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In vitro antifungal effect of potassium sorbate and sodium benzoate on the growth of fungi causing sinusitis

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The study involved 70 nasal swab collected from people suffering from sinusitis and consulted the Ear, Nose and Throat Unit in Al-Diwaniya Teaching Hospital, Iraq during the period from 12 June to 12 August 2016 with ages ranging from 0-65 years old. The results showed that the number of nasal swabs that gave fungal growth was 36/70 (51.428%); highest percentage of fungal infection was recorded in ages ranging from 11-20 years old 8(80%), followed by above 60 years old 7(70%) and lowest infection was in 51-60 years old 3(30%) when compared. A total of 43 fungal isolates were identified and the isolates belong to 7 genera comprising of *Penicillium* spp. with 16 isolates, *Aspergillus* spp. with 14 isolates (mainly *A. niger*= 7, *A. flavus*= 5 and *A. fumigates*= 2), *Cladosporium* spp. with 4 isolates, *Rhizopus spp.* 3 isolates, *Sporothrix schenckii* 3 isolates, *Mucor* spp. 2 isolates and *Fusarium* spp. 1 isolate. *Penicillium* spp. occurred with the highest frequency percentage of (37.209%), followed by *A. niger* (16.279%) and *A. flavus* (11.627%) with significant differences when compared with other fungi isolated in this study while the lowest percentage frequency was recorded for *Fusarium* spp. (2.325%). Potassium sorbate and Sodium benzoate have high inhibitory activity against the growth of all fungi studied and increases in their concentration caused corresponding increases in their percentage inhibition of fungal growth which ranged at concentration 1000 mg/L of potassium sorbate and sodium benzoate between (90.7-94.5%) and (91.2-95.8%) respectively.

Key words: Antifungal, potassium sorbate, sodium benzoate, sinusitis.

INTRODUCTION

Sinuses are cavities filled with air surrounding the eyes, nose inside the skull bones are associated cavity nose through small openings and these cavities lined with a membrane thinning secrete mucus and the lattice cells remove mucus to get rid of foreign particles such as viruses, bacteria, fungi and dust (Rabago et al., 2005). Mucus discharge occurs naturally through small openings between the sinuses and nose, however sinusitis these

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natural openings may be blocked and stops discharge process (Sari-Aslani et al., 2006; Nagar and Gautam, 2015). Sinusitis can be defined as an inflammation of the mucous layer lining the sinus due to bacterial or fungal infection in the upper respiratory tract which can also occur secondary to viral infection of the upper respiratory tract which creates favorable conditions for the growth of bacteria and fungi (Wald, 1998). Fungal sinusitis can be classified according to the causal factor into two types: Noninvasive fungal sinusitis which occurs in two forms, the first form is Allergic fungal sinusitis caused by fungi such as *Aspergillus fumigatus*, *A. niger*, *Penicillium* spp, *Curvularia lunata, Sporothrix schenckii* and *Rhinosporidium* spp, and is characterized by the presence of a black-green material known as Allergic mucin. The allergic mucin form calcified polyps mass which prevents sinus drainage. The second form of Noninvasive fungal sinusitis is called Mycetoma or Fungal ball, which is caused by *A. fumigatus* that grows on one side of the maxillary sinus producing purulent material like ceramic clay. The second type of sinusitis is Invasive fungal sinusitis which also occurs in two forms: Acute fulminant form which is a dangerous infections that have high mortality rate if not treated early and the etiology include some fungi such as *Mucor* spp., *Rhizopus* spp., *Absidia* spp., *Rhizomucor* spp. and *Mortierella* spp. The second form of Invasive fungal sinusitis is called chronic invasive fungal sinusitis that is caused by *A. flavus* and *A. fumigates* (Gillespie et al., 1998; Schubert, 2004; Aribandi et al., 2007; Jiang et al., 2008; Thiagarajan and Ramamoorthy, 2013).

