

## Antibacterial effect and healing potential of nutmeg oil for chemically induced oral ulcerations in rabbits

Received: 4/10/2011

Accepted: 29/7/2012

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### Abstract

**Background and objective:** *Myristica fragrans* is an aromatic green tree usually growing to around 5-13 meters high, and their seeds (nutmegs) is firm broadly ovoid. The objectives of the study were to find the concentration of nutmeg oil that has maximum antibacterial action against *Staphylococcus aureus* and to evaluate the healing potential of this concentration on a chemically induced oral ulceration in the rabbit's cheek mucosa.

**Methods:** Different concentrations of nutmeg oil were prepared and their antibacterial activity against *Staphylococcus aureus* was determined by disk diffusion method. The chemically induced oral ulcerations in rabbit's cheek mucosa were achieved by ethanol, and then these ulcerations were irrigated with 0.5ml (5%) of nutmeg oil twice daily. Biopsies were taken after four and eight days of the experiment, processed and stained by hematoxylin and eosin.

**Results:** The concentration 100% of nutmeg oil and the dimethyl sulfoxide; which was used as diluents for preparing different concentrations of nutmeg oil, showed no inhibition zone for *Staphylococcus aureus*. The concentration (5%) showed maximum inhibitory zone (16.8mm) for the micro-organism which was parallel to the effects of amoxicillin (positive control), both with highly significant action ( $P < 0.05$ ). The histopathological pictures showed a delay in the healing process of oral ulcers in rabbits treated by this concentration.

**Conclusion:** The concentration of nutmeg oil (5%) has a good antibacterial action against *Staphylococcus aureus*, but it causes a delay in the healing process of oral ulcerations.

**Keywords:** Nutmeg, essential oils, *myristica fragrans*.

### Introduction

The scientific name of nutmeg is *Myristica fragrans* houtt of the family myristicaceae, it comes from the tree *Myristica fragrans*, which originates from the Indonesian Banda Islands, and its therapeutic applications have been recorded by Arab physicians since the seventh century C.E, after that nutmeg appeared in Europe<sup>1, 2</sup>. In medicine the nutmeg was prescribed for headache, fevers, mouth-sores, excessive heat of the body, foul breath, diarrhea, intestinal weakness, and used as analgesic, sedative and as an anti-inflammatory<sup>3</sup>. The seed extracts of nutmeg possess a potent hepatoprotective activity<sup>4</sup>. The reported functional, medicinal and narcotic properties of nutmeg are due to myrsiticin.

Myrsiticin when taken in large dosages (about 15-25g) can cause hallucinations, vomiting, and epileptic symptoms<sup>5</sup>. The oral cavity is colonized by a diverse range of microorganism, these comprises 300–500 species of bacteria, fungi and protozoa. Bacteria that are potentially pathogenic and that are sometimes found in the oral cavity include *Staphylococcus aureus*, *Enterococcus faecalis*, *S. pneumoniae*, *Streptococcus pyogenes*, *Neisseria meningitidis*, and members of the family Enterobacteriaceae, *Haemophilus influenzae* and actinomycetes<sup>6</sup>. Of the bacteria that are easily recovered upon routine culture, the *Staphylococcus aureus* are among the most frequent isolates. The macelignan isolated from *Myrsiticin fragrans* possessed

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preferential activity against oral microorganisms such as *Staphylococcus aureus*, *Streptococcus sobrinus*, *Streptococcus salivarius*, *Streptococcus sanguis*, *Lactobacillus acidophilus* and *Lactobacillus casei*<sup>7,8</sup>. Nutmeg has been reported as an anti-plaque and anticariogenic activity against a cariogenic bacterium, *Streptococcus mutans*<sup>7</sup>. Nutmeg oil also showed effective antibacterial activity against 25 different bacteria, including animal and plant pathogens, food poisoning and spoilage<sup>9,10,11</sup>, and antifungal activity against several pathogenic fungi including *Aspergillus flavus* and *Candida albicans*<sup>12</sup>. Nutmeg contains 5%-15% essential oil, which mostly composed of camphene or sabinene, d-pinene, dipentene, d-linalool, d-borneol, i-terpineol, geraniol, myristicine, safrole, eugenol, and iso eugenol<sup>13</sup>. An important characteristic of essential oils and their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable. Extensive leakage from bacterial cells or the exit of critical molecules and ions lead to death<sup>14</sup>. Olajide *et al* (1999) found that nutmeg oil can be used for treatment of different types of oral sores because of its anti-inflammatory and antimicrobial activity<sup>3</sup>. This study was aimed to found the concentration of nutmeg oil that has maximum inhibition activity against *Staphylococcus aureus*, and to study the effect of this concentration on the healing potential of chemically induced oral ulcerations in the rabbit's cheek mucosa.

