

**Analysis of Antifungal Activity of Essential Oil from Orange Peels**

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**Abstract**

The largest fruit in Nepal is citrus, which takes up 2684 of the country's total fruit-growing land. 71.65% of the nation's citrus-growing land is devoted to the planting of citrus called mandarin oranges. Pectin is a structural hetero-polysaccharide that is integral to the motion of terrestrial flora. The fact some components are becoming resistant to specific fungal infections and illnesses has become among the biggest obstacles in treating them at the moment. This necessitated the employment of novel medications to treat infectious disorders brought on by these microbes. Additionally, they are exceedingly low in toxicity and abundant sources of active chemicals. Soxhlet can be used to easily extract the essential oil. The end result confirms that essential oils were successfully extracted as pectin solutes, which is particularly helpful for pectin extraction in industrial sectors both economically and for environmental health.

**Keywords:** Alcoholic extract, antifungal activity, essential oil, *Citrus reticulata balnco*

**Introduction**

The largest fruit in Nepal is citrus, which takes up 2684 of the country's total fruit-growing land. 71.65% of the nation's citrus-growing land is devoted to the planting of citrus called mandarin oranges (*Citrus reticulata balnco*). (MOAD,2013) The major citrus cultivar groupings farmed worldwide are those of *C. sinensis*, which accounts for over 70% of the total yearly production of citrus species (Flamini et al., 2003).

The essential oil's smell character can be noticeably altered by even minor compositional alterations. The amount of chemicals found in the essential oil seems to be significantly influenced by the analysis technique. The bitter orange essential oil is said to be influenced by the growing environment, the cultivar, the ripening stage, the storage environment, and the extraction techniques. Bitter orange peel oil should be kept at a temperature of 21°C to avoid compositional changes and the production of artefacts when kept for a few months. (Gaff et al., 2020). Legal restrictions frequently make it difficult to dispose of waste products like peels, and the treatment procedure itself is more expensive. The production of usable items from this trash could lower the cost of global treatment. So, the goal of our experimental work was to turn sweet lime peel into essential oil, which is highly sought-after in the culinary, pharmaceutical, and cosmetic industries (Kadapure et al., 2022).

Pectin is a structural hetero-polysaccharide that is integral to the motion of terrestrial flora. The pectin crew of complicated polysaccharides acts as a hydrating agent and a binding agent for the cellulose region in the transportable partitions of massive plants. Pectin and pectino are the Greek phrases which means "congealed and curdled" (Thakur et al., 1997)

The name "pectin," is a heteropolysaccharide which constitutes the majority of the cell walls of terrestrial vegetation, comes from the Greek for "congealed and curdled." Orange trees are principally and extensively bred in tropical and subtropical climates for the flavorful fruit, which is peeled or smashed (to prevent the acidic rind) and consumed completely, processes to extract orange juice, and also for the fragment peel. In the cellular shells of higher plants, a family of complicated polymers known as pectin acts as a hydrating agent and a cement for the cellulose matrix. Pectin also has a variety of special qualities that make it useful as a matrix for delivering and/or trapping various medications, proteins, and cells.

Pectin is a naturally occurring biopolymer that is increasingly being used in the biotechnology and pharmaceutical sectors. It has been used successfully for many years as a colloidal stabilizer, thickening agent, and gelling agent in the food and beverage sector. The pulp and peels (rind skin) make up the majority of an orange's two constituent sections. The

**Article information**

Received: 7 September 2023

Accepted: 22 September 2023

Published: 9 October 2023



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pulp, which is the portion of the fruit that may be eaten, and the peels, which are excellent sources of pectin, can be easily distinguished from one another. A naturally occurring biopolymer called pectin is being used more frequently in fields like biotechnology and pharmaceuticals.

