Antibacterial activity of *Ocimum sanctum* L. fixed oil

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_Ocimum sanctum_ fixed oil showed good antibacterial activity against *Staphylococcus aureus*, *Bacillus pumilus* and *Pseudomonas aeruginosa*, where _S. aureus_ was the most sensitive organism. Sesame and soyabean oils also showed moderate activity against _S. aureus_. Higher content of linolenic acid in _O. sanctum_ fixed oil could contribute towards its antibacterial activity. The antibacterial activity combined with anti-inflammatory and analgesic activities of the oil, could make it useful in inflammatory disorder resulting from staphylococcal infection.

**Keywords:** Antibacterial, Linolenic acid, *Ocimum sanctum* fixed oil, _S. aureus_.

*Ocimum sanctum* L. (Labiatae) commonly known as “Holy basil” is a herbaceous plant found throughout India. Different parts of the plant have been claimed to be valuable against a number of diseases1. In earlier studies, _O. sanctum_ fixed oil was found to possess significant anti-inflammatory2, antipyretic, analgesic, antiarthritic and antiulcer activity3. The fixed oil contains five fatty acids viz. palmitic (11.69%), stearic (3.19%), oleic (13.82%), linoleic (32.23%) and linolenic acid (16.63%)4 and linolenic acid appears to be responsible for the anti-inflammatory activity of the oil4. In the present study, antibacterial activity of _O. sanctum_ fixed oil has been evaluated against a number of microorganisms and the same has been compared with sesame and soyabean oils, which contain varying proportions of unsaturated fatty acids.

Dried seeds of _O. sanctum_ were collected from Maidan Garhi (New Delhi, India) and were authenticated by a resident botanist of the Department of Genetics, Indian Agricultural Research Institute, New Delhi and voucher samples were deposited in the Department of Genetics. The seeds were crushed and cold macerated in petroleum ether (40°-60°C) (S.D.Fine Chemicals Ltd., India) for 3 days. The petroleum ether was evaporated from the extract and oil was filtered to clarity (weight per ml at 25°C is 0.8750). The fixed oil thus obtained was tested for its antibacterial activity. Refined food grade soyabean (Alpine Industries Limited, Madhya Pradesh, India) and sesame (Ahmed Mills, Mumbai, India) oils used in the study were procured locally. The bacterial strains used in the study were received from Indian Drugs & Pharmaceuticals Ltd., Gurgaon, India.

**Antibacterial activity**—Antibacterial activity of _O. sanctum_ fixed oil was evaluated against *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Micrococcus luteus*, *Bacillus pumilus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The microorganisms were maintained on agar slants made of antibiotic assay medium A (Hi-Media, Mumbai, India) making monthly transfers. Antibacterial activity was evaluated by paper disc diffusion method5. Overnight slant of the microorganism was washed with sterile saline and the cell suspension was further diluted with sterile saline so that when read in a colorimeter at 530 nm it produced 25% light transmission. The cell suspension (0.1 ml) was used to inoculate 100 ml molten antibiotic assay medium A (sterile). This inoculated medium was poured in 9 cm petridishes (Borosil) and the medium was allowed to solidify. Sterile paper discs of 4 mm diameter (made from Whatman no.1 filter paper) were soaked in the oil and each disc in triplicate was placed on the inoculated media contained in the petridish. Each petridish was incubated at 37°C. After 18 hr of incubation, the clear zone of inhibition in each petridish was measured in mm. Antibacterial activities of soyabean and sesame oil were evaluated against *Staphylococcus aureus*, *Bacillus pumilus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* using the same method described above.

**Determination of inhibitory concentration** of _O. sanctum_ fixed oil—_O. sanctum_ fixed oil was incorporated in molten antibiotic assay medium to final concentrations of 0.1, 0.5 and 1.0% (v/v). The

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medium was then shaken for 20 sec and cooled under running cold water with rotation until the temperature was about 40°C. The resultant dispersion was poured in 20 ml quantities in 9 cm petridishes. The plates were dried for 3 hr at 37°C and then inoculated with cultures (Staphylococcus aureus or Bacillus pumilus) diluted sufficiently to give isolated colonies. The end point (MIC) was defined as the concentration of oil required to inhibit the growth of 95% of the inoculated organism after incubation at 37°C for 24 hr.

