

ANTIMICROBIAL ACTIVITY OF FULLER'S EARTH, TURMERIC, AND SANDALWOOD AGAINST STREPTOCOCCUS MUTANS, MICROCOCCI AND COAGULASE NEGATIVE STAPHYLOCOCCI -AN IN VITRO STUDY

RUNNING TITLE: Antimicrobial activity of fullers earth, turmeric, and sandalwood against *Streptococcus mutans*, *Micrococci*, and *Coagulase-negative staphylococci* - an in vitro study

ABSTRACT:

INTRODUCTION:

Sandalwood powder (*Santalum alba*) is used if the skin is oily for removing dark spots on the skin. Sandalwood has an anti-tanning and anti-aging property . Turmeric powder (*Curcuma longa*) is mainly used to rejuvenate the skin. It helps to delay aging like wrinkles and also possesses other properties like antibacterial, antiseptic, and anti-inflammatory, and many other properties. Multani mitti helps the skin in many different ways like the reduction of pore size pore sizes, removing blackheads and removing whiteheads fading freckles, soothing sunburns, cleansing skin, improving blood circulation, complexion, reducing acne and blemishes, and gives a glowing effect to your skin as they contain many-particles which can inhibit various organisms.

MATERIALS AND METHODS:

In this investigation, the antimicrobial viability of Turmeric, sandalwood and Multani Mutti was tested against *Streptococcus mutans*, *Micrococci*, and *coagulase-negative staphylococcus*. The microorganisms were filled in strong media, and culture containing stock suspensions was made and, in this way, standard strains of *Streptococcus mutans*, *Micrococci*, and *Coagulase-negative staphylococci* were obtained. Microorganisms were subcultured in fitting society media to affirm their virtue.

RESULTS:

The zone of inhibition of turmeric against *Streptococcus mutans* is about 11mm. The zone of inhibition of turmeric against *Micrococci* is about 9 mm . The zone of inhibition of turmeric against *Coagulase-negative staphylococci* is about 13mm. There was no zone of inhibition for both sandalwood and Multani mitti for *Coagulase-negative staphylococci*, *Streptococcus mutans*, and *Micrococci*

CONCLUSION:

The study proves the turmeric has antimicrobial potential against *Coagulase-negative staphylococci*, *Streptococcus mutans*, and *Micrococci* with good prospects of development into antimicrobial face pack or face wash

Keyword: Antimicrobial activity - fullers earth - turmeric - sandalwood - *Streptococcus mutans* - *micrococcus* - ecofriendly - *Coagulase-negative staphylococci* - an in vitro study

INTRODUCTION:

Microbial activity is important for a series of soil reactions and functions, including organic matter decomposition, humus formation, nutrient cycling, aggregate formation, and stabilization (1). Microbial activity in the final product may be derived from contamination sources located in the production line even preprocessing such as pre-drying is carried out in a hygienic condition. Contamination sources may be established at any site where temperature and moisture provide conditions that are conducive for the multiplication of microorganisms (2). The English word “fuller”, comes from “foulon” in French or “fullone” in Italian, which derives from the Latin term “fullo”, indicating a person who degreases and thickens clothes. Fuller’s Earth can be also used to decontaminate skin and it has various other properties. Fuller's Earth resembles clay in texture and form (3,4)

Fullers Earth more commonly known as ‘Multani mitti’ is used for healing purposes, especially of the skin. It is natural and has a variety of effects on metabolism in our body (5). Multani mitti is a clay deposit found in Pakistan, it is found in plenteous deposits, which are located in Multan. Scientifically, Multani mitti is a mineral of montmorillonite smectite and bentonite clay, From the ancient Chinese, Mesopotamians, Egyptian, and Indian civilizations, it is easy to find out various beneficial and curative effects of clay. (6). Clay has a variety of applications i.e. aesthetic medicine, dietary supplements, therapeutics, healing, and cleaning agents (7). It is a superabsorbent form of aluminum silicate. Mostly composed of silica, magnesium, iron, and aluminum, it has been used to absorb dirt and oil used by people throughout many years. . Recent developments of procedures for cultivating and identifying microorganisms are aiding microbiologists in their assessment of the earth's full range of microbial diversity(8)*Fuller's earth*, a grey-green powder, is hydrated aluminum silicate and aluminum-magnesium sulfate. Employed for its absorbing properties in cleansing products (9). Sandalwood powder (*Santalum alba*) is used if the skin is oily for removing dark spots on the skin. Sandalwood has an anti-tanning and anti-aging property. It also helps the skin in many ways like toning effect, emollient, antibacterial properties, cooling astringent property, soothing and healing property.