One-third of the dry substance in higher plants' walls of cells is made up of a substance called pectin, a complicated combination of polysaccharides. Small plants, like grasses, have much lower concentrations of these chemicals in their cell walls. As one moves through the primary wall toward the plasma membrane and away in middle of the lamella, pectin concentration gradually decreases. A refined carbohydrate compound is found in the orange peels' interior portion is where the mentioned substance. The main component is polygalacturonic acid which has partially been methoxylated. In case of appropriate circumstances, pectin can produce gels with acid and glucose. It is nearly always generated in plant cells of all species and is useful in the professional production of sweet jellies and products based on apple pomace and citrus peels.

Despite being a common component of the majority of plant tissues, where it serves as a cement in the middle lamella and a thickening agent for the cell wall, there are very few sources from which pectin can be synthesized for use in the commercial sector. *Citrus* pectin is either pale cream or light yellow in hue, but apple pectin is typically darker. The pectin separates as a stringy, gelatinous substance that needs to be squeezed, dried, and crushed after being washed to remove the mother liquor. The majority of pectin's carbohydrates are linear. This plant polysaccharide is polydisperse and polymolecular, and its composition is influenced by its sources and isolation conditions.

The molecular mass and quantity for particular sub-units will vary from molecule to molecule in each sample of pectin. It is extremely difficult to determine the basic composition of pectin since the component known as proteins is susceptible to change throughout the processing and storage of plant material. Additionally, the primary element may be accompanied by contaminants. Currently, it is believed that D-galacturonic acid (GalA) units, linked together in chains by  $\alpha$ -(1-4) glycosidic linkage, make up the majority of pectin's.

Essential oils are extracted by cold pressing and their antifungal effectiveness is determined using an agar dilution process the effects spotlight orange peels essential oil extremely good antifungal performance pomelo essential oil used to be as quickly as most exquisite in opposition whereas lime essential oil used to be most immoderate high-quality in opposition these effects recommend that natural

antifungal redress made from citrus indispensable oils can be used in the food pharmaceutical and attractiveness industries (Van Hung et al., 2013) One of the great current challenges in the treatment of fungal diseases has been the resistance they have acquired to certain compounds. This requires the use of new drugs for the treatment of infectious diseases caused by these microorganisms (Bastos *et al.* 2011; Newman & Cragg 2016). It also boosts the body's production of antibodies and interferon which helps to protect the body from viral invaders and cancerous cells.

### Literature Review

Diffusion and dilution (direct contact) or vapor-phase (gaseous contact) procedures are the most often used techniques to assess the antibacterial activity of essential oils in vitro. The available techniques for extracting essential oils, their mathematical modeling, and their usage as antimicrobial agents are the main topics of this paper. (Alvi et al., 2022)

Because of the not appearance of water-dissolvable molecules in cold-pressed oils and acid-catalyzed reactions in hydrodistilled oils including break down of linalyl acetate creating types of terpenes bi-product the releasing system may also be to blame for some compositional discrepancies (Gaff et al., 2020)

In order to maintain the standard and safety of the produced goods and also the wellness as well as activity of the animal's orange peel extracted oil may be used as a natural feed additive for ewes (Kotsampasi et al., 2018).

we determined that the production of 100-grade pectin from the smooth peel that was leached in the lab was once as soon as 656 and 559 for lime and lemon respectively when lemon peel was dried to its final moistures of 15-20 8-12 and 3-7 it yielded yields of 411 411 and 25 respectively (Crandall et al., 1978) The movement of land flora depends on the constitutional hetero polysaccharide pectin. The complicated polysaccharide pectin group serves as a humidify and binding factor for the cellulose local in the transportable divisions of large plants these two phrases "congealed and curdled" are translated by a language called Greek language as pectin and pectin (Thakur et al., 1997). Pectin was taken out of peel scraps during the current studies after essential oil was taken out. Fruits were gathered in five different altitude ranges, namely 800-1000 m, 1000 m-1200 m, 1200 m-1400 m, and >1600 m (Pradhan et al., 2019)

The minerals as well as nutrients constitutional material inside the respective pattern flour as well as antifungal hobby of the respective pattern crude ethanol (95%) and water extracts been decided through general methods. Each extract (one hundred mg/ml) become examined towards fungi – *Candida*

*albicans* and *Aspergillus flavus* (Egbuonu, A. C. C., and Amadi, C. C. 2016).