### Methods

16 male rabbits were used (*Oryctolagus cuniculus*), around four months old, and weighting (1-1.2 kg), all rabbits were housed under similar environmental conditions, with free access to food and water. The study was conducted in the period from September/2010 to March/2011, and the research project was approved by the Scientific Committee at the College of

Dentistry/Hawler Medical University. The animals were randomly distributed in to two groups: the control group (8 animals); they did not receive any treatment for their chemically induced oral ulcerations, and the study group (8 animals); in which their chemically induced oral ulcerations were treated by nutmeg oil. Nutmeg oil was obtained from the local market in Erbil city. Six different concentrations [ 1:1 (50%), 1:5 (20%), 1:10 (10%), 1:20 (5%), 1:40 (2.5%), and 1:60 (1.6%) ] of the oil were prepared to find the appropriate concentration which shows maximum inhibitory activity against *Staphylococcus aureus*, by diluting them with 10% aqueous dimethyle sulfoxide solution-DMSO( a powerful organic solvent that enhance the penetration of the oil through the mucous membranes), dispensed into dark bottles, and stored at 4°C<sup>14,15</sup>.

**Pilot study:** Creation of oral ulceration was done by intra mucosal injection of 0.1 ml (96%) of ethanol at right cheek mucosa of the rabbits. \_A swab from the chemically induced oral ulcer, in the second day of the experiment was taken and cultured on a conventional media (Blood agar) under aerobic condition at 37°C for 24 hrs. After the incubation period; the identification of *Staphylococcus* isolates was based on colonial morphology and Gram stain characteristics<sup>(6)</sup>. The selected bacteria (*Staphylococcus aureus*) were inoculated into 10 ml of sterile nutrient broth, and incubated at 37 °C for 18 hours. Using a sterile cotton swab, the nutrient broth cultures were swabbed on the surface of sterile nutrient agar plates. The antibacterial activity of nutmeg oil was determined by the disk diffusion method<sup>16,17</sup>. A sterile filter paper discs (6mm in diameter) impregnated with 50 µl of dimethyl sulfoxide (negative control), 100% nutmeg oil, and with different concentrations of nutmeg oil, were placed on a surface of nutrient agar plate for sensitivity testing. Standard 6 mm discs containing amoxicillin 25 µg/disc were used as positive controls. All petri dishes were sealed with sterile laboratory

parafilms, and the plates were left at ambient temperature for 30 min to allow good diffusion of the oil prior to incubation at 37 °C for 24 hour<sup>17</sup>. After that the size of inhibition zone was measured by a caliper. The studies were performed in triplicate, and mean values were calculated. Data analysis was made using ANOVA test. The comparisons between groups were done using Duncan test.  $P < 0.05$  was considered as statistically significant.

**The result of pilot study:** The different concentrations of nutmeg oil used showed different sizes of inhibition zones, and the concentration 1:20 (5%) showed maximum inhibitory zone (16.8mm) for the micro-organism Figure-1, which was parallel to the effect of amoxicillin on staphylococcus bacteria ( $P < 0.05$ ). The other concentrations of nutmeg oil [1:1 (50%), 1:5 (20%), 1:10 (10%), 1:40 (2.5%), and 1:60 (1.6%)] showed less size of inhibition zones [mean size 7.7, 8.2, 8.5, 7.9 mm in diameters respectively] as shown in Figure 2. Dimethyl sulfoxide solution and 100% nutmeg oil showed no inhibition zones against *Staphylococcus aureus*.

**Animal grouping and biopsy procedure:** After intra mucosal injection of ethanol at right cheek mucosa of the rabbit, the ulcer appear round in shape, white in color and surrounded by red halo after 24 hours from the injection time. The oral ulcerations of the study group were treated by irrigation with 0.5ml of (5%) nutmeg oil two times/day, which represent the best concentration with maximum inhibition zone. Because the chemically induced ulcer healed normally after nine to ten days, so in the fourth day of the experiment, eight animals (4 from control group and 4 from study group) were anesthetized with subcutaneous injection of Ketamin (40 mg/kg) and Xylazine (4mg/kg) for the biopsy procedure, and the biopsy from the other eight rabbits (4 from control group and 4 from study group) were taken after eight days. Using ocular grid, the mean number of neutrophils, lymphocytes, plasma cells,

histiocytes, and fibroblasts in the connective tissue in five microscopic fields per slide were calculated at 40 x magnifications, and the mean number of blood vessels were also calculated in five randomly selected microscopical fields but at 20 x magnifications.

