Moestopo International Review on Societies, Humanities, and Sciences (MIRSHuS)
Vol. 4, No. 1 (2024) pp. 78-87
ISSN: 2775-9601

STUDY IN THE POTENTIAL OF CHEMICAL COMPOUNDS OF ROSELLA FLOWER (HIBISCUS SABDARIFFA LINN) EXTRACT AS A PREVENTION OF RELAPSE AFTER ORTHODONTIC TREATMENT

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ARTICLE INFO
Article History:
received: 21/03/2024
revised: 22/04/2024
accepted: 30/04/2024

Keywords:
Flavonoids, tannins, saponins, phytochemicals, orthodontic

DOI:
10.32509/mirshus.v4i1.71

ABSTRACT

In dentistry, we can correct malocclusion and malposition by moving the teeth with orthodontic treatment. After the orthodontic treatment is done there'll still be a possibility of relapse. Relapse is when the teeth return to their original position before the orthodontic treatment was done. The prevalence of relapse after orthodontic treatment is still quite high. Relapse prevention can be achieved through the use of mechanical devices but, they have some limitations. Natural materials can be used as an alternative as the prevention method that can prevent the relapse from happening. Roselle extract contains chemical compounds that have the potential to prevent relapse after the orthodontic treatment is done. To observe the potential of chemical compounds in roselle flower extract as a relapse prevention method after orthodontic treatment. This research was a laboratory experiment. The research phase began with the extraction of rosella flowers *(Hibiscus sabdariffa Linn)*, then continued with the second stage, namely the phytochemical screening of rosella flowers *(Hibiscus sabdariffa Linn)* using the color reaction method. The phytochemical tests showed that the roselle flower extract contains chemical compounds such as flavonoids, tannins, and saponins. **Conclusion:** The roselle flower extract contains chemical compounds such as flavonoids, tannins, and saponins, which have anti-inflammatory and antioxidant properties, making it potentially effective in preventing relapse after orthodontic treatment.

INTRODUCTION

The condition of oral health in Indonesia and world wide remains a serious issue. According to the Global *Burden of Disease Study*, approximately 3.5 billion people worldwide suffer from oral health problems. Between 2013 and 2018, the oral health problems in the Indonesian population increased from 25.9% to 57.6%. One of the oral health problems with a high prevalence is malocclusion (Suala et al., 2021)(James et al., 2018). Malocclusion is common in Indonesia, accounting for 80% of cases (Ratya Utari & Kurnia Putri, 2019). According to Syada, malocclusion is a condition in which the teeth are not arranged in a normal position in the jaw arch, or a state of abnormal occlusion caused by a mismatch in the relationship between the antagonist teeth (Badaring et al., 2020). Malocclusion requires proper handling, specifically orthodontic treatment. (Satria Darwis et al., 2018). Malocclusion urgently require proper treatment by orthodontic treatment (Satria Darwis et al., 2018). Orthodontic treatment aims to correct incorrect occlusion (malocclusion) in the form of dental abnormalities, jaw relationship abnormalities, facial bone growth abnormalities, or soft tissue abnormalities in the mouth. Orthodontic treatment involves alveolar bone remodeling. This process can be stimulated by mechanical forces generated by the activation of orthodontic appliance components that press on the teeth and are transmitted to the tissues surrounding the teeth, including the gingiva, periodontal ligament, and alveolar bone. Orthodontic tooth movement occurs as a result of alveolar bone remodeling, which combines resorption and apposition processes. Orthodontic pressure triggers the release of inflammatory mediators such as interleukin-1β (IL-1β) from the periodontal ligament and alveolar bone, leading to bone resorption (Amin & Permatasari, 2016). Preventing teeth from returning to their original position is the biggest challenge after orthodontic treatment.(Littlewood et al., 2017) According to Proffit, even if the patient believes that treatment is complete when the appliance is removed, the tooth may still be in an unstable position, with continued pressure from the surrounding soft tissues leading to a tendency for relapse (Goenharto et al., 2017). Relapse is defined as a complete or partial return of tooth position to the initial form of malocclusion (Edrizal et al., 2021). The prevalence of relapse after orthodontic treatment is quite high. According to Edrizal et al., there were 93% cases of relapse after orthodontic treatment and 7% cases of no relapse after orthodontic treatment (Dianastesi, 2016). Sheibani *et al*., showed a relapse prevalence of 61.5%. The results of Dianastesi's study found 70.83% of cases of relapse after orthodontic treatment (Ratya Utari & Kurnia Putri, 2019). Many factors can contribute to relapse, including the pull on the periodontal ligament, growth changes, bone adaptation, muscle stress, failure to eliminate causative factors, the role of third molar teeth, and bad habits (Ratya Utari & Kurnia Putri, 2019). Based on data from the University of Washington, research conducted by Riedel and Little, more than 800 cases after orthodontic treatment showed signs of relapse, then Little stated that a reliable way to overcome relapse cases is the use of long-term retention or lifetime use (Andriekute et al., 2017). Retention can be achieved by using one of two types of retention devices: permanent/adhesive retainers or removable retainers (Arowoogun et al., 2021). Fixed retainers made of stainless steel wire are generally used canine to canine. The disadvantage of fixed retainers is that they can cause plaque and calculus accumulation so oral hygiene must be maintained properly. Levin *et al.* showed that fixed retainers cause increased plaque accumulation, gingival recession, and bleeding on probing. Meanwhile, Pandis *et al.* reported that the result of long-term use of fixed retainers caused an increase in socket depth, marginal gingival recession, and calculus accumulation. Calculus accumulation occurs because the interproximal area under the retainer is difficult to clean (Kartal & Kaya, 2019).