After three weeks, the growth of the fungus was inhibited in Petridis which had been sprayed with essential oil. The essential oil-free Petridis had been seen to spread fungus with multiplication. Lemon peel was used to extract the pectin, which was calculated to weigh 200g (Singh, N. P., & Paudel, N. 2023).

### Materials and Methods

This research study was based on experimental research which was done at Dhawalagiri Multiple Campus, Baglung. The oranges are selected randomly from Pang ward 1, Kusma, Parbat and Bhansa-6, Baglung, Baglung

#### Collection and Preparation of Materials

The fresh unaffected citrus fruits; Oranges (*C. reticulata*) were collected from Pang-1, Kusma, Parbat and Bhansa-6, Baglung randomly then were washed and peeled. The fresh orange peel was dried in the sunlight and grinded into powder which was then ready for the experiment.

#### Methods of Essential Oil Extraction:

Oranges that had just been picked had been purchased, taken from the orange gardens in the Pang-1 Kushma Municipality Parbat and Baglung Municipality, and thoroughly washed. The peels were then manually removed, and they were allowed to air dry for 6 to 8 days at room temperature. The obtained dry barks were then ground using an electronic blender and kept in an airtight container for later usage. For the extraction, ethanol and acetone were used as two distinct solvents. 250 ml of solvent (acetone or ethanol) and approximately 10 grams of peel powder were added to a soxhlet. Up to 78°C of heat was applied to the solvent (ethanol or acetone). The thimble is a device used to gather essential oil (Hussian et al., 2008).

#### Methods of Analysis of Antifungal Activity

PDA media was made ready in the botanical laboratory using the powder of the given PDA (1 litre water, 2 gm potato, 20 gm dextrose and 20 gm agar) then took 45 minutes in an autoclave, finally, the media was ready. Sprit was used for disinfection of the surrounding area. Then, the 6 plates smoothly filled with media and made hole. The *Fusarium* and *Aspergillus* were dipped by needle into them but one of them was sprayed essential oil in the Botany laboratory DMC, Baglung which was left for 3 weeks for incubation at 25°C. The presence or absence of clear zones was observed after 48 hours. (Pinontoan et al., 2019)

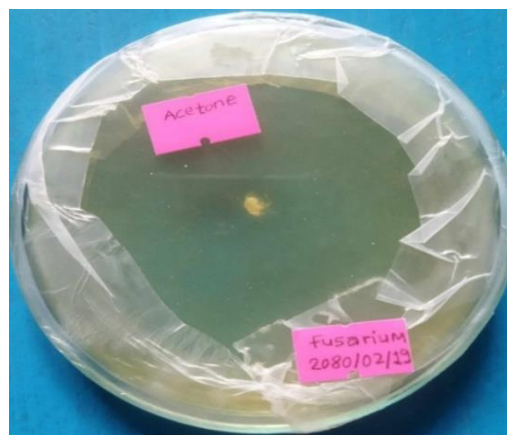
### Methods of Extraction of Pectin from the Prepared Sample

100 gm dried peels were separately transferred into a beaker containing 500 ml of water, and 2.5 ml of hydrochloric acid was added to give a PH of 2.2. Each of the fruits was then boiled for 45 minutes separately. Thereafter, the peels were removed from the extracts by filtering through a filter paper study. The cake was washed with 250 ml of boiled water and the combined filter was allowed to cool to 25°C to minimize heat degradation of the pectin. The exerted pectin was precipitated by adding 200ml which means 95 % ethanol to 100ml of the extracted pectin with stirring, left for 30 minutes to allow the pectin to float on the surface. The gelatinous substances flocculants were then skimmed off. The extracted pectin was purified by washing with 200ml ethanol and then pressed on a nylon cloth to remove the residual HCl and universal salt. The resulting pectin was weighed and shredded into small pieces and weighed and was air-dried. Finally, the dried pectin was further reduced into smaller pieces using a pestle and mortar and weighed using a digital weighing balance. The percentage yield of pectin from initial wet peels will be then determined on both wet and dry weight basis (Bagde et al., 2017).

