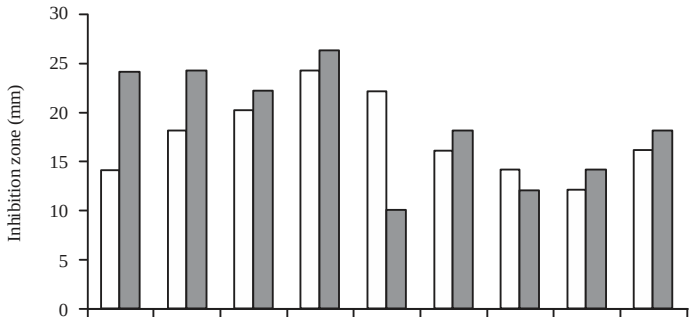
Probiotic bacteria: Positive effect of *Saussurea lappa* and *Ziziphus spina-christi* L., on the growth rate of some probiotic bacteria was depicted in (Fig. 3), the results revealed that *Saussurea lappa* has pronounced effect on enhancement of the benefit bacteria than *Ziziphus spina-christi* L. this may be due to it is richer with phenolic components than *Ziziphus spina-christi* L. These findings were in agreement with that previously recorded where phenolic compounds enhanced probiotic bacteria, that explain the safety of using plant extract in the growth of some probiotic bacteria that have a big role in microbial balance especially in children[22].



	Streptococus thermophilus	*Lactobacillus bulgaricus*	*Lactobacillus acidophilus*	*Lactobacillus jensenii*	*Lactobacillus casei*
Control	0.022	0.03	0.044	0.034	0.062
Saussrea lappa	0.037	0.037	0.052	0.044	0.068
Ziziphus spina-christ L.	0.033	0.033	0.049	0.044	0.065

Probiotic bacteria

Fig. 3: Biomass of probiotic bacteria strains in the MRS broth with added *Saussurea lappa* and *Ziziphus spina-christi* L. extract in a concentration of 25% after 24 h



	B. s	St.	S. l	E. col	P. a	K. p	C. a	A. f	A. niger
Ziziphus spina-christ L.	14	18	20	24	22	16	14	12	16
Saussrea lappa	24	24	22	26	10	18	12	14	18

Microorganismes

Fig. 4: Antimicrobial activities were expressed as inhibition zones
B.s: *Bacillus subtilis* ATCC6633, St: *Staphylococcus aureus* ATCC29213, S.l: *Sarcina lutea* ATCC27853, *E. coli*: *Escherichia coli* ATCC259, P.a: *Pseudomonas aeruginosa* ATCC27953, K.p: *Klebsiella pneumoniae* ATCC 27736, C.a: *Candida albicans* NRRL Y-477, *A. niger*: *Aspergillus niger* NRRL-3 and A.f: *Aspergillus flavus* ATCC 16883

Antimicrobial effect: The *in vitro* antimicrobial activities of the *Saussurea lappa* and *Ziziphus spina-christi* plant extracts are shown in Fig. 4 the presented results showed that water extract of *Saussurea lappa* has antibacterial activity against *Escherichia coli*, *Bacillus subtilus*, *Staphylococcus aureus* and *Sarcina lutea* (26, 24, 24 and 22 mm respectively). These findings run partially with previous study of Borchardt which recorded *Saussurea lappa* having antibacterial activity against four bacterial strains (*Lactobacillus subtilis*, *Bacillus thureogenesis*, *Corney bacterium* and *Escherichia coli*)[29]. This study is correlated with the work of Yang *et al.*[30] and Adzu *et al.*[31] in which they studied the antibacterial activity of *Saussurea lappa* with *Escherichia coli* and *Bacillus* strains.

However, *Aspergillus niger* showed the good inhibition with Saussurea *lappa* extract in and *Ziziphus spina christi* L. (18 and 16 mm, respectively), but it was moderated effect using *Candida albicans* (14 and 12 mm, respectively). Finally, the *Aspergillus flavus* did not exhibited good response by using both extracts. Antimicrobial activity may involve complex mechanisms, like the inhibition of the synthesis of cell walls and cell membranes, nucleic acids and proteins as well as the inhibition of the metabolism of nucleic acids[32]. Both bacteria and fungi were affected by *Ziziphus spina-christi*[10]. *Ziziphus spina-christi* was shown to be scavenging activity[11]. Plant leaves were used in medicine as an anti-inflammatory agent, antiseptic and antifungal for

diseases of skin[12]. The saponin fraction of the leaves has an antimicrobial activity against *Candida albicans*[13]. Phytochemical analysis of the ethanolic and water crude extracts for the leave of *Ziziphus spina-christi* showed presence of alkaloids, tannins, saponins, glycosides, flavonoids, steroids and terpenoids[33].

The overuse of antibiotics leads to produce multidrug resistant microorganism. In the current scenario herbal products are considered as safe alternatives of synthetic drugs. The common effect of medicine plant has acceleration the interest of scientists and industrialists to focus on herbal medicine and other economic products[34].