Commonly used removable retainers are acrylic retainers and removable vacuum formed retainers/clear retainers. However, there are drawbacks to the use of removable retainers, acrylic retainers cause speech impairment at the beginning of use, unsatisfactory aesthetics and are highly dependent on patient co-operation in wearing the retainer. Patients generally prefer to wear clear retainers because they are invisible, but there are weaknesses to wearing clear retainers including that they can cause a slight open bite, are highly dependent on the patient's discipline in using them, in long-term use they can change color and cause unpleasant odors. A study conducted in Shanghai stated that there was a 24% incidence of broken clear retainers

(Goenharto et al., 2017). A study found that 50% relapse was seen at 2 years post retainer use, 28% relapse was seen at 2-5 years post retainer use, and 12% relapse was seen at 5-10 years post retainer use (Fathurrahman & Musfiroh, 2018). There are other options for preventing relapse, including natural ingredients. Novita *et al.* found that flavonoid compounds (anthocyanins) can be used as alternative materials to prevent relapse after orthodontic treatment.

Rosella *(Hibiscus sabdariffa Linn)* is known to have a variety of properties that have been utilized in the health sector. The chemical content contained in rosella petals are polyphenols, saponins, tannins, and flavonoids consisting of flavonol and anthocyanins (Yunitasari et al., 2015). Based on the description above, researchers want to further analyze the study of the potential of chemical compounds of rosella flower extract *(Hibiscus sabdariffa Linn)* as an antioxidant.

METHOD

This research was a laboratory experiment. The research phase began with the extraction of rosella flowers *(Hibiscus sabdariffa Linn)*, then continued with the second stage, namely the phytochemical screening of rosella flowers *(Hibiscus sabdariffa Linn)* using the color reaction method.

The population in this study was rosella flower petals *(Hibiscus sabdariffa Linn)* in Indonesia. The sample of this study was rosella flower extract *(Hibiscus sabdariffa Linn.*

The number of samples used for this research was 2 kg of dried rosella flowers which was extracted and produced a thick extract of 300.1 grams.

The rosella plant *(Hibiscus sabdariffa Linn)* has a single ovoid leaf with rays, a blunt tip, serrated edges, and a notched base. The leaf measures 6-15 cm in length and 5-8 cm in width. The rounded petiole is green and measures 4.7 cm in length. Rosella flowers *(Hibiscus sabdariffa Linn)* have brightly colored flowers, with dark red, bell-shaped, and not deciduous petals.

Screening tests were conducted to determine the content of secondary metabolite compounds in rosella flower ethanol extract. The tests carried out included flavonoid, tannin, and saponin tests.