### Result and Discussion

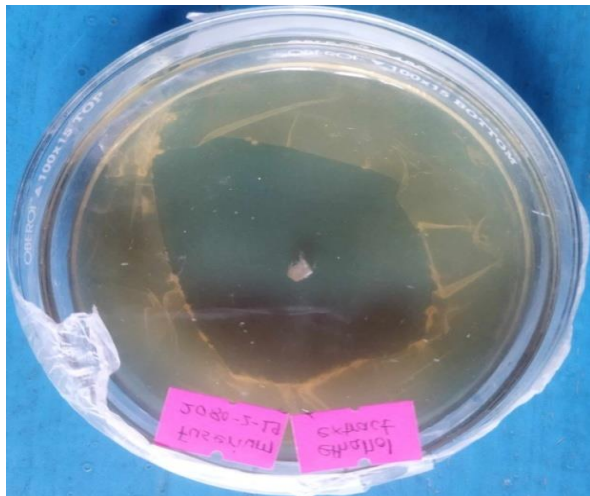
In antifungal activity fungi were killed by the extract essential oils, they are acetone and ethanol extract. They inhabit the growth of this fungi which convey as reported by Astana (2020). In our experiment as well fungus were also killed by dominating the inoculated *Eusarium spp.* and *Aspergillus spp.*,

**Fig. 1 :** *Petridis Antifungal Activity of Essential Oil of Acetone From Orange Peel on Fuserium*



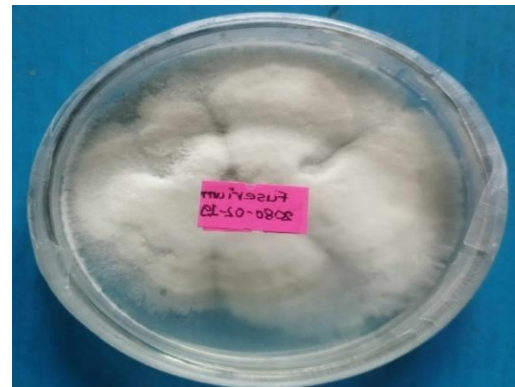


**Fig. 2 :** *Petridis Antifungal Activity of Essential Oil of Ethanol Extract From Orange Peel on Fuserium*



Antifungal activities that were done against the fungus *Aspergillus Fuserium* spp by using two essential oil extracts, acetone and ethanol extract from *Citrus reticulata* peels was similar as reported by Budhathoki and Paudel (2023).

**Fig. 5:** *Petridis For Fuserium without Essential Oil Essential Oil (Control)*



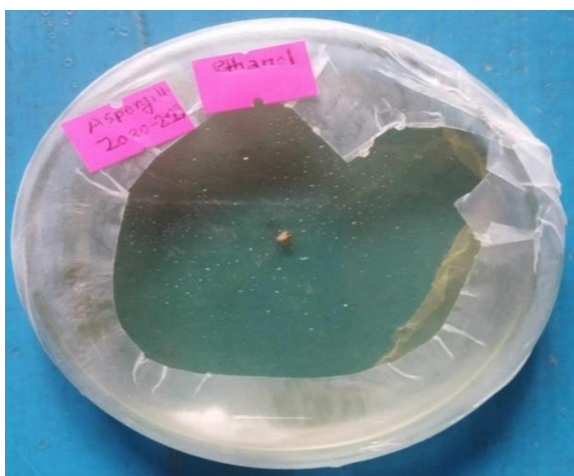
**Fig.3:** *Petridis Antifungal Activity of Essential Oil of Acetone Extract From Orange Peel on Aspergillus*



