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Calcium nanoparticles produced by *Acacia arabica* leaf extract and their influence on fresh-cut fruit quality features



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Keywords: Calcium Nanoparticles Acacia arabica Green synthesis Characterization Shelf life	Calcium oxide nanoparticles have possessed unique structural and numerous applications, including the food preservative, antimicrobial, and chemotherapeutic properties. The study's goal was to identify and create calcium oxide-derived leaf extract nanoparticles from <i>Acacia arabica</i> (AA) and assess their effectiveness on fresh-cut fruit quality attributes. The calcium oxide nanoparticles (AACaN) produced using AA leaf extract by the simple precipitation method and the AACaN obtained were characterised and confirmed using various analytical techniques. Freshly sliced apple fruit as well as unsliced blueberry and blackberry were immersed in AACaN (10 and 20 μ g/mL) at two different concentrations for five minutes before being put in packed polypropylene plastic bags and maintained at 5 °C. According to the quality evaluations of pH, DPPH, 2,2-diphenylpicrylhydrazyl (DPPH), hardness, total soluble solid content (TSS), and sensory analysis. AACaN at 20 μ g/mL has the ability to preserve freshly cut fruits of apples as well as unsliced blueberries and blackberries, thereby effectively extending fruits 20-day shelf life in comparison to control (untreated) fruits. This study has concluded that the AA is an ideal source to synthesis calcium oxide nanoparticles, and it was proved by materially characterizing. Moreover, the AACaN at 20 μ g/mL value while maintaining a storage quality.

1. Introduction

The fabrication of nanomaterials involved the influence of structures at the nanoscale, which have increased surface area, high surface potential, and distinctive surface characteristics (Roy et al., 2002). Since calcium oxide (CaO) has prospective agricultural uses, it is used as an inorganic antibacterial agent. In recent years, there has been a notable rise in calcium oxide nanoparticles (CaONPs) on a global level. Although the CaO nanoparticles may undergo numerous carbonation and decarbonation cycles, their relative adsorption effectiveness is low (Roy and Bhattacharya, 2011). Several horticultural crops grew better when exposed to calcium NPs. For instance, it has been demonstrated that calcium nanoparticles are beneficial for plants like lettuce (Lactuca sativa), Bengal gramme (Cicer arietinum L.), rice (Oryza sativa), and zucchini (Syu et al., 2020; Gandhi, 2020; Meier et al., 2020). Even though it has been said that CaO nanoparticles have a high adsorption efficiency, unprocessed CaO quickly loses its ability to go through many cycles of carbonation and decarbonation (Lu et al., 2006). Calcium chloride is an ionic compound and dissolved in water. It is used for various medicinal applications such as diabetics, cardiac problems, antioxidants, hyperkalemia, and the antimicrobial properties. Moreover, it is used as an antidote for intoxication of magnesium due to the high dose of magnesium sulfate. Therefore, it is very safe for us to consume a tolerable dose.

Apples (Malus domestica) are frequently kept for extended periods of time at low temperatures in climate-controlled environments. While in storage, fruit loses some of its nutritional value and quality. Apple planting, harvesting, and storage practices, among other things, all have an impact on how long apples stay fresh (Soliva-Fortuny et al., 2002). Blackberries and blueberries are summer fruits, and they have distinct applications for human health, especially for antioxidants, antiinflammatory, antibacterial, and cardiovascular issues, as well as helping to fight against Alzheimer's disease. Apples, blackberries, and blueberries are the most commonly likeable and high-nutrition fruits but

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the economically unreachable for most people's. Hence, we have chosen these life-time extension studies, and these results hopefully may lessen these fruits economies. Postharvest calcium dips could dramatically enhance content of calcium associated to preharvest sprays without hurting fruit, reliant on the calcium concentration and salt employed. The after harvest calcium treatment increases the life of fresh fruits (Picchioni et al., 1998). It does this by keeping cell membrane integrity, turgor, and tissue stiffness, and by decreasing membrane lipid catabolism. Plant cell walls are stabilised and protected from cell walldegrading enzymes by exogenously supplied calcium (White and Broadley, 2003).

Freshly cut fruits and vegetables, especially fresh apples, have recently gained popularity in restaurants, homes, and school lunch programs over the past few decades (Guan and Fan, 2010). Although in the current era of changing lifestyles, consumers have increased interest in healthy and nutrient-dense diets, it is challenging to preserve apples that have just been sliced because the fruit deteriorates quickly after being cut and also undergoes further decomposition. Due to tissue weakening, microbial growth, and enzymatic browning, the life of fruits is often short (Ruiz-Cruz et al., 2007). The most trustworthy, costeffective, and environmentally acceptable technique for resolving these problems was green nanoparticle production. Due to its safety, widespread application, and accessibility to plants, the synthesis of NPs by plants has attracted the interest of scientists and researchers from throughout the world (Zhang et al., 2011).

