



Extraction of polyphenols using afroyal gala and granny smith for antimicrobial activity

Narayana Rao K^{*1}, Pruthviraj P V¹, Senthamarai S², Subha V J²

¹Department of Pediatrics, Konaseema Institute of Medical Sciences Research Foundation, Amalapuram, Andhra Pradesh, India

²Department of Microbiology, Meenakshi Academy of Higher Education and Research, Chennai, Tamilnadu, India

Article History:

Received on: 15 Nov 2020
Revised on: 18 Dec 2020
Accepted on: 19 Dec 2020

Keywords:

Antimicrobial,
Royal Gala and Granny
Smith,
Polyphenol

ABSTRACT



Apples contain many useful human health-related contents. Polyphenols exhibit antimicrobial effects against human pathogens. In this study, the polyphenolic contents are removed by the skin of Royal Gala and Granny Smith, which are the two varieties of apples. The phenolic compounds were removed from fresh fruit peels of Royal Gala and Granny Smith with the solvents: A-(water), B- (ethanol: water). The antimicrobial happenings of the excerpts are verified in contradiction of *Escherichia coli* and *Staphylococcus aureus* bacteria by jelly healthy dispersion technique. The extracts from both apples by solvent A presented advanced anti micro bisection as associated with excerpts by solvent B. Among the 2 varieties of apples, the Granny Smith extracts presented a superior antimicrobial result as compared to Royal Gala extracts against the selected microorganisms E-coli and S-aureus. The zone of inhibition by the apple peel extracts is more in case of S-aureus compared with E-coli; this reveals that the organism S-aureus is more sensitive to polyphenolic compounds than *Escherichia coli*. With this study, it was concluded that the water extract of Granny Smith peels showed superior antimicrobial movement because of advanced amounts of polyphenolic mixtures as associated to Royal Gala and the best solvent to extract polyphenolic compounds from apple peels is water.

*Corresponding Author

Name: Narayana Rao K
Phone: 9032156365
Email: narayanarao65@gmail.com

ISSN: 0975-7538

DOI: <https://doi.org/10.26452/ijrps.v11iSPL4.4061>

Production and Hosted by

IJRPS | www.ijrps.com

© 2020 | All rights reserved.

INTRODUCTION

An antimicrobial is a mediator killing microorganisms or halts the development. Antimicrobial

medicines are collected by microorganism's by performance basically in contradiction of example, and antibacterials are used in contradiction of microbes and antifungal are exploited in contradiction of parasites. Likewise, be ordered by their size (Kanner *et al.*, 1994). Specialists that perform microorganisms are called microbicidal; though individuals that just suppress their growth are named biostatic. The use of antimicrobial prescriptions to indulgence disease is recognized as antimicrobial chemotherapy, though the use of antimicrobial meds to forestall pollution is recognized as antimicrobial prophylaxis (Frankel *et al.*, 1995). Phenolic mixes are notable for their medical advantages identified with cell reinforcement movement. Moreover, this sort of mixes can be separated from regular sources, for example, olives, grapes, apples, organic prod-

ucts, vegetables, rice, flavours, spices, tea and green growth (Mangas *et al.*, 1999). Truth be told, other significant attributes related to phenolic mixes are the antimicrobial movement, since phenolics have the limit of impeding the microbial intrusion in certain items and staying away from the rottenness of others, principally products of the soil (Eberhardt *et al.*, 2000). These possessions permit phenolic mixes to be reasonable for various food protection applications. Dynamic pressing is a creative system where phenolic mixes can assume a significant function for improving the worldwide evaluation and broaden the time span of usability of business products (van der Sluis *et al.*, 2001). 15 Polyphenols are grouped by the construction as phenolic acids derivates, flavonoids, and tannins. Apples comprise numerous sorts of phenolic derivates and flavonoids (flavan-3-ols, flavonols, procyanidins, chalcones, and anthocyanins). 3, 8 Apple skin contains the flavonoids, quercetin glycosides and cyanidin glycosides. 7 Epidemiological investigations partner phenolic utilization with lower mortality, particularly brought about by coronary illnesses (Alberto *et al.*, 2001). The current various organic possessions, the stare of developing enthusiasm for customers because of the high cell reinforcement, mitigating, against hypersensitive, hostile to apoplexy and antimicrobial activities. Furthermore, they go about as anti-deposit of fatty oil, anticholesterolemic, and antiviral specialists, between other phenolic mixes, may influence microbial development and digestion (Alberto *et al.*, 2002).