Turmeric powder (*Curcuma longa*) is mainly used to rejuvenate the skin. It helps to delay aging like wrinkles and also possesses other properties like antibacterial, antiseptic, and anti-

inflammatory, and many other properties. Turmeric in the powdered state has been in continuous use for its flavoring, as a spice in both veg and non-veg food, it also has digestive properties. It is the best source of a blood purifier. It is a very effective cure of acne due to its antiseptic antibacterial and antimicrobial properties that fight pimples to provide a shining glow to your skin. It also reduces the oil secretion which is produced by sebaceous glands. *Fuller's earth* (Multani mitti) helps the skin in many different ways like the reduction of pore size pore sizes, removing blackheads and removing whiteheads fading freckles, soothing sunburns, cleansing skin, improving blood circulation, complexion, reducing acne and blemishes, and gives a glowing effect to your skin as they contain many-particles which can inhibit various organisms. Multani mitti is rich in magnesium chloride (10). Our team has extensive knowledge and research experience that has translated into high quality publications(11–22),(23–27). (28) (29) (30) . The aim of the study is to find out the antimicrobial activity of fuller's earth against microbes present in the face like *Streptococcus mutans*, *Micrococci*, and *Coagulase-negative staphylococci*

Materials and methods

Preparation of Turmeric paste

Turmeric was purchased from the herbal care store and then 5 grams of turmeric was taken and soak it in water for about 1 hour and after which it is made into a paste with mortar and pestle and then transferred into a sterile test tube the test tube was kept in a hot air oven for sterilization after which was transferred into a sterile container

Preparation of Sandalwood paste

Sandalwood was purchased from herbal care store and then about 5 grams of the sandal was taken and soaked in water for about 1 hour and after which was made to paste with the help of mortar and pestle and then transfer into a sterile test tube which was kept in the hotter oven for sterilization after which it was transferred into a sterile container

Preparation of Multani Mutti paste

The Multani mitti powder was purchased from herbal care store after about 5 grams was taken made into a paste by adding water and mixing it in the mortar and pestle and that it was transferred into a sterile tissue which was kept in the hot air oven for sterilization and after it was transferred into a sterile container

Agar well diffusion method

Standard strains of *Streptococcus mutans*, *Micrococci*, and *Coagulase-negative staphylococci* grown on enriched media and incubated for 12 hours. These fresh cultures were used to make a suspension in saline with turbidity matching 0.5 Mcfarland standard, 50 microliters of the suspension was pipetted and placed on the surface of Mueller Hinton agar and uniformly spread using a sterile swab. Then using a sterile metal tube of 6 mm diameter 3 wells were cut on the

media. The wells are filled with fullers earth , turmeric, and sandalwood. After incubation, the zone of inhibition was measured in millimeters and tabulated. Measure the zone of inhibition after 12 hrs. Take care to avoid contamination.

RESULTS

The zone of inhibition of turmeric against *Streptococcus mutans* is about 11mm. The zone of inhibition of turmeric against *Micrococci* is about 9 mm . The zone of inhibition of turmeric against *Coagulase-negative staphylococci* is about 13mm. There was no zone of inhibition for both sandalwood and Multani mitti for *Streptococcus mutans*, *Micrococci*, and *Coagulase-negative staphylococci* . The highest zone of inhibition was noted for turmeric for *Coagulase-negative staphylococci* followed by the zone of inhibition of turmeric for *Streptococcus mutans*, and the zone of inhibition of turmeric for *Micrococci*.

	Coagulase-negative staphylococci	S.MUTANS	Micrococci
Turmeric	13 mm	11mm	9mm
Sandalwood	0	0	0
Multani mutti	0	0	0

TABLE 1: Table showing the zone of inhibition for fullers earth, turmeric, and sandalwood against *Streptococcus mutans*, *Micrococci*, and *Coagulase-negative staphylococci*



FIGURE 1: The image shows the zone of inhibition of turmeric against *micrococcus*



FIGURE 2: The image shows the zone of inhibition of turmeric against *Streptococcus mutans*,



FIGURE 3: The image shows the zone of inhibition of turmeric against *Coagulase-negative staphylococci*

