Pak. J. Biotechnol. Vol. 14 (2) 227 -231 (2017)
www.pjbt.org

ISSN Print: 1812- 1837
ISSN Online: 2312- 7791

STUDY OF CUMIN ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF ALCOHOLIC AND AQUEOUS EXTRACTS

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Article Received 5.5.2017, Revised 14.6.2017, Accepted 19.6.2017

ABSTRACT

Chemical composition and antibacterial activity of alcoholic and aqueous extracts of Cumin (*Cuminum cyminum*) studied on *Streptococcus mutans, Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa.* Study results showed that carbohydrate, protein, fat, moisture, ash and fiber percents in Cumin were 33.8, 16.3, 25.3, 6.1, 8.6 and 9.6 respectively.

Alcoholic extract of Cumin had higher antibacterial activities against all tested bacteria comparing to the aqueous extracts. Antioxidant activities of Cumin extracts were compared to butylated hydroxyanisole (BHA). Results showed that alcoholic extracts had the highest antioxidant activity comparing to the aqueous extracts.

INTRODUCTION

Medicinal plants are very important for human health, it acts as an antibacterial agent against pathogenic bacteria (Zaika, 1988). Plants regarded for a long time as a natural valuable source for human health maintenance (Tanaka *et al.,* 2006). The extracts of these plants have numerous health related effects such as antibacterial, antimutagenic anticarcinogenic, antithrombotic and vasodilator activities (Bidlack *et al.,* 2000).

Spices provide food with the desired taste, flavor and regards as active antimicrobial compounds (Kizil and Sogut, 2003). *Cuminum cyminum* L. (Cumin) from Apiaceae family is used as a traditional medicine and belongs to the Mediterranean region (Milan *et al.,* 2008: Sahana *et al.,* 2011). *Cuminum cyminum* is short leaves herbaceous annual plant (5-10 cm). Cumin fruit has a single yellow brown seed. Seeds are used in pickles, cheese, mixed soups, candies and meat (Taleb *et al.,* 1997).

Heri *et al.,* (2003) reported that the main active components of Cumin are cuminal and safranal 32.26% and 24.46% respectively. *Cuminum cyminum* seeds have showed diuretic, stomachic, astringent, carminative, fungicidal and bactericidal properties (Jirovetz *et al*., 2005, Singh *et al*., 2006; Gachkar *et al*., 2007).

Cumin (*Cuminum cyminum* L.) has broad spectrum antibacterial characteristics against gram-positive and gram-negative bacteria. It is aromatic plant used for medical preparations, food industries and as a flavor for foods (Iacobellis *et al.,* 2005). Cumin seeds have strong aroma and special flavor because of its content of essential oil (Gachkar *et al.,* 2007: Hajlaoui *et al.,* 2010).

Pathogenic bacteria are a serious threat to human health. The screening for antibacterial activity in plant extracts has revealed that plants are a potential source of novel antibiotic prototypes (Afolayan, 2003). Food rancidity reduced by antioxidants which forbidden toxic oxidation product formation, keeps the nutritional quality and prolong shelf life. Many studies discussed phenolic compounds and the activities of antioxidants of plants parts such as seeds, leaves and peels (Al-Juhaimi and Ghafoor, 2011; Ghafoor and Choi, 2009; Ghafoor *et al*., 2010). Consumers are looking for fresh foods in appearance and this led to use antioxidants from natural sources such as spices which are a good source of polyphenolic compounds that have antioxidant activities and might replace the food systems' synthetic antioxidants and provide extra health benefits (Shan *et al*., 2005).

The current study aimed to determine the potential antibacterial activity of alcoholic and aqueous extracts of Cumin (*C. cyminum* L.) against human pathogenic bacteria and to evaluate the antioxidant properties of Cumin.

MATERIALS AND METHODS

Plant material: Cumin seeds used in this study were collected from the Basrah local markets and crushed by electric grinder.