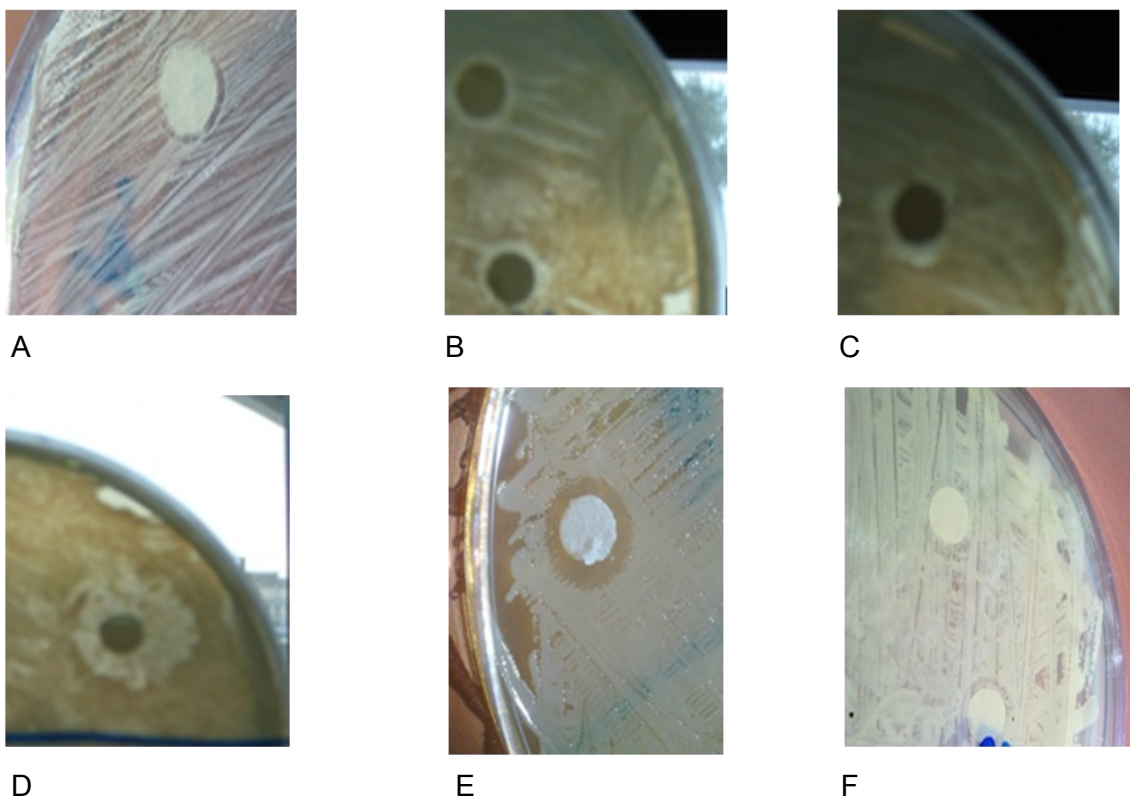
## Results

After laboratory processing of the biopsies which were taken from the oral ulcers in the control and study groups, the result showed that 5% nutmeg oil when used as a treatment for a chemically induced oral ulceration in the rabbit's cheek mucosa causes a delay in the healing process as seen in Table 1.