AA, also referred to as Indian gum, babul, or kikar, has become well known on a global level and is widely distributed in arid and semi-arid areas. It demonstrates that AA is effective in treating malaria, sore throats, and toothaches (Kubmarawa et al., 2007). Many Acacia species, including *A. senegal* and *A. nilotica*, have been used to biofabricate NPs, and this process has been thoroughly documented (Hayat et al., 2022). It has been used traditionally from the centuries for the treatment of many illnesses, such as antibacterial, antiviral, toothache, and malaria (14). In addition, it has been used to treat hypertension, diarrhoea, gastric problems, and constipation. Recently, AA has been used to treat diabetes, and it has proved well for improved glucose absorption as well as β -cell function (13). Therefore, the goal of the current work was to synthesis calcium oxide nanoparticles using aqueous AA leaf extracts and to ascertain the positive impacts of AACaN on the characteristics that affect fresh-cut fruit's ability to maintain quality over time.

2. Materials and Methods

2.1. Materials

AA leaves were gathered from a nearby farm, and analytical grades with 98 % purity of calcium nitrate Ca (NO3)2·.6H2O, deionized water grade I (Extra Pure), and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich.

2.2. Preparation of leaf extract

The healthy AA leaves were obtained from a local farm. To get rid of any dirt and pollutants, the leaves of AA were washed with flowing water from the faucet. To eliminate debris from the leaves, they were additionally washed three times in sterile distilled water. 10 g of leaves were used to make the leaf extract, which was then immersed for one day in 100 ml of purified water. Next, the filtrate was put to use to make the nanoparticles after it had been filtered using Whatmann No. 1 filter paper.

2.3. Nanoparticles made from calcium oxide

A simple precipitation method was used to make calcium oxide nanoparticles (AACaN) from the leafy extract of AA and 50 ml of a 50 mM calcium nitrate solution. After that, it was agitated in a magnetic stirrer for 30 to 45 min while 1 M sodium hydroxide was incorporated drop by drop until a white precipitate had formed. The precipitate was created by centrifugation, and the basicity of the precipitate was removed by repeatedly washing it in sterile water. The precipitate obtained was maintained for one hour in a hot air oven and then calcined for three hours at 400 °C in a muffle furnace. Eventually, the known AACaN was obtained through the characterizations and utilised in a variety of applications (Ashwini et al., 2016).

2.4. Calcium nanoparticles identification studies

Analytical tools like FTIR, Zeta potential, SEM, and EDX, as well as UV–Vis absorption spectroscopy, were used to figure out the size, functional group, and structure of the AACaN nanoparticles that were made. The AACaN nanoparticle was characterised by UV–Vis spectroscopy. The UV–Vis colour curve of the response mixture at different wavelengths was observed, which revealed the formation of calcium nanoparticles. AACaN UV–Vis spectra were collected in the 300–800 nm range. This experiment was conducted at 25 $^{\circ}$ C (1 cm optically) using quartz cuvettes.

The definition of the zeta potential analysis (ZPA) was used to determine the AACaN initial condition and to assess liquid equilibrium. Zeta Potential software from Malvern Instruments Ltd. was used to make the determination (Ver. 2.3). By absorbing light from the electromagnetic spectrum, the chemicals and functional groups contained in the sample are identified using FTIR and checked for vibrational frequencies. The sample was examined between 400 and 4000 cm, and the data were examined using conventional spectrum data. A SEM study was also conducted to ascertain the AACaN dimensions, morphology, and molecular information.

2.5. Evaluation of biosynthesized AACaN for maintaining shelf life characteristics of freshly cut fruit

Over the course of the 20-day storage period, measurements were made at first 3-day and then 5-day intervals for the TSS, 2,2-diphenylpicrylhydrazyl (DPPH), pH, hardness, weight reduction rate, and sensory analysis. The weight loss of freshly sliced apples, blueberries, and blackberries was determined using the Tefera et al., (2007) approach. A digital balance (A&D, Japan) was used to weigh the fruit with an accuracy of 0.01 g. A pressure meter (OSK 10,576 CO., Japan) equipped with a flat tip measuring 8 mm in diameter was used to gauge the firmness of fruit on two opposing peeled sides.

The firmness is quantified in kilogrammes (kg) as the average peak force of ten distinct fruits. Using a portable refractometer (NC-1, Atago Co., Japan), the percentage of total soluble solids in the apple extract was estimated at room temperature (Schirra et al., 2004). A pH metre was used to measure the juice's pH (Jenway, 3020). The antioxidant activity of AACaN in comparison to ascorbic acid was assessed using the free radical DPPH scavenging test. According to Brand-Williams et al., (1995), the 2,2-diphenyl-1-picrylhydrazyl (DPPH) study proved that the DPPH reagent was used to carry out the free radical scavenging activity. The decrease in absorbance at 515 nm was discovered using a spectro-photometer (Spectromax Plus 384, Molecular Devices, Sunnyvale, CA, USA) after 20 min of incubation at room temperature.