Test microorganisms: The bacterial isolates used in this study (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Streptococcus mutans*) were obtained from the stock cultures of Agriculture College, University of Basrah.

Chemical Content: The chemical content of Cumin seeds (carbohydrate, protein, fat, moisture, ash and fiber) was determined according to Egan *et al.* (1988).

Preparation of Plant extract: The ethanol extract of *Cumin* was done according to Parekh *et al* (2005) with few modifications. Electrical grinder was used to crush 10 grams of dried seeds and then extracted with 100 ml of 80% ethanol and kept for 24 h. on rotary shaker. Then filtered through Whatman No.1 filter paper, centrifuged at 5000 rpm for 15 min. After collecting the supernatant, the solvent was evaporated at 40ºC by rotary evaporator. The same procedure was carried out with aqueous extract by using distilled water instead of the ethanol.

Preparation of Bacterial Inoculums: Five ml of Muller Hinton Broth was inoculated with five pure culture colonies of the test bacteria and incubated at 37ºC for 24 h. Cultures' turbidity was compared to 0.5 Mcfarland standard to get 150x 10^6CFU/ml. Inoculum suspension was inoculated within 15-20 minutes (Saeed *et al*., 2005).

Antimicrobial activity of extract: Well diffusion technique: 0.1 ml of the prepared bacterial inoculum of each bacterium was seeded in Mueller Hinton Agar plates. A sterile glass spreader used to spread the inoculum over plate. The plates kept in incubator at 37ºC for 20 minutes and could dry. Uniform wells were cut on the surface of the Mueller Hinton Agar using 8 mm diameter cork borer, 100 µl of each extract was introduced in the wells. Inoculated plates were incubated at 37ºC for 24 h. Inhibition zones were measured to the nearest centimeter (cm) (Saeed & Tariq, 2005).

Antioxidative assay: Antioxidant activity of Cumin alcoholic and aqueous extract using linoleic acid system according to Osawa & Namiki (1981). Extracts and BHT samples were prepared (0-100 mg/ml) and dissolved in ethanol 98%. A mixture of 4.1 ml linoleic acid (2.5% ethanol conc.), 4 ml of each extract, 8 ml of phosphate buffer (0.05 M, pH 7) and 3.9 distilled water was prepared. The mixture was incubated in dark containers, tightly closed and kept on 40ºC for 24 h. Oxidation degree was determined using thiocyanate procedure: adding 0.1 ml of this mixture into 9.7 ml of ethanol (75% conc.) and 0.1 ml ammonium thiocyanate (30% conc.), after 3 minutes add 0.1 ml iron chloride $FeCl_3$ (20 mM in 3.5 HCl). Absorbance was measured on 500 nm, the inhibition percent of linoleic acid was calculated according to the following equation:

$$\text{Antioxidant Activity \%} = 1 - \frac{\textbf{AbsorbanceofSample}}{\textbf{AbsorbanceofControl}} \times 100$$

Control was prepared according to the previous steps except mixing 4ml of ethanol instead of extracts.

RESULTS AND DISCUSSION

Chemical Composition: Table -1 illustrates the percentages of Cumin chemical composition (carbohydrate, protein, fat, moisture, ash and fibers) which were 33.8, 16.3, 25.3, 6.1, 8.6 and 9.6 respectively.

Table -1: Cumin Chemical Composition

Composition	carbohydrate	protein	fat	moisture	ash	fibers
Percentages	33.8	16.3	25.3	6.1	8.6	9.6

Antibacterial Activity: Herbs have different chemical compounds such as lipids, tannins, alkaloids and volatile oils which presented in in their tissues and they are the main source of herbs' antimicrobial characteristics (Con *et al.,* 1998). Table 2 illustrates the antibacterial activity of aqueous and alcoholic extracts against *Streptococcus mutans, Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa.* Alcoholic extract of Cumin had higher antibacterial activity against all tested bacteria comparing to the aqueous extract, the reason behind to the weakness of aqueous extracts antibacterial activity may attributed to the non-extraction of spices antimicrobial components in aqueous phase such as lipophilic or may attributed to the loosing of essential oil components during grinding and the procedure of extraction because of its high volatile ability (Bhatia and Sharma, 2012), while Cumin oil and cuminaldehyde have been reported to exhibit strong larvicidal and antibacterial activity (Rathore*et al*., 2013)