The preparation of rosella flower extract started with rosella in the oven at 50°C for 24 hours until it was easily crushed when squeezed (moisture content ±8 percent). The dried rosella flowers were crushed with a blender until smooth and sieved through a 60 mesh sieve. Rosella flower extract *(Hibiscus sabdariffa Linn)* was prepared using the maceration method, which involved weighing 2 kg of rosella flower powder and placing it in an erlenmeyer flask with 96% ethanol solvent, the powder-to-ethanol ratio being 1:4, and macerating for 2 hours at 60°C. The solution was then filtered through a large cloth, the rosella extract was filtered through Whatman paper no. 1, and the mixture was concentrated using a rotary evaporator at a temperature of 40-50°C and a pressure of 10 mBar, yielding a thick extract of rosella petals of 300.1gram. The termination of the evaporation process was determined by the non-dripping of the solvent.

The flavonoid test is conducted by dissolving 10 grams of extract in 96% ethanol until dissolved. Tannin and saponin tests were performed by taking 10 grams of extract, adding 10 mL of hot water, and boiling for 10 minutes. Rosella extracts in the phytochemical screening process were tested as liquid extracts.

The flavonoid test was conducted by dissolving rosella extract in ethanol was placed in a test tube with 0.1 mg magnesium powder and 5 drops of 37% hydrochloric acid, shaken vigorously. A red or orange-colored solution indicated a positive reaction in the presence of flavonoids. 2N sulfuric acid reagent as much as 4 drops. A positive reaction for the presence of flavonoids was the formation of a red or orange-colored solution. Reagent 10% sodium hydroxide solution as much as 4 drops. A positive reaction for the presence of flavonoids was the formation of a red or orange-colored solution.

The tannin test was conducted when the rosella extract that had been dissolved with ethanol was put into a test tube and added with 4 drops of 1% iron (III) chloride.

The formation of a greenish-black colored solution indicated the presence of tannin compounds.

The saponin test was conducted when rosella extract that had been dissolved in ethanol was added to 1 ml of water and then heated for 15 minutes and then shaken vigorously for 30 seconds. Saponin compounds were positive if a stable foam was formed with a height of 1-10 cm with an interval of ± 10 minutes and did not disappear with the addition of 1 drop of 2N hydrochloric acid.

RESULT AND DISCUSSION

Phytochemical screening is one of the qualitative tests used to identify active compounds in specific extracts. Flavonoids, tannins, and saponins are the active or secondary metabolite compounds found in rosella flower extract *(Hibiscus sabdariffa Linn)* in a phytochemical screening test for relapse prevention. The results are shown in Table 1.

Table 1. Phytochemical Screening Test Results

Secondary Metabolites	Test Method	Test Results	Description
Flavonoids	Concentrated HCl + Mg Reagent H2SO4 2N Reagent NaOH 10% Reagent	+	Red/orange solution
Tannins	FeCl3 1% Reagent	+	Black-greenish solution
Saponins	HCl + H2O Reagent	+	Formed foam
Phenolic	FeCl3 5% Reagent	+	Thick black solution
Steroids	Lieberman-Burchard Reagent	-	Color change
Terpenoids	Lieberman-Burchard Reagent	-	Color change
Alkaloids	Wagner Reagent Dragendorff Reagent	+	Reddish brown/ orange brown Precipitate

Note: The presence of active compounds is marked (+)

The phytochemical test found that rosella flower extract contained flavonoids, tannins, saponins, phenolics, and alkaloids. Flavonoids, tannins, and saponins were the chemical compounds identified in this study as having the potential to prevent relapse. After reacting rosella extract with concentrated HCl + Mg reagent, 2N H2SO4 reagent, and 10% NaOH reagent, the color of the flavonoid test changed to red/orange. After reacting rosella extract with 1% FeCl3 reagent, the tannin test found a greenish black color change. The saponin test showed the formation of foam when rosella extract was reacted with HCl + H2O reagent. This can be seen in Figure 1-3.