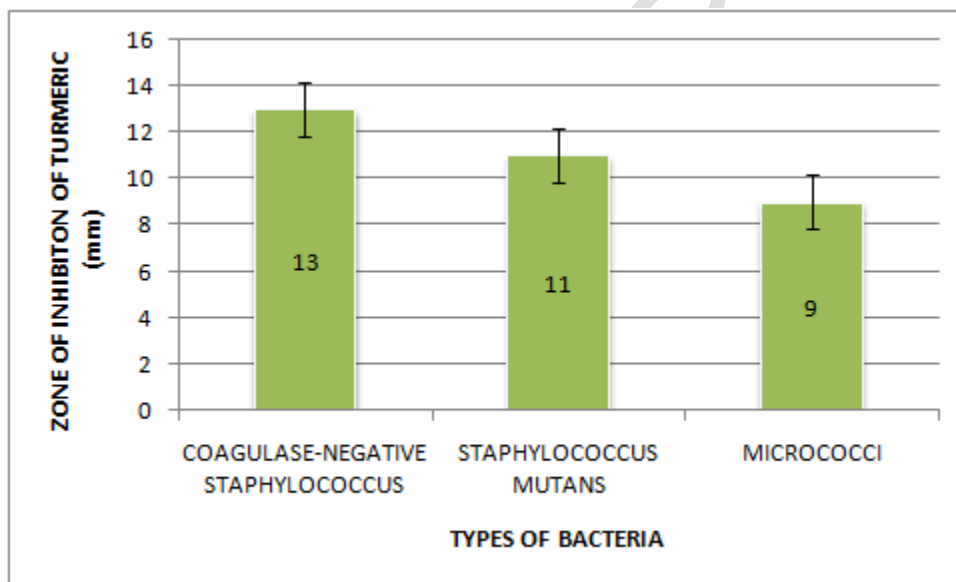


FIGURE 4: Bar Graph showing the zone of inhibition on *Streptococcus mutans*, *Micrococci*, and *Coagulase-negative staphylococci* on turmeric in which zone of inhibition of turmeric against *Streptococcus mutans* is about 11mm. The zone of inhibition of turmeric against *Micrococci* is about 9 mm . The zone of inhibition of turmeric against *Coagulase-negative staphylococci* is about 13mm

DISCUSSION

According to the study, The zone of inhibition of turmeric against *Streptococcus mutans* is about 11mm. The zone of inhibition of turmeric against *Micrococci* is about 9 mm . the zone of inhibition of turmeric against *Coagulase-negative staphylococci* is about 13mm. There was no zone of inhibition for both sandalwood and Multani mitti for *Coagulase-negative staphylococci*, *Streptococcus mutans*,, and *Micrococci*.

Turmeric powder (*Curcuma longa*) Turmeric is mainly used to rejuvenate the skin. It helps to delay aging like wrinkles and also possesses other properties like antibacterial, antiseptic, and anti-inflammatory, and many other properties. Turmeric in the powdered state has been in continuous use for its flavoring, as a spice in both veg and non-veg food, it also has digestive properties. In a previous study, (31) . The maximum zone of inhibition was 13.5 mm which was noted for *Staphylococcus aureus* and it showed a zone of inhibition of 11.5 mm in 12 μ g concentration in *Staphylococcus aureus*. Whereas it showed zero inhibition in *Candida albicans* and *Staphylococcus aureus* in water extracted samples. And zero zone of inhibition for water extracted samples in *Candida albicans* applied on both concentration Whereas in our study the maximum zone of inhibition was voted for turmeric in *Coagulase-negative staphylococcus* which was 13 mm and sandal and Multani mitti show zero inhibition in all three organisms. In a study done by (32) the zone of inhibition in turmeric against *Staphylococcus aureus* showed about 14 mm and the zone of inhibition in turmeric against *Escherichia coli* showed about 10 mm, compared to our study, our study Showed about in turmeric it showed 13 mm zone of inhibition in *Coagulase-negative staphylococci*, 11mm zone of inhibition in *Streptococcus mutans* and 9, mm zone of inhibition in *Micrococci* whereas in Zero zone of inhibition in sandalwood and Multani Mutti in all organisms .