Table -2: Antibacterial Activity (cm) of Cumin Alcoholic and Aqueous Extracts

Extracts	*Streptococcus mutans*	*E. coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
Aqueous	1.5	1.5	**1**	**0.2**
Alcoholic	**2.5**	**2**	**2**	**0.6**

Ouattara *et al.,* (1997) reported that the antibacterial activity of Cumin might be attributed to carvacrol and carvone contained in Cumin's volatile oil. Study results agreed with many researchers such as

Sagdic *et al.*, (2002) who reported that the antibacterial activity of Cumin extract on *Escherichia coli* 0:157 has been demonstrated in vitro. Cumin aqueous extract have been reported to have antimicrobial activity against many pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* species and *Bacillus cereus* (Stefanini *et al.*, 2003; Chaudhry & Perween, 2008 and Das *et al.*, 2012). Cumin aqueous extract has been reported to have antimicrobial activity against *E. coli* and *P. aeruginosa* (Stefanini *et al*., 2003). Cumin extract has exhibited antimicrobial activity against all the four tested bacteria, Cumin extract was effective against *E. coli*, *P. aeruginosa*, *S. aureus* and *B. pumilus* (Anita *et al*., 2013). Another study found that the essential oil of *Cyminum cuminum* was active against *streptococcus mutans* and *streptococcus pyogenes* (Shayegh*et al*., 2008).

The results showed that Cumin extract is effectively inducing cell damage in both gram negative as well as gram positive bacteria. Alcoholic extract of spices contains phytochemicals including polyphenols and are reported to exhibit considerably high free radical scavenging and peroxide inhibition activity indicating its reducing character, which may in part explain the inhibition of bacterial growth. Metal ion chelating property of the polyphenols in extracts of the spices may also be contributing to the antimicrobial properties by leading to the deficiency of essential metal ions in the growth medium.

Antioxidants Activity: Antioxidant activities of Cumin extracts were compared to a popular synthetic antioxidant which is butylated hydroxy toluene (BHT). Results illustrated in Figure -1 showed that alcoholic extract antioxidant activities were higher than aqueous extract in all different concentrations. Alcoholic extract of different spices including Cumin have been reported to possess antioxidants activities and polyphenolic compounds (Naveen *et al.*, 2011). The Cumin alcoholic extract also has high antioxidant activity mainly due to the presence of monoterpene alcohol (Gohari and Saeidnia, 2011). In fact, the antioxidant activity of Cumin alcoholic and aqueous extracts were close. These results suggest that Cumin or its extracts could potentially be used in food systems to prevent oxidative deterioration of foods.

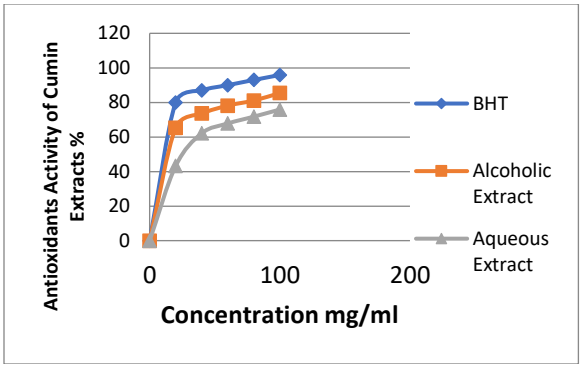

Figure -1: Antioxidant activity of Cumin extracts

CONCLUSION

The Cumin extracts have the antimicrobial activity against the tested pathogenic bacteria. Thus, the use of Cumin and its extracts as natural preservatives in food might be an alternative to chemical additives. Cumin extracts have a good antioxidant activity and could be the substitution for the synthetic antioxidants in foods to reduce oxidative deterioration.