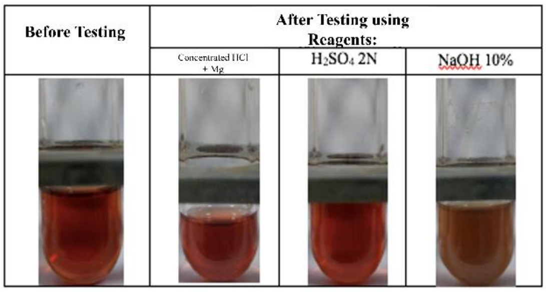

Figure 1. Documentation of Flavonoid Results in the Phytochemical Screening Test

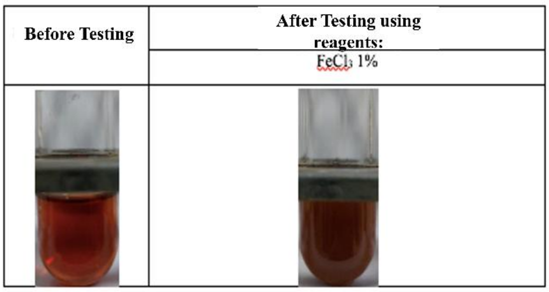

Figure 2. Documentation of tannin results in phytochemical screening test

Before Testing	After Testing using Reagents:
	$HCl + H_2O$
	

Figure 3. Documentation of Saponin Results in the Phytochemical Screening Test

Phytochemical screening is one method for determining the levels of secondary metabolite compounds in plants. Phytochemical screening is one method for identifying the presence of secondary metabolite compounds in natural materials. Phytochemical screening is a preliminary step that can provide an overview of the content of specific compounds in the natural materials being studied. Phytochemical screening can be done qualitatively, semi-quantitatively, or quantitatively, depending on the desired outcome. Color reactions with specific reagents can be used to conduct qualitative phytochemical screening. The most important factor influencing the phytochemical screening process is the solvent and extraction method used. Inadequate solvents cause the desired active compounds to not be attracted properly and perfectly (Vifta & Advistasari, 2018).

Phytochemistry in this study was conducted in a simple way, where qualitative color testing was conducted through the selection of color reagents and extraction methods.(Vifta & Advistasari, 2018) The selection of appropriate color reagents allowed the desired secondary metabolite compounds to be seen properly and perfectly. The extraction method functions in the separation of active compounds from a mixture using a solvent.(Ibrahim et al., 2016) The purpose of extracting natural materials was to obtain the chemical components contained within them. This extraction was based on the mass transfer principle, in which substance components began to transfer into the solvent in the interfacial layer before diffusing into it. Following sample preparation, the first step in the isolation of plant secondary metabolites was extraction. Time and temperature were critical components of the solvent extraction process. Ideally, increasing time and temperature improves the solubility of active compounds in solvents (Hikmawanti et al., 2021). One type of extraction method that can be used is maceration, which involved mixing simplisia and solvents in a closed container at room temperature. Maceration was a solvent-based extraction technique for solids. Methanol or ethanol were commonly used as solvents. The maceration process typically took up to six days. The process began with placing the sample in a closed container, then adding the required solvent in a 1:7 ratio and allowing it to stand for 6 days at room temperature, protected from light, with occasional stirring. After that, the liquid was separated, and the settled portion was removed (Atun, 2014).

Rosella extraction was carried out by maceration method using 96% ethanol solvent. In accordance with the research of Badaring *et al.,* (2020) the maceration process is a simple extraction technique without heating (cold extraction) in this process the sample and solvent do not go through a heating process so that they can be used in compounds that are not heat resistant (Badaring et al., 2020). Ismaningdyah *et al.* (2016) found that 96% ethanol was the most effective solvent for achieving the highest extract concentration (Kurniawati et al., 2016).