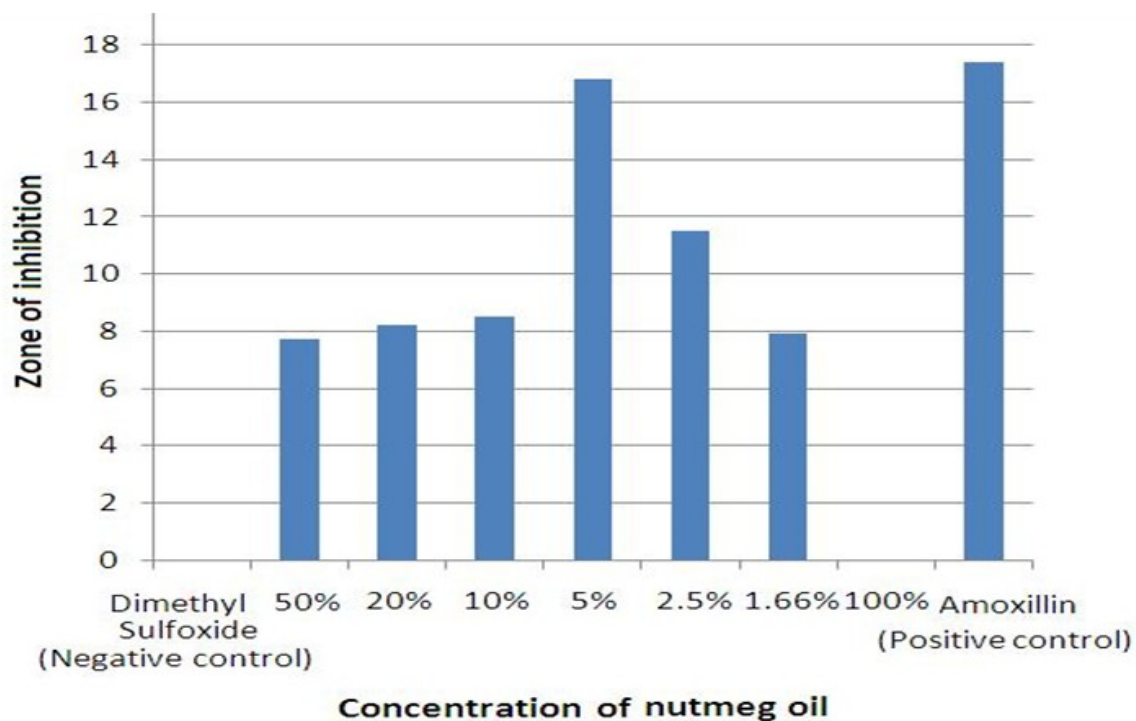
**Four days postoperatively:** Eight animals were scarified in the fourth day of the experiment (4 animals from the control group and 4 animals from the study group). The results showed that the number of neutrophils in the connective tissue of oral ulcer treated twice daily with 5% nutmeg oil was significantly higher than that of the control group ( $p < 0.05$ ), while the number of lymphocytes, plasma cells, histiocytes, and fibroblast cells were significantly less than that of the control group ( $p < 0.05$ ), but the reverse was true for the number of blood vessels.

### **Eight days postoperatively:**

Eight animals were scarified in the eight day of the experiment (4 animals from the control group and 4 animals from the study group). The results showed that the size of the ulcers in the study group were larger than that of the control group, this may be due to a delay in the growth of epithelial tongue (ET) which covers the surface of the ulcer gradually, in addition to that, areas of tissue necrosis at the site of ulcer were also seen within the connective tissue, Figure 3. The total number of inflammatory cells in the study group were significantly higher than that of the control group ( $p < 0.05$ ), and the number of fibroblast cells was less than that of the control group ( $p < 0.05$ ), but the number of blood vessels was nearly equal.



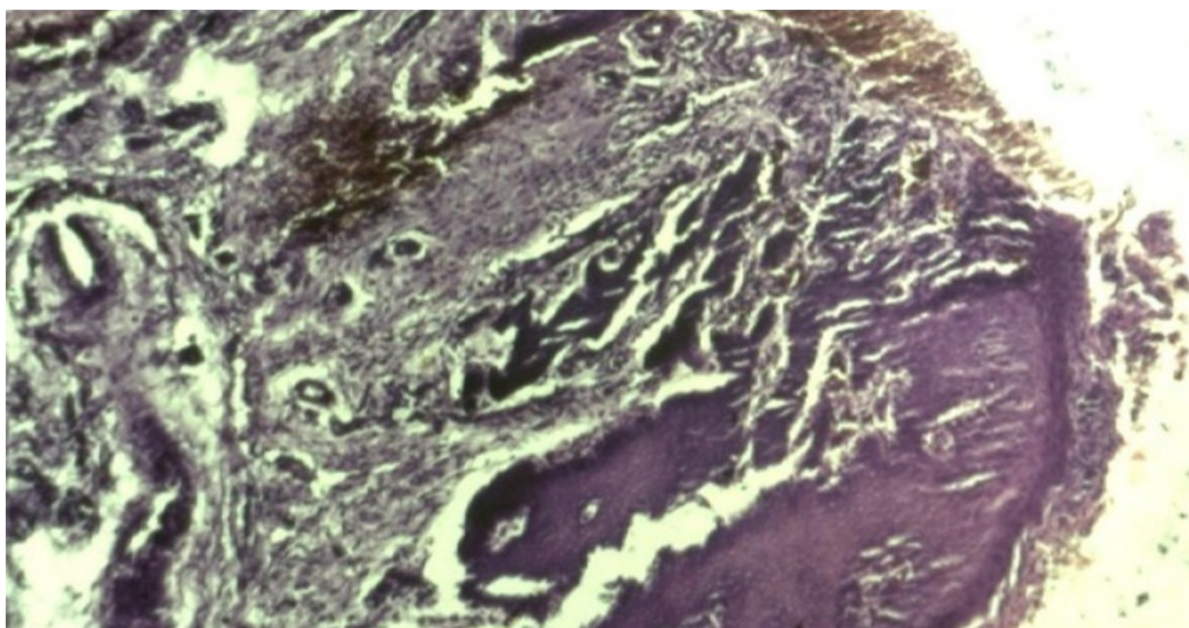
**Figure 1:** The mean size of inhibitory zone for the micro-organism in relation to different concentrations of nutmeg oil. A(50%), B(20%), C(10%), D(5%), E(2.5%), and F(1.6%).