The objectives of the present study are to examine the antimicrobial activity of phenolic mixtures by the skin of two apple diversities, Royal Gala and Granny Smith, in contradiction of pathogenic bacteria and to find out the variety of apple with superior activity, and to find out the preferable solvent for extraction of polyphenols from apple peels (Shoji *et al.*, 2003).

Royal Gala (yellowish-red skin)- Synonyms-Royal Gala, Local Name-Red apple, *malus pumila*, Biological Source-Dried ripe fruit of *malus pumila*, *malus sieversii*, Family-Rosaceae. Description -royal gala apples are canvassed in a flimsy yellow to orange skin, featured with pink to red stripes that change in shade subordinate upon the apple's development (Jayaprakasha *et al.*, 2003) in Figure 1.

Their thick substance is velvety yellow and fresh, offering a somewhat sweet flavour and botanical fragrance. Function's that are permitted to arrive at the pinnacle of their pleasantness on the tree will have a more profound red shade and a marginally better flavor (Shoji *et al.*, 2004).

Granny Smith (greenish-white skin)- Morphology

Synonyms-Granny smith, Local Name-Green apple, *malus pumila*, Biological Source-Dried ripe fruit of *malus pumila*, *malus sylvestris*, Family-Rosaceae. Granny Smith apples have brilliant green skin that is regularly spotted with faint white lenticels (spots). Medium to enormous in size and round fit as a fiddle, they are a firm and delicious apple with toughness. Their tissue is brilliant white and fresh in sweet surface flavor (Baydar *et al.*, 2004).

MATERIALS

Two Apple varieties one is Red apple- Royal Gala and another one is green apple-Granny Smith, agar, peptone, sodium chloride, Beef excerpt/Yeast excerpt, purified water, Ethanol, Micro-organisms-Gram-positive and Gram-negative (*E. coli*, *S. Aureus*) (Alberto *et al.*, 2004).



Figure 1: Royal Gala (yellowish-red skin) and granny smith



Figure 2: Peels and powder of Royal Gala and Granny Smith

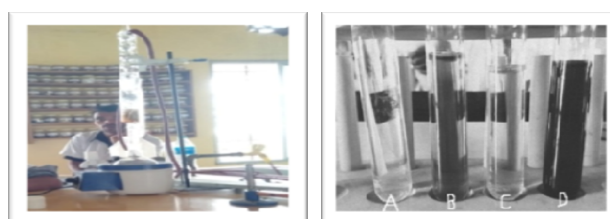


Figure 3: Extraction assembly & Extracts of Royal Gala & Granny Smith. A: Granny Smith water extract, B: Royal Gala water extract, C: Granny Smith ethanol: water extract, D: Royal Gala ethanol: water extract

Table 1: The consequence of the apple skin excerpts from *Royal Gala* and *Granny Smith* diversities in contradiction of bacterial pathogen

Excerpts		E coli	S. Aureus
Granny Smith	A	7.0	9.0
	B	2.0	3.0
Royal Gala	A	3.0	4.0
	B	1.0	2.0

A: water, B: ethanol: water. The diameter of the inhibition zone in mm

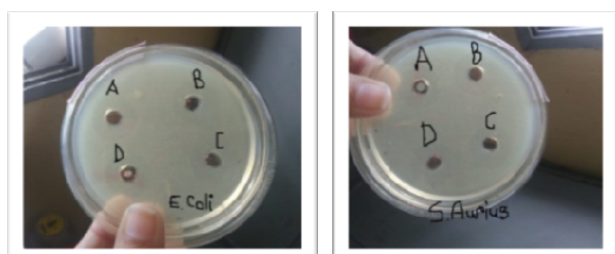


Figure 4: Zone of inhibition of E-coli and S-aureus (Diameter in mm). A: water extract (Granny Smith), B: ethanol: water (Granny Smith). C: water extract (Royal Gala), D: ethanol: water (Royal Gala)

Polyphenol removal

The strip of 6 kg of every apple assortment was hacked up finely. Removals are completed double for 8 hrs at 60°C (Jayaprakash, 2003) with 400 mL of the accompanying solvents: A, acetone:water: acetic corrosive; B, ethyl acetate:methanol: water and C, ethanol: water. Concentrates stayed then moved by ventilation in a revolutionary evaporator at 35°C to keep away from hydrolysis, redox and polymerization responses by adjusting the example synthesis. A while later, the amasses were resuspended in 50 ml methanol and kept at - 18°C, staying away from direct interaction by light and oxygen (Rojas-Graü *et al.*, 2006).