Fungal sinusitis occurs in young and medium-age individuals due to *A. niger*, *A. fumigatus* and *Penicillium* spp. infection especially in children or people who suffer from immunosuppression after exposure to spores that inhabit the respiratory tract while in the older age group, it is caused by *Candida albicans* and *S. schenckii* especially in people working in the mines, as well as peasants or people who use immunosuppressive drugs (Hildmann, 1991; Dosa et al., 2002). The potassium sorbate which is a potassium salt of sorbic acid and has a molecular weight of 150.22 g/mol and sodium benzoate which is a sodium salt of benzoic acid and has a molecular weight of 144.10 g/mol are considered as the most important chemicals used largely as materials for food preservation which are Generally Recognized as Safe (GRAS) and they have high inhibitory effect for the growth of many micro-organisms such as bacteria and fungi that contaminate food, and they are also used in cosmetics, pharmaceuticals and various medical preparations (Guynot et al., 2005; Esfandiari et al., 2013). Previous studies on the use of potassium sorbate or sodium benzoate as inhibitors for the growth of micro-organisms in non-food areas are scanty. This marks the stimulus for this study with the aim of determining the inhibitory effect of potassium sorbate and sodium benzoate *in vitro* against the growth of fungi that cause inflammation of the sinuses.

MATERIALS AND METHODS

Sample collection

Seventy (70) nasal swabs were collected from people suffering from sinusitis using sterile cotton swabs. The samples were taken from 12 June to 12 August 2016 from patients who consulted Ear, Nose and Throat Unit in Al-Diwaniya Teaching Hospital and were clinically diagnosed by a specialist doctor. The patients' ages ranged from 0-65 years old and patients' information, such as gender, age and infection status was recorded. All the swabs were transported immediately to the laboratory and cultured on the appropriate culture media.

Isolation and identification of fungi

The following culture media were used after sterilization with the aid of autoclave at 121°C for 15 min in order to isolate and diagnose fungi under study: Blood agar medium, Brain heart infusion agar and Sabouraud dextrose agar (SDA) containing (0.05 mg/ml) for both antibiotics Cycloheximide and Chloramphenicol. The Petri dishes containing Blood agar medium and Brain heart infusion agar were inoculated with nasal swabs and incubated at 37°C for 14 days in order to isolate the fungus *Sporothrix schenckii*. Dishes containing SDA were also inoculated with nasal swabs and incubated at 25°C for three to four days for the purpose of isolating other fungi. The fungal growths were identified after purification using morphological and microscopic features of their taxonomic placement (Gow et al., 1997; De Hoog et al., 2000; Watanabe, 2010). All pure fungal isolates obtained were saved in test tubes with a capacity of 20 ml containing slant SDA medium and maintained in refrigerator at 4°C until used.

Effect of potassium sorbate and sodium benzoate in the growth of fungal isolates

The method of Lopez-Malo et al. (2005) was used in this test. Spores suspensions of all pure fungal isolates under study was prepared, after cultivation on SDA slants and incubated at 30°C for seven days. The spores were harvested by adding 10 ml of 0.1% Twee 80 solution sterilized by filtration using Millipore filter paper 0.45 µm. The number of spores harvested was calculated using Haemocytometer and the spores suspensions were adjusted by adding the same solution to obtain a final concentration of 1×10^6 spore/ml. Then were prepared graded concentrations of potassium sorbate and sodium benzoate by added appropriate quantities of it to 10 flasks containing sterilized SDA separately and these concentrations were 200, 400, 600, 800 and 1000 mg/L, also used 1 flask containing sterilized SDA with antifungal Amphotericin B at 300 µg/ml, according to Shirazi et al. (2007), and then distributed all prepared media into Petri dishes with three replications for each concentration and then inoculated with 2 ml of spores suspensions of each fungus under study separately. After inoculation, the dishes were incubated at 30°C for 7 days; thereafter the inhibitions were evaluated by measuring the diameter of inhibition zone in millimeter.