CONCLUSION

The aqueous extract of costus (*Saussurea lappa*) was richer with phenolic compounds that has pronounced antioxidative effect and enhancement effect on probiotic bacteria than cidir (*Ziziphus spina-christi* L.). It also has antimicrobial effect against gram positive and gram negative bacteria. However, cidir (*Ziziphus spina-christi* L.) was more effective against fungi. The most active extracts can be subjected to isolation of the therapeutic antimicrobials to carry out further pharmacological evaluation.

SIGNIFICANCE STATEMENTS

- Antioxidant and anti-scavenging studies of aqueous extract of each of cidir and costus showed that *Saussurea lappa* was richer with phenolic compounds than cidir
- Significant growth of probiotic bacteria (*Lactobacillus acidophilus*, *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus casei* and *Lactobacillus jensenii*) was remarkable in aqueous extract of costus *Saussurea lappa* more than cidir
- Pathogenic Gram positive bacteria (*Staphylococcus aureus*, *Sarcina lutea* and *Bacillus subtilis*), Gram negative bacteria (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*) and fungi (*Aspergillus flavus*, *Aspergillus niger* and *Candida albicans*) were inhibited by aqueous extract of costus *Saussurea lappa* this is due to high content of phenolic compounds

ACKNOWLEDGMENTS

This Project was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under grant No. G-50-363-1436. The authors, therefore, acknowledge with thanks to DSR for technical and financial support.

REFERENCES

1. Hedge, P.K., H.A. Rao and P.N. Rao, 2014. A review on insulin plant (*Costus igneus* Nak). Pharmacogn. Rev., 8: 67-72.
2. Nandagoapalan, V., A. Doss and C. Marimuthu, 2016. Phytochemical analysis of some traditional medicinal plants. Biosci. Discov., 7: 17-20.
3. Yu, H.H., J.S. Lee, K.H. Lee, K.Y. Kim and Y.O. You, 2007. *Saussurea lappa* inhibits the growth, acid production, adhesion and water-insoluble glucan synthesis of *Streptococcus mutans*. J. Ethnopharmacol., 111: 413-417.
4. Matsuda, H., I. Toguchida, K. Ninomiya, S. Kageura, T. Morikawa and M. Yoshikawa 2003. Effects of sesquiterpenes and amino acid-sesquiterpene conjugates from the roots of *Saussurea lappa* on inducible nitric oxide synthase and heat shock protein in lipopolysaccharide-activated macrophages. Bioorg. Med. Chem., 11: 709-715.
5. Rao, K.S., G.V. Babu and Y.V. Ramnareddy, 2007. Acylated flavone glycosides from the roots of *Saussurea lappa* and their antifungal activity. Molecules, 12: 328-344.
6. Patil, S.M., M.B. Patil, G.N. Sapkale and R.P. Umbare, 2009. Antimicrobial activity of *Saussurea lappa* Clarke roots. Res. J. Pharmacogn. Phytochem., 1: 51-53.
7. Khalid, A., U. Rehman, A. Sethi, S. Khilji and U. Fatima *et al*., 2011. Antimicrobial activity analysis of extracts of *Acacia modesta*, *Artemisia absinthium*, *Nigella sativa* and *Saussurea lappa* against gram positive and gram negative microorganisms. Afr. J. Biotechnol., 10: 4574-4580.
8. Karaman, I., F . Sahin, M. Gulluce, H. Ogutcu, M. Sengul and A. Adiguzel, 2003. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. J. Ethnopharmacol., 85: 231-235.
9. Khalil, A., B.F. Dababneh and A.H. Al-Gabbiesh, 2009. Antimicrobial activity against pathogenic microorganisms by extracts from herbal Jordanian plants. J. Food Agric. Environ., 7: 103-106.
10. Shekhawat N. and R. Vijayvergia, 2010. Evaluation of antimicrobial potential of some medicinal plants against plants and human pathogen. J. Pharm. Res., 3: 700-702.
11. Doughari, J.H., 2006. Antimicrobial activity of *Tamarindus indica* Linn. Trop. J. Pharm. Res., 5: 597-603.
12. Al-Kattan, M.O., 2013. Anti-bacterial effect of Indian Costus and sea-qust and their water extracts on some pathogenic bacteria of the human respiratory system. J. Med. Plants Res., 7: 1418-1423.
13. Al-Mutairi M.H., S. Ali, A.S. Mesalhy and Y. Aldebas, 2016. Antibacterial activity of sider (*Ziziphus spina-christi*), leaves extract against selected pathogenic bacteria. Eur. J. Pharm. Med. Res., 3: 138-144.
14. Iwu, M.M., 2014. Handbook of African Medicinal Plants. 2nd Edn., CRC Press, USA., ISBN-13: 9781466571976, pp: 464.
15. Michel, C.G., 1993. A pharmacognostical study of *Ziziphus spina-christi* growing in Egypt. Ph.D. Thesis, Faculty of Pharmacy, University of Cairo, Egypt.