**Fig. 6** *Petridis For Aspergillus without Essential Oil (Control)*



**Fig. 4:** *Petridis Antifungal Activity of Essential Oil of Ethanol Extract From Orange Peel on Aspergillus*



**Conclusion**

Citrus fruits, in short, are widely consumed over the world. The number of pharmacological and therapeutic compounds in their peels is astounding. They also contain a lot of active component sources and are extremely low in toxicity. Soxhlet is an easy method for obtaining the essential oil. The analysis shows that effective essential oil extraction has numerous economic and environmental benefits, including the antifungal activity of alcoholic extracts.

**Acknowledgement**

My deepest gratitude is extended to Teaching Assistant Roshan Chhetri, for his assistance with antifungal analysis, and to Lecturer Yadunath Paudel, who helped classify *Citrus reticulata* botanically. Special gratitude is out to all the staff at DMC

Baglung, including the professors, students, and experts.

**Funding:**

**Ethical Approval for Research:** Not applicable:

**Conflict of Interest:**

No conflict of interest

**Ethical Conduct of Research:**

I declare that this work has been ethically conducted

**References**

- Alvi, T., Asif, Z., & Khan, M. K. I. (2022). Clean label extraction of bioactive compounds from food waste through microwave-assisted extraction technique-A review. *Food Bioscience*, 101580.
- Arora, M., & Kaur, P. (2013). Phytochemical screening of orange peel and pulp. *International Journal of Research in Engineering and Technology*, 2(12), 517-522.
- Bagde, P. P., Dhenge, S., & Bhivgade, S. (2017). Extraction of pectin from orange peel and lemon peel. *International Journal of Engineering Technology Science and Research*, 4(3), 1-7
- Bastos GM, Nogueira NAP, Soares CL, Martins MR, Rocha LQ, Teixeira AB. 2011. *In vitro* determination of the antimicrobial potential of homemade preparations based on medicinal plants used to treat infectious diseases. *Revista de Ciências Farmacêuticas Básica e Aplicada* 32: 113-120
- Bhudhathoki, N.P., Shukla, R., Prakash, B., Kumar, A., Singh, S., Mishra, P. K., & Dubey, N. K. (2010). Chemical profile, antifungal, antiaflatoxic and antioxidant activity of Citrus maxima Burm. and Citrus sinensis (L.) Osbeck essential oils and their cyclic monoterpene, DL-limonene. *Food and Chemical Toxicology*, 48(6), 1734-1740.
- Budhathoki N. P., & Paudel, Nabin (2023). Analysis of Pectin and Essential Oil Citrus L. Lemon Peel. *Journal of Tikapur Multiple Campus*, 160-175.
- Crandall, P. G., Braddock, R. J., & Rouse, A. H. (1978). Effect of drying on pectin made from lime and lemon pomace. *Journal of Food Science*, 43(6), 1680-1682
- Del Carmen Razola-Díaz, M., Guerra-Hernández, E. J., García-Villanova, B., & Verardo, V. (2021). Recent developments in extraction and encapsulation techniques of orange essential oil. *Food Chemistry*, 354, 129575.
- Egbuonu, A. C. C., & Amadi, C. C. (2016). Some nutritive and antifungal properties of *Citrus sinensis* (sweet orange) peels and seeds. *American Chemical Science Journal*, 14(2), 1-7.
- El Asbahani, A., Miladi, K., Badri, W., Sala, M., Addi, E. A., Casabianca, H., ... & Elaissari, A. (2015). Essential oils: From extraction to encapsulation. *International journal of pharmaceuticals*, 483(1-2), 220-243.
- Evans WC. Trease and Evans Pharmacology 14th edition. Harcourt Brace and Company. Asia. Pvt. Ltd. Singapore. 1997.
- Flamini, G.; Cioni, P.L.; Morelli, I., (2003). Use of solid-phase micro-extraction as a sampling technique in the determination of volatiles emitted by flowers, isolated flower parts and pollen. *J. Chromatogr. A*, 998,229–233.
- from Citrus reticulata L. Blanco collected from different altitudes of Sikkim Himalaya. *Journal of Applied and Natural Science*, 11(1), 168-181.
- Gaff, M., Esteban-Decloux, M., & Giampaoli, P. (2020). Bitter orange peel essential oil: a review of the different factors and chemical reactions influencing its composition. *Flavour and fragrance journal*, 35(3), 247-269.
- Hussain, A. I., Anwar, F., Sherazi, S. T. H., & Przybylski, R. (2008). Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depend on seasonal variations. *Food chemistry*, 108(3), 986-995.
- Kadapure, S. A., Kadapure, P., Anjali, C., Akansha, B., Sabera, B., Sanket, M., & Shet, A. (2022). Extraction of Essential Oil Form Sweet Lime Orange Peel: A Comparison Study. In *Materials Science Forum* (Vol. 1048, pp. 468-475). Trans Tech Publications Ltd.
- Kokate CK. Practical pharmacognosy.4th edition. Vallaph prakashan publication, New Delhi, India. 1999.
- Kotsampasi, B., Tsiplakou, E., Christodoulou, C., Mavrommatis, A., Mitsiopoulou, C., Karaiskou, C., ... & Zervas, G. (2018). Effects of dietary orange peel essential oil supplementation on milk yield, composition, and blood and milk antioxidant status of dairy ewes. *Animal Feed Science and Technology*, 245, 20-31.
- Mace, M. E. (1963). Histochemical localization of phenols in healthy and diseased banana roots. *Physiologia plantarum*, 16(4), 915-925.
- MOAD (2013): Statistical Information on Nepalese Agriculture 2012/13. Government of Nepal. Ministry of Agriculture Development. Agriculture Business Promotion and Statistical Division, Statistics Section, Singhadurbar, Kathmandu, Nepal.
- Negi, K., Asthana, A. K., & Chaturvedi, P. (2020). GC-MS analysis and antifungal activity of acetone extract of *Conocephalum conicum* (L) Underw (liverwort) against aflatoxins-producing fungi. *South African Journal of Botany*, 131, 384-390.