Statistical analysis and equations

The percentages of fungal infection and frequency of fungi isolates of the same genera were calculated using the following equations:

$$\text{Fungal infection } \% = \frac{\text{The number of swabs that gave growth}}{\text{The total number of swabs}} \times 100$$

$$\text{Frequency of isolates } \% = \frac{\text{The number of isolates of the same genus or species}}{\text{The total number of isolates}} \times 100$$

The percentage of inhibition was calculated as described by Combina et al. (1999) using the equation:

$$\text{Inhibition } \% = \frac{\text{Diameter of colony control} - \text{Diameter of colony treatment}}{\text{Diameter of colony control}} \times 100$$

One way analysis of variance (ANOVA) was used to test significant differences between the rates by Duncan multiple range test at 0.05 probability level (Wayne, 1983).

RESULTS

Percentages of fungal sinusitis infection by age

The results of the current study showed that the number of nasal swabs that gave fungal growth was 36 swabs of the total number of 70 swabs (51.428%), also found that highest percentage of fungal infection occurred in ages ranging from 11-20 years old, reaching 80%, followed by ages above 60 years old with 70% compared with other ages (Table 1).

Isolation and identification of fungi

In this study, 43 fungal isolates were obtained and identified as belonging to 7 genera comprising of *Penicillium* spp. with 16 isolates, *Aspergillus* spp. 14 isolates (*A. niger*= 7, *A. flavus*= 5 and *A. fumigatus*= 2), *Cladosporium* spp. 4 isolates, *Rhizopus* spp. 3 isolates, *Sporothrix schenckii* 3 isolates, *Mucor* spp. 2 isolates and *Fusarium* spp. 1 isolate. The highest frequency percentage of fungi was recorded for *Penicillium* spp. (37.209%) followed by *A. niger* and *A. flavus* with (16.279%) and (11.627%) respectively with significant differences compared with other fungi studied. The lowest frequency percentage was recorded for *Fusarium* spp. (2.325%) (Table 2).

Effect of potassium sorbate and sodium benzoate in the growth of fungal isolates

The results showed that the potassium sorbate and sodium benzoate have high inhibitory activity against the growth of all fungi studied and increasing their concentrations resulted in increased percentage of inhibition. At highest concentration of 1000 mg/L of potassium sorbate and sodium benzoate, the percentages of inhibition ranged between (90.7-94.5%) and (91.2-95.8%) respectively differing significantly ($p<0.05$) from other concentrations used. There was no significant differences ($p>0.05$) between inhibition by Amphotericin B (90.5-94.7%) at a concentration level of 300 µg/ml against all fungi studied and inhibitions by other two substances (Table 3).

DISCUSSION

The high percentages of the fungal sinusitis infection in both, children and adolescents regarding to very high activity of the age group being frequently involved in outdoor activities thereby exposing them to contaminated environment. Under this condition, they inhale spores in large quantities into the upper and lower respiratory tract were the fungi establishes and grow to cause disease (Grewal et al., 1990; Manning and Holman, 1998). While in the older age the infection occur due to a defect in the immune system especially those who suffer from chronic diseases, who are taking immunosuppressive drugs and due to physiological changes such as dryness of mucous membranes lining the nose (which extends to the sinuses) with advancing age and thus increasing the possibility of the transmission of infection from the nose to the sinuses (Gungor and Adusumilli, 1998; Montanaro, 2000; Prateek et al., 2013). The fungi *Penicillium* spp. and *Aspergillus* spp. were the most frequent isolates in fungal sinusitis infections in the USA (Liza et al., 1997). Also Tekin (2008) found that the fungus *Penicillium* spp. was the first pathogen associated with fungal sinusitis in Turkey with a score of 17 cases, followed by *Aspergillus* spp. with 15 cases, which this is consistent with the results of this study. Previous reports on the inhibitory effect of potassium sorbate and sodium benzoate against growth of fungi and production of toxins, showed that these substances have inhibitory effect on the growth of fungi by inhibiting both spores germination and growth of mycelium (Tsai et al., 1984; Marshall and Bullerman, 1986; Palou et al., 2002; Heydaryinia et al., 2011). Similarly, Sofos and Busta (1991) found that the materials used in food preservation such as potassium sorbate and sodium benzoate control the growth of fungi by reducing its metabolism, denaturing fungal cell proteins or by changing the permeability of the cell membrane. In a recent study, potassium sorbate was found to have inhibitory effect on the growth of all fungi isolated from indoor airborne samples and the number of colony forming units was reduced by more than 88% compared with control media (Chin Ming et al., 2016).