In a previous study by (33) the turmeric oil was effective in inhibiting the three pathogenic bacteria with zone of inhibition of 7 mm, 8 mm, and 9 mm against *S. mutans*, *Enterococcus*, and *S. aureus*, respectively, in the concentration of 25 μ g/ml. In 50 μ g/ml, the zone of inhibition was found to be 8 mm, 5 mm, and 7 mm against the oral pathogens, respectively. For 100 μ g/ml, it was 8 mm, 5 mm, and 12 mm against *S. mutans*, *Enterococcus*, and *S. aureus*, whereas in our study our study Showed about in turmeric it showed 13 mm zone of inhibition in *Coagulase-negative staphylococci*, 11mm zone of inhibition in *Streptococcus mutans* and 9, mm zone of inhibition in *Micrococci* . In a study done by (34) 100 μ g/ml curcumin extracted into the culture plates of *S. mutans*, it shows 78.35% of inhibition of the biofilm formation. From the result, it is evident that curcumin has a very good inhibitory effect on *S. mutans* growth similar to our study which is zone of inhibition in *Coagulase-negative staphylococci*, 11mm zone of inhibition in *Streptococcus mutans* and 9, mm zone of inhibition in *Micrococci* whereas in Zero zone of inhibition in sandalwood and Multani Mutti in all organisms.

Sandalwood powder (*Santalum alba*) is used if the skin is oily for removing dark spots on the skin. Sandalwood has an anti-tanning and anti-aging property. It also helps the skin in many ways like toning effect, emollient, antibacterial properties, cooling astringent property, soothing and healing property. In a previous study by (35,36) *Santalum alba* exhibited the highest antimicrobial activity against one strain of *S. hominis* which was 8.67 mm. Whereas in another previous study by (37) the zone of inhibition of sandalwood is 6.25 mm which was detected against *S. capitis* and all strains of *S. hominis* which showed contrasting findings with our results because in our study sandalwood showed zero zone of inhibition against all 3 organisms which are *Streptococcus mutans*, *Micrococci*, and *Coagulase-negative staphylococci*. In a previous study done by (35) the ethanolic extracts of sandalwood, seeds were shown to have no antibacterial properties (Patil et al. 2011), thereby indicating that the seeds do not accumulate such principles, whereas the aqueous extracts from leaves and stem did show antimicrobial potential whereas in our study the sandalwood paste which was done in sandalwood stem also showed zero zones of inhibition and no antimicrobial activity.

Multani mitti helps the skin in many different ways like the reduction of pore size, removing blackheads and removing whiteheads, fading freckles, soothing sunburns, cleansing skin, improving blood circulation, complexion, reducing acne and blemishes, and gives a glowing effect to your skin as they contain many particles which can inhibit various organisms. A study done by Silver nanoparticles of biogenic materials inhibits bacterial growth by giving a clear inhibition zone. Different sizes of cubic AgNPs were estimated in which small cubic AgNPs of Multanimitti which showed the mean size 4.6 nm which showed the strongest antibacterial activity, whereas in our study Multani Mutti showed zero zones of inhibition against all 3 organisms which is *Streptococcus mutans*, *Micrococci*, and *Coagulase-negative staphylococci*. (37,38)

CONCLUSION

Thus, from the current study, Turmeric is found to have a very good antimicrobial effect on *Streptococcus mutans*, *Micrococci*, and *Coagulase-negative staphylococci*, and It can recommend to be made into a face pack, face wash for people with rashes, pimples, and dark skin as a remedy.

REFERENCE

1. Research on Nitrification and Related Processes, Part B [Internet]. Methods in Enzymology. 2011. Available from: <http://dx.doi.org/10.1016/c2010-0-66910-0>
2. Horn JM, Meike A. Microbial activity at Yucca Mountain [Internet]. 1995. Available from: <http://dx.doi.org/10.2172/177391>