Diagnostic judgments

Complete phenolic satisfied is acquired with Folin-Ciocalteu reagent (Singleton and Rossi, 1965). The flavonoid division is acquired by blending 10 mL of the concentrate in by 10 ml of weakened HCl (1:3) and 5 ml of an 8 mg/mL formaldehyde arrangement (Martillanes *et al.*, 2017). The combination is left to encourage for 24 hrs and afterwards separated. The non-flavonoid phenol substance was resolved in the deposit utilizing Folin-Ciocalteu reagent. The flavonoid contented was acquired by the distinction among complete phenol and non-flavonoid contented. The phenol contented is communicated as what could be compared to a gallic corrosive per gram of apple skin (mg GAE/g). All judg-

ments are completed in three-fold.

Concentrate explanation

The explanation was done by 1 g of actuated carbon in 20 ml of every concentrate. Concentrates stayed surprised and afterwards layered separated (0.45 μ). The explained separates deprived of phenol mixes are utilized as undesirable panels in the antimicrobial movement measures. All concentrates were channel sanitized (0.22 μ film) and reserved at - 18°C.

METHODOLOGY

Collection of peels

Healthy Apples of *Royal Gala* and *Granny Smith* were collected and washed with distilled water. The fresh peels of *Gala* and *Granny Smith* were collected and authenticated by Botanist. Spread the peels on the sterile clean green net in a well-ventilated room in Figure 2. Peels completely dry within 4 days, the dried peels are grinded in the mortar by pestle to make powder, then this peel powder dried at 50°C for 30 minutes to diminish humidity satisfied and protected from light and humidity (Chandrakant, 2013).

Polyphenol abstraction

The skin powder of 6 g of respectively apple variability is hacked up outstandingly. Withdrawals are approved out double for 8 hrs at 60°C with 40 ml of the subsequent diluents: A, water; B, ethanol: water (1:1) then Extracts were then concentrated in an evaporator by avoiding direct contact with light and oxygen.

Excerpt illumination

Illumination is approved out by 1 g of stimulated carbon in 20 ml of every excerpt. Excerpts are surprised and formerly membrane-filtered in Figure 3.

Antimicrobial activity

Agar plate media was prepared by suspending 28 g of nutrient agar precipitate in 1 liter of purified water and animated this combination though

inspiring to fully liquify all mechanisms. Autoclaved the softened combination at 1210 C for 15 minutes. When the nutrient agar is autoclaved, allowable it to cool than not solidify. Then inoculated the selected microorganism into nutrient agar medium and poured into plates and set aside on the sterile superficial ending the agar has hardened.

In this cup-plate method/agar well diffusion method, when the agar has solidified, holes about 9 mm in diameter are cut in the medium with a sterile corn borer. The antimicrobial agents (apple peel extracts) directly placed in the holes. The plates are incubated at 30 to 35°C for 2 to 3 days. The zone of inhibition is observed after the development phase, the distance of the zone of the reserve is restrained in millimetres . The incubated plates shown Figure 4.

RESULTS AND DISCUSSION

Zone of Inhibition

In Table 1. The values of the zone of inhibition by aqueous and alcoholic extracts of two apple varieties are given. It was observed that the major repressive consequences for mutually diversities due to the polyphenolic mixtures present in apple peels. It is a known fact that the zone of inhibition is directly protonate.

d to the antimicrobial activity. So These results show that when compare with water extract and water: ethanol extract, water extracts showing more antimicrobial effect than water: ethanol extracts. Water extract of Granny Smith is more effective than that of Royal Gala.

The zone of inhibition is more in *S. aureus* inoculated plates compare with *E-coli* inoculated plates, so this indicates the *S-aureus* is more susceptible microorganism to phenolic compounds than *E-coli*. The Granny Smith and Royal Gala are more effective in water extract, but Granny Smith is having more activity than the Royal Gala in both extracts.