**Figure 2:** The concentrations of nutmeg oil and the size of the inhibition zone.

**Table 1:** The mean number of neutrophils, lymphocytes, plasma cells, histiocytes, fibroblast cells, and blood vessels in the connective tissue of the oral ulcers for the control and study groups after four and eight days respectively.

Groups		Mean No. of inflammatory cells				Mean No. of fibroblast cells	Mean No. of blood vessels
		Neutrophils	Lymphocyte	Plasma cells	Histiocyte cells		
Control Group	After 4 Days	20	35	12	10	6	2
Study Group	After 4 Days	30	6	2	4	Zero	4
Control Group	After 8 Days	2	5	1	5	12	1
Study Group	After 8 Days	5	6	3	6	7	2



**Figure 3:** The histopathological picture after eight days of oral ulcer in the study group showing a delay in the growth of epithelial tongue (ET), large number of inflammatory cells in the connective tissue, and areas of necrosis (H&Ex20).

## Discussion

The present study showed that 5% concentration of nutmeg oil had the maximum antibacterial activity against the *Staphylococcus aureus* which is parallel to the effect of amoxicillin. This comes in agreement with the results of other studies<sup>8,18,19</sup>, but disagrees with the results of Seenivasan *et al* (2006), they showed that 5% nutmeg oil showed no inhibition zone against *Staphylococcus aureus* and the concentration 50% showed maximum inhibition zone of 11.7mm<sup>14</sup>; and the study of Charu *et al* (2008), they found that 12.5% of nutmeg oil showed maximum inhibition zone (14mm) against *Staphylococcus aureus*<sup>11</sup>. In addition to that Smith-Palmer *et al* (2004) found that the oil of nutmeg showed antibacterial activity at 1% (v/v) concentration when inoculated with 10 µl of an overnight culture of *Staphylococcus aureus*<sup>10</sup>. The differences in the results could be due to different methods of nutmeg oil extraction, different concentrations used, and different sources of the plant. The antibacterial activity of nutmeg oil is mainly related to its components, mainly camphene (which is about 50% of the essential oil), eugenol, thymol and linalool are also known for their good antimicrobial activity<sup>11,13,20,21</sup>. Even the dimethyl sulfoxide has been shown to help in treating ulcerations (taking around 500 mg of dimethyl sulfoxide four times per day can help in treating peptic ulcer)<sup>22</sup>, the result found that a concentration of 5% nutmeg oil cause a delay in the healing process of chemically induced oral ulceration. In the fourth day of the experiment, the histopathological pictures of the ulcers in the study group showed that the number of chronic inflammatory cells (lymphocytes, plasma cells and histiocytes) in the connective tissue were less than that of the control group, this could be due to the anti-inflammatory effect of nutmeg oil<sup>3</sup>. but there was a large number of neutrophils, this means that the ulcer was still in the acute stage; this may be due to the

continuous irritation by the chemical constituents of the nutmeg like the eugenol and iso eugenol. Maralhas *et al*(2006) found that toxic properties have been linked to eugenol<sup>23</sup>. These results disagree with the study of Olajide *et al* (1999) in which they found that nutmeg oil can be used for treatment of different types of oral sores because of its anti-inflammatory and antimicrobial activity<sup>3</sup>. This disagreement could be due to differences in the cause of oral ulceration, or the concentration and the source of nutmeg used.

## Conclusion

The 5% concentration of nutmeg oil has a good antibacterial action against *Staphylococcus aureus*, and can be used with the dental floss, tooth wedges, and for irrigation of the root canals, but not for treatment of oral ulcerations, because it retards the healing process.

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