REFERENCES:

Afolayan, A.J., Extracts from the shoots of Arctotis artotoides inhibit the growth of bacteria and fungi. Pharm. Biol. 41(1): 22-25 (2003).

AL-Juhaimi, F. and K. Ghafoor, Total phenolics and antioxidant activities of leaf and stem extracts from coriander, mint and parsley grown in Saudi Arabia. Pakistan Journal of Botany 43: 2235-2237 (2011).

Bhatia M. and A. Sharma, Brassica nigra and *Cuminum cyminum*. Inhibitors of food borne pathogens, International Journal of Applied Biology and Pharmaceutical Biotechnology 3(3): (2012).

Bidlack, W.R., S.T. Omaye, M.S. Meskin and D.K. W. Topham, Phytochemicals as Bioactive Agents. Lancaster PA: Technomic Pp. 241-270 (2000).

Chaudhry, N.M.A. and P. Tariq, In vitro antibacterial activities of Kalonji, cumin and poppy seed. Pak. J. Botany 40(1): 461-467 (2008).

Con, A.H., A. Ayar and H.Y. Gokalp, Antimicrobial activity of the essential oils extracted from some spices. Food 23: 171-175 (1998).

Das, S., C. Anjeza and S. Mandal, Synergistic or additive antimicrobial activities of Indian spice and herbal extracts against pathogenic probiotic and food-spoiler micro-organisms. Int. Food Res J. 19(3): 1185-1191 (2012).

Dua A., G. Gaurav, S. Balkar and R. Mahajan, antimicrobial properties of methanolic extract of cumin (cuminum cyminum) seeds. International Journal of Research in Ayurveda and Pharmacy 4: 104-107 (2013).

Egan, H., R.S. Kirk and R. Sawyer, Pearsons chemical analysis of food. 8Sh ed. Longman Scientific and Technical Pp. 591 (1988).

Gachkar, L., Y. Davood, B.R. Mohammad, T. Masood, A.A. Shakiba and R. Iraj, Chemical and biological characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* and essential oils. Food Chemistry 102: 898-904 (2007).

Ghafoor, K. and Y.H. Choi, Optimization of ultrasound-assisted extraction of phenolic compounds and antioxidants from grape peel through response surface methodology. Journal of Korean Society of Applied Biological Chemistry 52: 295–300 (2009).

Ghafoor, K., J. Park and Y.H. Choi, Optimization of supercritical carbon dioxide extraction of bioactive compounds from grape peel (Vitis labrusca B.) by using response surface methodology. Innovative Food Science and Emerging Technologies 11: 485-490 (2010).

Gohari, A.J. and S. Saeidnia, Review on Phytochemistry of *Cuminum cyminum* seeds and its Standards from Field to Market. Pharmacognosy Journal 3(25): 1-5 (2011).

Hajlaoui H., H. Mighri, E. Noumi, M. Snoussi, N. Trabelsi, R. Ksouri, et al., Chemical composition and biological activities of Tunisian *Cuminum cyminum* L. essential oil: a high effectiveness against Vibrio spp. strains. Food Chem. Toxicol. 48(8-9): 2186–92 (2010).

Heri, Y.J., T. Wen, Z. Ming and D. Ninghua. Determination of chemical components of volatile oil from *Cuminum cyminum* L. by gas chromatography - mass spectrometry. Peop. Rep. China, 414000 (2003).

Iacobellis S.N., L.P. Cantore, F. Capasso and F. Sentore, Antibacterial activity of *Cuminum cyminum* L. and Carumcarvi L. essential oils. Journal of Agricultural and Food Chemistry: 53: 57-61 (2005).