CONCLUSION

Grounded on study consequences it is decided the best solvent for removal of polyphenols by apples is water, the Granny Smith peels excerpts are additional operative antibacterial negotiators by Royal Gala because here is a straight association among phenolic satisfied and antibacterial effect. And it also reported that *S. aureus* is more susceptible than *E- coli* to polyphenols. The antibacterial consequence of apple polyphenols established in this education is extra to the previously recognized helpful belongings of apple by human health.

Funding Support

The authors declare that there is no funding support for this research work.

Conflict of Interest

The authors declare that there is no conflict of interest among the authors and research.

REFERENCES

- Alberto, M. R., Farías, M. E., de Nadra, M. C. M. 2001. Effect of Gallic Acid and Catechin on *Lactobacillus hilgardii* 5w Growth and Metabolism of Organic Compounds. *Journal of Agricultural and Food Chemistry*, 49(9):4359–4363.
- Alberto, M. R., Farías, M. E., et al. 2002. Effect of Wine Phenolic Compounds on *Lactobacillus hilgardii* 5w Viability. *Journal of Food Protection*, 65(1):211–213.
- Alberto, M. R., Gómez-Cordovés, C., de Nadra, M. C. M. 2004. Metabolism of Gallic Acid and Catechin by *Lactobacillus hilgardii* from Wine. *Journal of Agricultural and Food Chemistry*, 52(21):6465–6469.
- Baydar, N. G., Özkan, G., Sağdıç, O. 2004. Total phenolic contents and antibacterial activities of grape (*Vitis vinifera* L.) extracts. *Food Control*, 15(5):335–339.
- Chandrakant, K. 2013. Pharmaceutical microbiology principle and application. pages 11–17, Pune. Ninth edition, Nirali Prakashan, Pune, January. ISBN: 9788185790612.
- Eberhardt, M. V., Lee, C. Y., Liu, R. H. 2000. Antioxidant activity of fresh apples. *Nature*, 405(6789):903–904.
- Frankel, E. N., Waterhouse, A. L., Teissedre, P. L. 1995. Principal Phenolic Phytochemicals in Selected California Wines and Their Antioxidant Activity in Inhibiting Oxidation of Human Low-Density Lipoproteins. *Journal of Agricultural and Food Chemistry*, 43(4):890–894.
- Jayaprakasha, G. K., Selvi, T., Sakariah, K. K. 2003. Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. *Food Research International*, 36(2):117–122.
- Kanner, J., Frankel, E., Granit, R., German, B., Kinsella, J. E. 1994. Natural antioxidants in grapes and wines. *Journal of Agricultural and Food Chemistry*, 42(1):64–69.
- Mangas, J. J., Rodríguez, R., Suárez, B., Picinelli, A., Dapena, E. 1999. Study of the Phenolic Profile of Cider Apple Cultivars at Maturity by Multivariate Techniques. *Journal of Agricultural and Food*

- Chemistry*, 47(10):4046–4052.
- Martillanes, S., Rocha-Pimienta, J., Cabrera-Bañegil, M., Martín-Vertedor, D., Delgado-Adámez, J. 2017. Application of phenolic compounds for food preservation: Food additive and active packaging. . pages 39–58. Phenolic compounds–Biological activity. London, UK: IntechOpen. ISBN: 9789535129608.
- Rojas-Graü, M. A., Avena-Bustillos, R. J., Friedman, M., Henika, P. R., Martín-Belloso, O., McHugh, T. H. 2006. Mechanical, Barrier, and Antimicrobial Properties of Apple Puree Edible Films Containing Plant Essential Oils. *Journal of Agricultural and Food Chemistry*, 54(24):9262–9267.
- Shoji, T., Akazome, Y., Kanda, T., Ikeda, M. 2004. The toxicology and safety of apple polyphenol extract. *Food and Chemical Toxicology*, 42(6):959–967.
- Shoji, T., Mutsuga, M., Nakamura, T., Kanda, T., Akiyama, H., Goda, Y. 2003. Isolation and Structural Elucidation of Some Procyanidins from Apple by Low-Temperature Nuclear Magnetic Resonance. *Journal of Agricultural and Food Chemistry*, 51(13):3806–3813.
- van der Sluis, A. A., Dekker, M., de Jager, A., Jongen, W. M. F. 2001. Activity and Concentration of Polyphenolic Antioxidants in Apple: Effect of Cultivar, Harvest Year, and Storage Conditions. *Journal of Agricultural and Food Chemistry*, 49(8):3606–3613.