The oil showed good antibacterial activity against S. aureus, B. pumilus and Ps. aeruginosa where S. aureus was the most sensitive organism (zone of inhibition 8.0 mm). The oil however was less active against E. coli, K. pneumoniae, S. typhi and S. epidermidis and inactive against B. subtilis and M. luteus. Sesame and soyabeen oil also showed moderate antibacterial activity against S. aureus and both had lesser activity against B. pumilus, Ps. aeruginosa, E. coli and K. pneumoniae (Table 1).

It is believed that antibacterial activity of fatty acids is related to their degree of unsaturation. The inhibitory capacity might increase with the degree of unsaturation so that linolenic acid (which contains three double bonds) is the most inhibitory, linoleic (two double bonds) less so, the oleic (one double bond) and stearic (saturated) with insignificant antibacterial properties. Kabara et al., have reported linoleic acid as more inhibitory to bacteria than linolenic while Lacey and Lord described linolenic acid as more inhibitory to S. aureus. In the present study, O. sanctum fixed oil has been found to be most inhibitory (zone of inhibition 8 mm) against S. aureus followed by sesame (zone of inhibition 6.5 mm) and soyabean oils (zone of inhibition 5.5 mm). O. sanctum fixed oil contains linoleic (52.23%) and linolenic acid (16.63%), soyabean oil also contains linoleic acid (50%) and linolenic acid (7.0%), while sesame oil contains linolenic acid (43%) only. Sesame oil does also contain a phenolic constituent called sesamol.

Thus the superior antibacterial activity of O. sanctum fixed oil could be due to its higher content of linolenic acid. It also appears that both linoleic acid and sesamol contribute towards antibacterial activity of sesame oil.

The minimum inhibitory concentration (MIC) of O. sanctum fixed oil against B. pumilus was found to be 0.1% (v/v). But with S. aureus 0.1% (v/v) concentration of oil caused 95% inhibition whereas 0.5-1.0% (v/v) concentration provided 52% inhibition of the organism. Thus MIC of the oil against S. aureus is difficult to define. Some more studies are needed to ascertain the fact. It would be worthwhile to mention here that such a paradoxical effect whereby a higher concentration is less inhibitory than a low one, has also been observed by Lacey and Lord with linoleic acid and linolenic acid against certain strains of S. aureus.

Efficacy of O. sanctum fixed oil has been reported against bovine mastitis, a disease caused mostly by S. aureus infection of the udder resulting in inflammation. It has been observed that the oil given by intramammary injection (3 ml/quarter) daily, for 5 days, can cure mastitis in buffaloes. Combination of the oil with cloxacillin sodium (200 mg), a beta-lactamase resistant penicillin, however produced curative effect within 3 days while cloxacillin alone needed 4 days to cure the condition. Thus the in vivo efficacy of the oil against mastitis compliments its in vitro antibacterial activity against S. aureus.

On the basis of results available it can therefore be concluded that O. sanctum fixed oil possesses good antibacterial activity against S. aureus, B. pumilus and Ps. aeruginosa where S. aureus appears to be the most sensitive organism. Sesame and soyabeen oils

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>O. sanctum</th>
<th>Soyabean</th>
<th>Sesame</th>
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</thead>
<tbody>
<tr>
<td>Staphylococcus aureus (ATCC 29737)</td>
<td>+++</td>
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<tr>
<td>Bacillus pumilus (ATCC 14884)</td>
<td>+++</td>
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<tr>
<td>Escherichia coli (ATCC 10536)</td>
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<tr>
<td>Klebsiella pneumoniae (ATCC 10031)</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Pseudomonas aeruginosa (ATCC 235619)</td>
<td>+++</td>
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<tr>
<td>Salmonella typhi (NCTC 786)</td>
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<tr>
<td>Staphylococcus epidermidis (ATCC 12228)</td>
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<tr>
<td>Bacillus subtilis (ATCC 6633)</td>
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<td>Micrococcus luteus (ATCC 10240)</td>
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Activity of oil (inhibition zones): - absent, + slight (4-4.5 mm), ++ moderate (5.5-6.5 mm), +++ good (7.0-8.0 mm)
also possess moderate anti-staphylococcal activity. Higher content of linolenic acid in *O. sanctum* fixed oil could contribute towards its antibacterial activity. The antibacterial activity combined with the anti-inflammatory and analgesic activities of the oil could make it useful in inflammatory disorder resulting from staphylococcal infection.

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