Jirovetz L., G. Bushbauer, A.S. Stoyanova, E.V. Georgiev and S.T. Damianova, Composition, quality control and antimicrobial activity of the essential oil of the cumin seeds from Bulgaria that had been stored for up to 36 years. Journal of Flavor and Fragrance: 21: 186-191 (2005).

Kizil, S. and T. Sogut, Investigation of antibacterial effects of spices. Crop Research 3: 86-90 (2003).

Milan K.S.M., H. Dholakia, P. Tiku and P. Vishveshwaraiah, Enhance¬ment of digestive enzymatic activity by cumin (*Cuminum cyminum* L.) and role of spent cumin as a bionutrient. Food Chem. 110(3): 678–83 (2008).

Naveen S., S.M. Siddalinga and F. Khanum, Antioxidant potential of some common plant sources. Int J Pharma Res Develop. 3(1); 154-174 (2011).

Osawa, T. and M. Namiki, A novel type of antioxidant isolated from leaf wax of Eucalyptus leaves. Agric. Biol. Chem., 45: 735-739 (1981).

Ouattara, B., R.E. Simard, R.A. Holley, G.J.P. Piette and A. Begin, Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. Int. J. Food Microbiol. 37: 155-162 (1997).

Parekh, J., R. Nair and S. Chanda. Preliminary screening of some folkloric plants from western India for potential antimicrobial activity. Indian J. Pharmacol., 68(6): 832-834 (2005).

Rathore S.S., S.N. Saxena and B. Singh, Potential Health Benefits of Major Seed Spices. International J. Seed Spices 3: 1-12 (2013).

Saeed S. and P. Tariq, Antibacterial activities of *Mentha piperita*, *Pisum sativum* and *Momordica charantia*. Pak. J. Bot. 37(4): 997-1001 (2005).

Saeed, S., P. Tariq and N. Fatima, Antibacterial activity of rhizome of *Zingiber officinale* against species of oral viridans streptococci. Int. J. Biotechnol. 2: 913-916 (2005).

Sagdic, O., A. Kuscu, M. Ozcan and S. Ozcelik, Effects of Turkish spice extracts at various concentrations on the growth of Escherichia coli O157:H7. Food Microbiol, 19: 473-480 (2002).

Sahana K., S. Nagarajan, L.J.M.Rao, Cumin (*Cuminum cyminum* L.) Seed Volatile Oil: Chemistry and Role in Health and Disease Prevention, Nuts and Seeds in Health & Disease Prevention. Academic Press. London, 417–427 (2011).

Shan B., Y.Z. Cai, M. Sun and H. Corke. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. J. Agric. Food Chem. 53: 7749-59 (2005).

Shayegh S., I. Rasooli, M. Taghizadeh and S.D.S. Astaneh, Phytotherapeutic inhibition of supra-gingival dental plaque. *Nat Prod Res*. 22(5): 428-439 (2008).

Singh G, P. Marimuthu and M.P. Lampasona, *Cuminum cyminum* L. Chemical constituents, antioxidant and antifungal studies on its volatile oil and acetone extract. Indian Perfumer 50:31-39 (2006).

Stefanini M.B., R.O.Figueiredo, L.C. Ming and A. F. Junior, Antimicrobial activity of the essential oils of some spice herbs. Proc Int. Conf. on MAP Eds. J. Bernath et al. Acta Hort. 597: ISHS; 215-216 (2003).

Taleb, C., M. Petit and P. Pileni, Optical Properties of Self- Assembled 2D and 3D Super lattices of Silver Nanoparticles Chem. Mater. 9, 950 (1997).

Tanaka, J.C.A., C.C. Deilva, A.J.B. DeOliveir, C. V. Nakamura and B.P.D. Filho, Antimicrobial activity of indol and alkaloids from *Aspidosperma ramiflorum*. Braz. J. Med.Biol. Res. 39: 387-391 (2006).

Zaika, L.L., Spices and herbs: Their antimicrobial activity and its determination. J. Food. Safety 9: 97- 118 (1988).