Conclusion

Based on the results of this study, potassium sorbate and sodium benzoate at concentration 1000 mg/L have high inhibitory activity against the growth of all fungi isolated from patients with sinusitis and gave a similar effect to the effect of antifungal Amphotericin B at 300 µg/ml. Therefore, it is possible to use these substances in inhibiting the growth of pathogenic fungi in addition to the main use in food preservation.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

Table 1. Percentages of fungal sinusitis infection by age of patients.

Ages in years	Number of swabs taken	Number of swabs that gave fungal growth	Fungal infection (%)
0-10	10	5	50
11-20	10	8	80
21-30	10	4	40
31-40	10	5	50
41-50	10	4	40
51-60	10	3	30
Above 60	10	7	70
Total	70	36	51.428

Table 2. Percentages of frequency of fungi isolated from sinusitis infection.

Fungi	Number of isolates	Percentages of frequency
Aspergillus flavus	5	11.627% ^{c}
Aspergillus fumigatus	2	4.651% ^{f}
Aspergillus niger	7	16.279% ^{b}
Cladosporium spp	4	9.302% ^{d}
Fusarium spp	1	2.325% ^{g}
Mucor spp	2	4.651% ^{f}
Penicillium spp	16	37.209% ^{a}
Rhizopus spp	3	6.976% ^{e}
Sporothrix schenckii	3	6.976% ^{e}
Total	43	-

Percentages of frequencies with the same letters did not differ significantly ($p>0.05$) between species of isolates.

Table 3. Percentages of growth inhibition of fungal isolates using different concentrations of potassium sorbate and sodium benzoate.

Fungi	Percent growth inhibition for potassium sorbate concentrations (mg/L)					Percent growth inhibition for sodium benzoate concentrations (mg/L)					Percent growth inhibition for Amphotericin B (µg/ml)
	200	400	600	800	1000	200	400	600	800	1000	300
Aspergillus flavus	23.8^{h}	37.6^{g}	51.4^{e}	82.7^{c}	93.8^{a}	24.6^{h}	42.2^{f}	57.3^{d}	88.2^{b}	94.3^{a}	94.6^{a}
Aspergillus fumigatus	19.6^{h}	25.8^{f}	61.9^{d}	78.6^{c}	92.2^{a}	22.7^{g}	35.6^{e}	62.8^{d}	83.5^{b}	92.5^{a}	92.8^{a}
Aspergillus niger	25.4^{g}	34.4^{f}	60.5^{d}	86.3^{b}	93.9^{a}	25.7^{g}	41.8^{e}	66.8^{c}	87.1^{b}	95.8^{a}	94.7^{a}
Cladosporium spp	27.9^{h}	39.3^{g}	52.6^{e}	79.2^{c}	94.5^{a}	28.2^{h}	43.9^{f}	58.8^{d}	82.0^{b}	94.0^{a}	92.3^{a}
Fusarium spp	18.3^{i}	25.0^{g}	57.9^{e}	80.5^{c}	94.0^{a}	23.3^{h}	38.4^{f}	65.3^{d}	86.4^{b}	93.8^{a}	94.2^{a}
Mucor spp	26.5^{g}	35.4^{f}	60.4^{d}	77.9^{b}	92.6^{a}	25.7^{g}	39.5^{e}	67.0^{c}	79.3^{b}	92.9^{a}	91.3^{a}
Penicillium spp	20.6^{g}	24.8^{f}	67.7^{d}	81.1^{c}	90.9^{a}	21.0^{g}	28.7^{e}	66.9^{d}	84.9^{b}	91.6^{a}	91.6^{a}
Rhizopus spp	36.2^{h}	44.5^{g}	56.1^{e}	76.0^{c}	91.9^{a}	34.8^{h}	50.2^{f}	63.9^{d}	81.3^{b}	91.2^{a}	92.4^{a}
S. schenckii	24.1^{i}	32.1^{g}	62.3^{e}	80.7^{c}	90.7^{a}	28.0^{h}	37.6^{f}	68.1^{d}	84.2^{b}	91.7^{a}	90.5^{a}