3. Sanford PG. The Fullers Earth of Nutfield [Internet]. Vol. 6, Geological Magazine. 1889. p. 526–526. Available from: <http://dx.doi.org/10.1017/s0016756800189642>
4. Fullers Earth [Internet]. Vol. 11, Scientific American. 1856. p. 179–179. Available from: <http://dx.doi.org/10.1038/scientificamerican02161856-179a>
5. Thakur P, Garg RK. New developing reagent for latent fingermark visualization: Fuller's earth (Multani Mitti) [Internet]. Vol. 6, Egyptian Journal of Forensic Sciences. 2016. p. 449–58. Available from: <http://dx.doi.org/10.1016/j.ejfs.2016.11.007>
6. Waheed S, Siddique N, Faiz Y. Rare Earth and High Field-Strength Elements in the Multani Mitti Clay: A Study Using INAA [Internet]. Vol. 37, Geostandards and Geoanalytical Research. 2013. p. 197–205. Available from: <http://dx.doi.org/10.1111/j.1751-908x.2012.00186.x>
7. Waheed S, Faiz Y, Rahman S, Siddique N. Toxic element composition of multani mitti clay for nutritional safety [Internet]. Vol. 295, Journal of Radioanalytical and Nuclear Chemistry. 2013. p. 143–50. Available from: <http://dx.doi.org/10.1007/s10967-012-1876-x>
8. Waheed S, Rahman S, Faiz Y, Siddique N. Neutron activation analysis of essential elements in Multani mitti clay using miniature neutron source reactor [Internet]. Vol. 70, Applied Radiation and Isotopes. 2012. p. 2362–9. Available from: <http://dx.doi.org/10.1016/j.apradiso.2012.06.030>
9. Malik S, Lal K, Fatima NG, Samo A, Haque S. Congenital constriction ring of limbs in subjects with history of maternal substance use. J Coll Physicians Surg Pak. 2015 May;25(5):383–5.
10. S.k. R, Rubina SK. FORMULATION AND EVALUATION OF NATURAL HERBAL FACE PACK [Internet]. World Journal of Pharmaceutical Research. 2017. p. 1561–73. Available from: <http://dx.doi.org/10.20959/wjpr20178-9030>
11. Priyadharsini JV, Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. In silico analysis of virulence genes in an emerging dental pathogen *A. baumannii* and related species [Internet]. Vol. 94, Archives of Oral Biology. 2018. p. 93–8. Available from: <http://dx.doi.org/10.1016/j.archoralbio.2018.07.001>
12. Vijayashree Priyadharsini J. In silico validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens. J Periodontol. 2019 Dec;90(12):1441–8.
13. Paramasivam A, Vijayashree Priyadharsini J, Raghunandhakumar S. N6-adenosine methylation (m6A): a promising new molecular target in hypertension and cardiovascular diseases. Hypertens Res. 2020 Feb;43(2):153–4.
14. Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. An insight into the emergence of *Acinetobacter baumannii* as an oro-dental pathogen and its drug resistance gene profile - An in silico approach. Heliyon. 2018 Dec;4(12):e01051.

15. Paramasivam A, Vijayashree Priyadharsini J. Novel insights into m6A modification in circular RNA and implications for immunity. *Cell Mol Immunol.* 2020 Jun;17(6):668–9.
16. Paramasivam A, Priyadharsini JV, Raghunandhakumar S. Implications of m6A modification in autoimmune disorders. *Cell Mol Immunol.* 2020 May;17(5):550–1.
17. Girija ASS, Shankar EM, Larsson M. Could SARS-CoV-2-Induced Hyperinflammation Magnify the Severity of Coronavirus Disease (CoViD-19) Leading to Acute Respiratory Distress Syndrome? *Front Immunol.* 2020 May 27;11:1206.
18. Jayaseelan VP, Arumugam P. Exosomal microRNAs as a promising theragnostic tool for essential hypertension. *Hypertens Res.* 2020 Jan;43(1):74–5.
19. Ushanthika T, Smiline Girija AS, Paramasivam A, Priyadharsini JV. An in silico approach towards identification of virulence factors in red complex pathogens targeted by reserpine. *Nat Prod Res.* 2021 Jun;35(11):1893–8.
20. Ramalingam AK, Selvi SGA, Jayaseelan VP. Targeting prolyl tripeptidyl peptidase from *Porphyromonas gingivalis* with the bioactive compounds from *Rosmarinus officinalis*. *Asian Biomed.* 2019 Oct 1;13(5):197–203.
21. Kumar SP, Girija ASS, Priyadharsini JV. Targeting NM23-H1-mediated inhibition of tumour metastasis in viral hepatitis with bioactive compounds from *Ganoderma lucidum*: A computational study. *pharmaceutical-sciences* [Internet]. 2020;82(2). Available from: <https://www.ijpsonline.com/articles/targeting-nm23h1-mediated-inhibition-of-tumour-metastasis-in-viral-hepatitis-with-bioactive-compounds-from-ganoderma-lucidum-a-comp-3883.html>
22. Mathivadani V, Smiline AS, Priyadharsini JV. Targeting Epstein-Barr virus nuclear antigen 1 (EBNA-1) with *Murraya koenigii* bio-compounds: An in-silico approach. *Acta Virol.* 2020;64(1):93–9.
23. Samuel SR, Kuduruthullah S, Khair AMB, Shayeb MA, Elkaseh A, Varma SR. Dental pain, parental SARS-CoV-2 fear and distress on quality of life of 2 to 6 year-old children during COVID-19. *Int J Paediatr Dent.* 2021 May;31(3):436–41.
24. Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? *Int J Paediatr Dent.* 2021 Mar;31(2):285–6.
25. Barma MD, Muthupandiyan I, Samuel SR, Amaechi BT. Inhibition of *Streptococcus mutans*, antioxidant property and cytotoxicity of novel nano-zinc oxide varnish. *Arch Oral Biol.* 2021 Jun;126:105132.
26. Teja KV, Ramesh S. Is a filled lateral canal - A sign of superiority? *J Dent Sci.* 2020 Dec;15(4):562–3.
27. Reddy P, Krithikadatta J, Srinivasan V, Raghu S, Velumurugan N. Dental Caries Profile and Associated Risk Factors Among Adolescent School Children in an Urban South-Indian