Phytochemical tests were conducted to determine the type of secondary metabolite compounds found in rosella extract *(Hibiscus sabdariffa Linn)*. Phytochemical screening includes tests for flavonoids, tannins, and saponin compounds. Rosella extract *(Hibiscus Sabdariffa Linn)* changes color in a phytochemical test, indicating that it contains flavonoid compounds (Oktapiya et al., 2022); (Pratiwi et al., 2022); (Fitriaturosidah et al., 2022). This is in line with phytochemical research conducted by Tira risa *et al.,* (2022), Zuliayu *et al.,* (2022), and Fitriaturosidah *et al.,* (2022), who found that rosella contains flavonoid compounds. Flavonoids are secondary metabolites of polyphenols found in plants and foods, with antiviral, anti-inflammatory, and antioxidant properties. (Wang et al., 2018). Flavonoid compounds are polyphenolic compounds with 15 carbon atoms arranged in the C6-C3-C6 configuration, which means the carbon skeleton is made up of two C6 groups (substituted benzene rings) connected by a three-carbon aliphatic chain.(Wang et al., 2018) Flavonoids are present in all green plants, so they can be found in any plant extract. Flavonoids are a group of compounds that are abundant in nature. Plants contain flavonoids, which help to produce yellow, red, orange, blue, and purple pigments from their fruits, flowers, and leaves. Flavonoids are water-soluble polyphenols.(Wang et al., 2018) Flavonoids can protect bone health through five reaction mechanisms: reducing bone resorption through antioxidant activity, reducing bone resorption through anti-inflammatory activity, increasing osteoblastogenesis activity, suppressing osteoclastogenesis activity, and osteoimmunological activity. Flavonoids also help to regulate bone mass by increasing osteoblastogenesis, suppressing osteoclastogenesis, and reducing bone resorption activity. According to Novita *et al.,* flavonoid-derived compounds, specifically anthocyanins, can be used as alternative materials to prevent relapse following orthodontic treatment because they are inexpensive and readily available in nature. Anthocyanins can help prevent post-orthodontic relapse by inhibiting osteoclast differentiation while increasing osteoblast differentiation. Anthocyanins are non-toxic and not mutagenic in vitro. Systemic anthocyanin injection in vivo had no teratogenic or mutagenic effects. In human clinical trials, only 4% of participants experienced adverse effects. Anthocyanin compounds have the potential to prevent relapse following orthodontic treatment by inhibiting osteoclastogenesis.

Ikalinus *et al.,* in the research of Tira risa *et al.,* (2022) explained that flavonoids are included in the group of phenol compounds that had many OH groups which were characterized by a high electronegativity difference, so they were polar. This group of compounds is easily extracted using a polar solvent such as ethanol, which allows hydrogen bonds to form. The addition of metal Mg and HCl to the flavonoid test reduces the benzopyrone nucleus within the flavonoid structure, resulting in the formation of a red or orange flavylium salt (Oktapiya et al., 2022). Flavonoids exhibit various biological activities such as antioxidant, anti-inflammatory, analgesic and antimicrobial. Research by Majeed *et al.,* (2018) on the application of flavonoid extracts of rosella for rat paw defects showed that flavonoids had potential activity in the process of bone defects by suppressing osteoclast activity and increasing osteoblast formation (Majeed & Ghani, 2018). Raut *et al.* also mentioned that flavonoids have anti-inflammatory properties to fight bone loss. Rosella extract *(Hibiscus Sabdariffa Linn)* in this study was positively identified as containing tannins because of the color change in the extract solution to greenish black. This is in line with research conducted by Tira risa *et al.,* (2022) and Zuliayu *et al.,* (2022) which stated that rosella positively contains tannin compounds (Oktapiya et al., 2022). Tannins are phenol compounds with high molecular weight that contain a hydroxy group as well as other related groups such as carboxyl, allowing them to form effective and strong complexes with proteins and macromolecules. Chemically, there are two types of tannins: condensed tannins and hydrolyzed tannins. Condensed tannins are formed by polymerization (condensation) reactions between flavonoids, whereas hydrolyzed tannins are formed by esterification reactions between phenolic acids and sugars (glucose). Tannins are easily oxidized, so the amount of time the substance is exposed to hot water or air determines how much tannic acid is produced. Tannic acid is an example of hydrolyzed tannins. Tannic acid is a polymer of gallic acid and glucose. Tannic acid is an amorphous, lustrous, yellow-white to light brown powder with a distinct odor. Tannic acid contains antibacterial, antienzymatic, antioxidant, and antimutagenic properties. The antioxidant effect has been extensively studied for its effect on bone metabolism by inhibiting osteoclast activity and increasing osteoblast activity (Fathurrahman & Musfiroh, 2018).

According to Jones *et al.,* in Sulistyarini's research (2019) tannin is a compound that is polar because there is an OH- group, therefore when the sample is added to $FeCl_3$ 10% there will be a color change to dark blue or greenish black which indicates the presence of tannin compounds while according to Sangi *et al.,* tannin compounds with $FeCl_3$ will hydrolyze to form a blue-black colour (Sulistyarini et al., 2019). Research conducted by Sukmana *et al.* (2017) stated that mango kasturi bark extract *(Mangifera casturi)* contains tannin compounds that are able to reduce IL-1β expression during bone remodeling and increase BMP-2 expression during bone remodeling where both of these are very influential in the process of bone density. IL-1β plays a role in inflammatory cells at the stage of bone resorption and BMP-2 induces the formation of osteoblasts (Sukmana et al., 2017). Phytochemical screening also showed that rosella flower extract positively contained saponin compounds because foam was formed. In accordance with research conducted by Tira risa *et al.,* (2022) and Zuliayu *et al.,* (2022) found that rosella positively contains saponin compounds (Kurniawati et al., 2016).