Percentage inhibitions by various species of fungi with the same letter at a particular concentration and between concentrations are not significantly different ($p>0.05$) at 95% confidence level.

REFERENCES

Aribandi M, McCoy VA, Bazan CI (2007). Imaging feature of invasive and noninvasive Fungal Sinusitis: A review. Radiographics 27:1283-1296.

Chin Ming ER, Sunar NM, Leman AM, Othman N, Kalthsom U, Jamal NA, Ideris NA (2016). The biocidal effect of potassium sorbate for indoor airborne fungi remediation Desalin. Water Treat. 57(1):288-293.

Combina M, Dalcero AM, Varsavsky E, Chulze S (1999). Effects of food preservatives on *Alternaria alternata* growth and tenuazonic acid production. Food. Addit. Contam. 16:433-437.

De Hoog GS, Gene JC, Figueras MJ (2000). Atlas of clinical fungi. 2nd ed. centraalbureeauVoor Schimmelculturees. Utrecht. Netherlands.

Dosa E, Doczi I, Mojzes L, Molnar EG, Varga J, Nagy E (2002). Identification and incidence of fungal strains in chronic rhinosinusitis patients. Acta. Microbiol. Immunol. Hung. 49(2-3):337-346.
Esfandiari Z, Badiey M, Mahmoodian P, Sarhang-pour R, Yazdani E, Mirlohi M (2013). Simultaneous determination of sodium benzoate, potassium sorbate and natamycin content in Iranian yoghurt drink (Doogh) and the associated risk of their intake through Doogh consumption. Iran. J. Public Health. 42:915-920.
Gillespie MB, O'Malley BW, Francis HW (1998). An approach to fulminant invasive fungal rhinosinusitis in the immunocompromised host. Arch Otolaryngol. Head. Neck. Surg. 124(5):520-526.
Gow NA, Robson GD, Gadd GM (1997). The fungal colony. Published of social Mycology. Cambridge University. Vol 21.
Grewal RS, Khurana S, Aujla KS, Goyal SC (1990). Incidence of fungal infections in chronic maxillary sinusitis. Ind. J. Pathol. Micobiol. 33:339-343.
Gungor A, Adusumilli V (1998). Fungal sinusitis: Progression of diseases in immunosuppression a case report. Ear. Nose. Throat. J. 77:207-215.
Guynot ME, Ramos AJ, Sanchis V, Marin S (2005). Study of benzoate, propionate, and sorbate salts as mould spoilage inhibitors on intermediate moisture bakery products of low pH (4.5- 5.5). Int. J. Food. Microbiol. 101:161-168.
Heydaryinia A, Veissi M, Sadadi A (2011). A comparative study of the effects of the two preservatives, sodium benzoate and potassium sorbate on *Aspergillus niger* and *Penicillium notatum*. Jundishapur. J. Microbiol. 4(4):301-307.
Hildmann H (1991). Sinusitis in the children from the ENT Specialty point of view. Fortschr. Med. 109(19):397-400.
Jiang RS, Liang KL, Shiao JY, Lin JF, Su MC, Hsin CH (2008). Ethmoid sinus mycology of chronic rhinosinusitis. Eur. J. Clin. Microbiol. Infect. Dis. 27(4):253-257.