City. *Oral Health Prev Dent*. 2020 Apr 1;18(1):379–86.

28. Jayaseelan VP, Paramasivam A. Emerging role of NET inhibitors in cardiovascular diseases. *Hypertens Res*. 2020 Dec;43(12):1459–61.
29. Iswarya Jaisankar A, Smiline Girija AS, Gunasekaran S, Vijayashree Priyadharsini J. Molecular characterisation of *csgA* gene among ESBL strains of *A. baumannii* and targeting with essential oil compounds from *Azadirachta indica*. *Journal of King Saud University - Science*. 2020 Dec 1;32(8):3380–7.
30. Girija AS. Fox3 (+) CD25 (+) CD4 (+) T-regulatory cells may transform the nCoV's final destiny to CNS! COMMENT. WILEY 111 RIVER ST, HOBOKEN 07030-5774, NJ USA; 2021.
31. Teow S-Y, Liew K, Ali SA, Khoo AS-B, Peh S-C. Antibacterial Action of Curcumin against : A Brief Review. *J Trop Med*. 2016 Nov 13;2016:2853045.
32. Kačániová M, Terentjeva M, Štefániková J, Žiarovská J, Savitskaya T, Grinshpan D, et al. Chemical Composition and Antimicrobial Activity of Selected Essential Oils against *Staphylococcus* spp. Isolated from Human Semen [Internet]. Vol. 9, *Antibiotics*. 2020. p. 765. Available from: <http://dx.doi.org/10.3390/antibiotics9110765>
33. Ortiz M. Antimicrobial Activity of Onion and Ginger against two Food Borne Pathogens *Escherichia Coli* and *Staphylococcus Aureus* [Internet]. Vol. 1, *MOJ Food Processing & Technology*. 2015. Available from: <http://dx.doi.org/10.15406/mojfpt.2015.01.00021>
34. G G, Gokul G, Rv G. EFFECT OF CURCUMA LONGA EXTRACT ON BIOFILM FORMATION BY STREPTOCOCCUS MUTANS [Internet]. Vol. 10, *Asian Journal of Pharmaceutical and Clinical Research*. 2017. p. 186. Available from: <http://dx.doi.org/10.22159/ajpcr.2017.v10i7.18161>
35. Jirovetz L, Buchbauer G, Denkova Z, Stoyanova A, Murgov I, Gearon V, et al. Comparative study on the antimicrobial activities of different sandalwood essential oils of various origin [Internet]. Vol. 21, *Flavour and Fragrance Journal*. 2006. p. 465–8. Available from: <http://dx.doi.org/10.1002/ffj.1625>
36. Misra BB, Dey S. Comparative phytochemical analysis and antibacterial efficacy of in vitro and in vivo extracts from East Indian sandalwood tree (*Santalum album L.*) [Internet]. Vol. 55, *Letters in Applied Microbiology*. 2012. p. 476–86. Available from: <http://dx.doi.org/10.1111/lam.12005>
37. Misra BB, Dey S. Biological Activities of East Indian Sandalwood Tree, *Santalum album* [Internet]. Available from: <http://dx.doi.org/10.7287/peerj.preprints.96>
38. Supplemental Information 1: Phylogenetic analysis of C-type LecRLKs from *Arabidopsis thaliana*, apple (*Malus domestica*), potato (*Solanum tuberosum*), rice (*Oryza sativa*) and tomato (*Solanum lycopersicum*) [Internet]. Available from: <http://dx.doi.org/10.7717/peerj.9310/supp-1>

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