- Newman DJ, Cragg GM. 2016. Natural products as sources of new drugs from 1981 to 2014. *Journal of Natural Products* 79: 629-661.
- Pinontoan, S., Sunati, N., Febriana, B., & Rambli, E. V. (2019). Antifungal activity of essential oil extract of lemon cui (*Citrus microcarpa*) skin against *Trichophyton rubrum* Growth. *International Journal of Pharma Medicine and Biological Sciences*, 8(2), 40-44
- Pradhan, A., Sharma, L., Tiwari, A., & Chettri, P. (2019). Characterization of pectin extracted
- Ramakrishnan SK, Prasannan G, Rajan R. Textbook of medical biochemistry. Orient Longman, New Delhi. India. 1994, 582
- Thakur, B. R., Singh, R. K., Handa, A. K., & Rao, M. A. (1997). Chemistry and uses of pectin—A review. *Critical Reviews in Food Science & Nutrition*, 37(1), 47-73.
- Thakur, B. R., Singh, R. K., Handa, A. K., & Rao, M. A. (1997). Chemistry and uses of pectin—A review. *Critical Reviews in Food Science & Nutrition*, 37(1), 47-73.
- Tongnuanchan, P., & Benjakul, S. (2014). Essential oils: extraction, bioactivities, and their uses for food preservation. *Journal of Food Science*, 79(7), R1231-R1249.
- Van Hung, P., Chi, P. T. L., & Phi, N. T. L. (2013). Comparison of antifungal activities of Vietnamese *Citrus* essential oils. *Natural product research*, 27(4-5), 506-508.
- Wagner HXS, Bladt Z, Gain EM. Plant drug analysis. Springer Verlag. Berlin. Germany. 1996.

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