Liza A, Donald C, Laurie A, David W, Kathleen T (1997). *In situ* hybridization for *Aspergillus* and *Penicillium* in allergic fungal sinusitis: A rapid means of speciating fungal pathogens in tissues. Laryngoscope 107(2):244-240.
Lopez-Malo A, Alzamora SM, Palou E (2005). *Aspergillus flavus* growth in the presence of chemical preservatives and naturally occurring antimicrobial compounds. Int. J. Food. Microbiol. 99:119-128.
Manning SC, Holman M (1998). Further evidence for allergic pathophysiology in allergic fungal sinusitis. Laryngoscope. 108:1485-1496.
Marshall DL, Bullerman LB (1986). Effect of sucrose esters in combination with selected mold inhibitors in growth and aflatoxin production by *Aspergillus parasiticus*. J. Food. Prot. 49:378-382.
Montanaro A (2000). Allergic disease management in the elderly: A wakeup call for the allergy community. Ann. Asthma. Allergy. Immunol. 85(2):85-86.
Nagar AC, Gautam A (2015). Maxillary sinusitis of odontogenic origin: A review. Int. J. Adv. Res. 3(9):1323-1328.
Palou L, Usall J, Smilanick JL, Aguilar MJ, Viñas I (2002). Evaluation of food additives and low-toxicity compounds as alternative chemicals for control of *Penicillium digitatum* and *Penicillium italicum* on citrus fruit. Pest. Manag. Sci. 58:459-566.
Prateek S, Banerjee G, Gupta P, Singh M, Goel MM, Verma V (2013). Fungal rhinosinusitis: A prospective study in a University hospital of Uttar Pradesh. Ind. J. Med. Microbiol. 31:266-269.
Rabago D, Pasic T, Zgierska A, Mundt M, Barrett B, Maberry R (2005). The efficacy of hypertonic saline nasal irrigation for chronic sinonasal symptoms. Otolaryngol. Head. .Neck. Surg. 133(1):3-8.
Sari-Aslani F, Khademi B, Vatanibaf M, Noroozi M (2006). Diagnosis of allergic fungal rhinosinusitis. Iran. J. Med. Sci. 31(4):200-203.
Schubert MS (2004). Allergic fungal sinusitis: Pathogenesis and management strategies. Drugs 64(4):363-374.
Shirazi MA, Stankiewicz JA, Kammeyer P (2007). Activity of nasal amphotericin B irrigation against fungal organisms *in vitro*. Am. J. Rhinol. 21(2):145-148.
Sofos JN, Busta F (1991). Antimicrobial activity of sorbate. J. Food. Prot. 44:614-621.
Tekin M (2008). Microbiology evaluation for fungal involvement of the paranasal sinuses in Turkey. J. Chin. Clin. Med. 10(3):1-10.
Thiagarajan B, Ramamoorthy G (2013). Fungal sinusitis An Overview. Otolaryngol. Online. J. 3(2):1-12.
Tsai WY, Shao KP, Bullerman LB (1984). Effects of sorbate and propionate on growth and aflatoxin production by sublethally injured *Aspergillus parasiticus*. J. Food. Sci. 49:86-90.
Wald ER (1998). Microbiology of acute and chronic sinusitis in children and adults. Am. J. Med. Sci. 316(1):13-20.
Watanabe T (2010). Pictorial atlas of soil and seed fungi morphologies of cultured fungi and key to species.3rd ed. CRC press Washington.
Wayne WD (1983). Biostatistics: A Foundation for Analysis in the Health Science. 3rd ed. USA.