Saponins are one of the secondary metabolite compounds contained in plants. According to Dumanau *et al,* this type of compound belongs to a group of organic components that have good steroid capacity. All plant organs such as fruit, flowers, leaves, stems and roots can be found with secondary metabolic compounds of saponins. The molecular structure of saponins consisting of a series of C and H atoms makes this compound have biological activity as an antibacterial. Saponin compounds are applied in pharmacy because they are known to have activities as antifungal, antibacterial, antitumor and antioxidant drugs (Ngginak et al., 2021).

Saponins have a molecular weight of 414.6231 grams/mol and a molecular formula of $C_{27}H_{42}O_3$. Saponins have a high boiling point, reaching 158°C and a density of 0.5 g/cm3 at 20°C. Saponins can dissolve in various solvents such as water, ethanol and also methanol. Some are also soluble in ether, chloroform, benzene, ethyl acetate or acetic acid (Santosa et al., 2018).

According to the Ministry of Health in the research of Sulistyarini *et al.,* (2019) the presence of positive saponins in the tested sample because it forms a foam as high as 1-10 cm, with an interval of ± 10 minutes. According to Harborne, in the study of Sulistyarini *et al.* (2019), the addition of HCl can make the foam more stable. Saponin compounds have the appearance of foam because they contain both water-soluble (hydrophilic) and nonpolar solvent-soluble (hydrophobic) surfactants that can reduce surface pressure (Sulistyarini et al., 2019). Saponins were reported by Lin Tao Hang *et al.* (2013) in the study of Xiang Ying *et al.,* which had anti-inflammatory properties (Kong et al., 2015).

The results of phytochemical screening tests that have been carried out show that rosella flower extract *(Hibiscus Sabdariffa Linn)* was positive for flavonoids, tannins, and saponins which had anti-inflammatory and antioxidant activities. Flavonoids reduced the release of inflammatory mediators. The anti-inflammatory activity of Flavonoids was achieved by inhibiting cyclooxygenase and lipoxygenase, resulting in a shorter inflammatory reaction, while the proliferative ability of Transforming Growth Factor was unaffected, allowing the proliferation phase to occur immediately. Flavonoids can also influence bone mass by stimulating osteoblasts. Bone formation can reduce bone resorption and prevent orthodontic relapse (Lai et al., 2014).

Franzen et al. found that alveolar bone remodeling plays an important role in orthodontic relapse in their animal study (Franzen et al., 2014). The bone remodeling process is controlled by osteoclast cells, which absorb bone, and osteoblast cells, which produce new bone (Martin, 2014). All these cells communicated and collaborated to achieve bone remodeling. Bone remodeling was resulted in changes to the alveolar bone through bone formation in the pull area and resorption in the pressure area. Bone remodeling occurred in three cycles, beginning with osteoclasts initiated bone resorption, followed by a transition from bone resorption to new bone formation, and finally by osteoblasts forming new bone. On days 3-5, there was initial resorption, followed by the healing phase on days 5-7, and the final stage of bone formation on days 7-14; a typical time in bone remodeling was 2-8 weeks. Post-orthodontic relapse can be effectively prevented by activities that can inhibit bone resorption and stimulate bone formation. These results showed that controlling alveolar bone after active orthodontic tooth movement is an important method in preventing post-orthodontic relapse (Schneider et al., 2015).

CONCLUSION

Based on the above research, the results of phytochemical tests found that rosella flower extract positively contains flavonoids, tannins, saponins, phenolic compounds, and alkaloids. In this study, the chemical compounds that have the potential to prevent relapse are flavonoids, tannins, and saponins. The compound content of rosella flower extract contains flavonoid, tannin, and saponin chemical compounds that have anti-inflammatory and antioxidant properties so they have the potential to prevent relapse after orthodontic treatment. Further research is needed to investigate rosella flower extract as a prevention of relapse after orthodontic treatment.

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