16. Dangoggo, S.M., L.G. Hassan, I.S. Sadiq and S.B. Manga, 2012. Phytochemical analysis and antibacterial screening of leaves of *Diospyros mespiliformis* and *Ziziphus spina-christi*. J. Chem. Eng., 1: 31-37.
17. Garcia, V.M.N., G. Rojas, L.G. Zepeda, M. Aviles, M. Fuentes, A. Herrera and E. Jimenez, 2006. Antifungal and antibacterial activity of four selected Mexican medicinal plants. Pharm. Biol., 44: 297-300.
18. Kaur, R., G. Rampal and A.P. Vig, 2011. Evaluation of antifungal and antioxidative potential of hydrolytic products of glucosinolates from some members of Brassicaceae family. J. Plant Breed. Crop Sci., 3: 218-228.
19. Zhou, K. and L. Yu, 2006. Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in Colorado. LWT-Food Sci. Technol., 39: 1155-1162.
20. Anyasor, G.N., K.O. Ogunwenmo, O.A. Oyelana and B.E. Akpofunure, 2010. Phytochemical constituents and antioxidant activities of aqueous and methanol stem extracts of Costus afer Ker Gawl. (Costaceae). Afr. J. Biotechnol., 9: 4880-4884.
21. Ozsoy, N., A. Can, R. Yanardag and N. Akev, 2008. Antioxidant activity of *Smilax excelsa* L. leaf extracts. Food Chem., 110: 571-583.
22. Saddiq, A.A. and E.N. Danial, 2014. Effect of Propolis as a food additive on the growth rate of the beneficial bacteria. Main Group Chem., 13: 223-232.
23. Fratianni, F., R. Pepe and F. Nazzaro, 2014. Polyphenol composition, antioxidant, antimicrobial and quorum quenching activity of the Carciofo di Montoro (*Cynara cardunculus* var. *scolymus*) global artichoke of the Campania region, southern Italy. Food Nutr. Sci., 5: 2053-2062.
24. Fratianni, F., F. Nazzaro, A. Marandino, M.D.R. Fusco, R. Coppola, V. de Feo and L. de Martino, 2013. Biochemical composition, antimicrobial activities and anti-quorum-sensing activities of ethanol and ethyl acetate extracts from *Hypericum connatum* Lam. (Guttiferae). J. Med. Food, 16: 454-459.
25. Rani, I., S. Akhund and H. Abro, 2008. Antimicrobial potential of seed extract of *Raphanus sativus*. Pak. J. Bot., 40: 1793-1798.
26. Borchardt, J.R., D.L. Wyse, C.C. Sheaffer, K.L. Kauppi and R.G. Fulcher *et al*., 2008. Antioxidant and antimicrobial activity of seed from plants of the Mississippi river basin. J. Med. Plants Res., 2: 81-93.
27. Ahmad, N., F. Anwar, S. Hameed and M.C. Boyce, 2011. Antioxidant and antimicrobial attributes of different solvent extracts from leaves and flowers of Akk [*Calotropis procera* (Ait.) Ait. F.)]. J. Med. Plants Res., 5: 4879-4887.
28. Sharma, V.K., M. Tiwari, N.S. Chauhan and R. Nema, 2012. Phytochemical investigation on the ethanolic extract on the leaves of *Ziziphus xylopyrus* (Retz.) Willd. Int. J. Agron. Plant Prod., 3: 26-37.
29. Cho, J.Y., K.U. Baik, J.H. Jung and M.H. Park, 2000. *In vitro* anti-inflammatory effects of cynaropicrin, a sesquiterpene lactone, from *Saussurea lappa*. Eur. J. Pharmacol., 398: 399-407.
30. Yang, M., C.M. Wang, Q. Zhang, Y.F. Han and Z.J. Jia, 2004. Sesquiterpenes, lignans and other constituents from *Saussurea macrota*. Die Pharmazie-Int. J. Pharm. Sci., 59: 972-976.
31. Irshad, S., M. Mahmood and F. Perveen, 2012. *In-vitro* anti-bacterial activities of three medicinal plants using Agar well diffusion method. Res. J. Biol., 2: 1-8.
32. Adzu, B., S. Amos, S. Dzarma, C. Wambebe and K. Gamaniel, 2002. Effect of *Ziziphus spina-christi* willd aqueous extract on the central nervous system in mice. J. Ethnopharmacol., 79: 13-16.
33. Nayagam, J.R., 2015. Plantation Technology for Seven Tropical Tree Species. Lambert Academic Publishing, Germany, ISBN-13: 978-3-659-40484-9, pp: 342-343.
34. Alaagib, R.M.O. and S.M.H. Ayoub, 2015. On the chemical composition and antibacterial activity of *Saussurea lappa* (Asteraceae). Pharm. Innov. J., 4: 73-76.