Five individuals were given instructions to identify and grade the characteristics of apple slices. Freshly sliced fruit's visual appeal, aroma, texture, and decay were graded on a scale from 1 (completely deficient or soft) to 9 (totally indicative of fresh). A comparable scale was used to assess the product's general acceptability, with 1 being unconsumable, 3 being poor, 5 being fair (the upper limit of marketability), 7 being acceptable, and 9 being excellent. Additionally, it was graded on a five-point scale, with 1 signifying a complete lack of softness and 5 signifying that it was completely fresh. A similar scale with the following values: 1 for unusable, 3 for poor, 4 for fair (limit of marketability), 4.5 for acceptable, and 5 for exceptional.

2.6. Statistical analysis

In order to calculate the ANOVA analyses of variance, SPSS software (SPSS Inc., version 13.0, Chicago, IL, USA) was used. Duncan's multiple range tests were used to determine the significance between mean values. At 0.05, the significance level was established.

3. Results

3.1. Characterization of AACaN

Zeta potential was used in our investigation to test the stability of AACaN, as shown in Fig. 1. The calcium phosphate nanoparticles' -20.25 mV Zeta potential showed that the calcium phosphate colloid is stable and that the particles do not agglomerate (Kilic et al., 2016). The particle surface is negatively charged, as shown by the negative zeta potential, because of OH, carboxylic groups, and other molecules.

In Fig. 1, results from the SEM-EDX pictures show that the AACaN particles produced in this experiment were produced through biological processes, as shown by the EDX diffractogram and SEM image analysis above. Their sizes were uniformly distributed, allowing them to be classified as nanoparticle material (less than 100 nm). In accordance with the EDX diffractogram, the synthesised CaO material mostly consists of 41 (w/w), C-52.13 %, Ca-29 %, O-13.22 %, and 3.44 % of Sn-L.

In this study, FTIR analysis was also used to show that Ca ions interacted with the OH functional group of the secondary chemical made from *Acacia arabica* leaf extracts, and these results are shown in Fig. 1. It exhibited O–H, C–H, and C–C vibrations with peaks at 3694.44, 3427.02, 1629.56, 14.77.16, 1415.44, 1069.48, and 869.85 cm⁻¹.

3.2. Effect of AACaN on the various qualities attributes of freshly cut fruits

In the current investigation, the pH variations of freshly cut apples, blackberries, and blueberries were stored at 5° C up to 20 days, and the results are shown in Fig. 2. Apples, blackberries, and blueberries are assumed to have a pH of 4 to 5, making them technically moderately acidic. It turned out that the pH of fresh-cut fruits treated with AACaN

 $(T1-10 \mu g/mL \text{ and } T2-20 \mu g/mL) \text{ and } TS1 (2 \% CaCl₂) was different from the control after 10 to 20 days. The breakdown of acids during respiration during storage would result in an increase in pH compared to an untreated control.$

Fig. 2. shows whether AACaN affected the weight loss of stored freshcut fruits of apples, blackberries, and blueberries. According to the findings, AACaN (T1-10 μ g/mL and T2-20 μ g/mL), Ts1 (2 % Cacl2), and fresh-cut fruits avoided weight loss compared to an untreated control.

In this study, the tissue stiffness of freshly cut fruits of fruits of apples, blackberries, and blueberries treated with T2 (20 μ g/mL) (AACaN) and TS1 (2 % CaCl2) was much higher than in the control group (Fig. 3). Moreover, the study found that AACaN (20 μ g/mL) produced firmer results than 2 % calcium chloride.

In comparison to the control, all treatments produced minimal TSS (Fig. 3). Additionally, TSS decreased as the AACaN concentration rose to 20 μ g/mL, then 2 % calcium chloride. TSS often rises when fruits ripen and the starch is converted into soluble sugars. The fruits wrapped in air showed a declining trend in the antioxidant activity of DPPH. AACaN (20 μ g/mL) and 2 % calcium chloride-treated fruits, however, have greater antioxidant activity than untreated fruits (Fig. 4).

The appearance evaluation of apple, blueberry, and blackberry treated with AACaN ($20 \mu g/mL$) and 2% calcium chloride was improved when compared to the control, and the results are shown in Fig. 4. The apple slices were just below the point at which they could be sold after 25 days, and this outcome was mirrored by Aguayo et al. (2010). The aroma in apple, blueberry, and blackberry slices treated with AACaN and CaCl2 had a noticeable difference when compared to the control (Fig. 5). The texture in apple, blueberry, and blackberry treated with AACaN and CaCl2 had exhibited good texture during the entire storage time (Fig. 5). The degradation of apple, blueberry, and blackberry was reduced in treated with AACaN and CaCl₂ after 20 days, once linked by untreated fruits and the outcomes are exposed in Fig. 6.

4. Discussion

Calcium (Ca^{2+}) is a mineral that is required for life. Calcium oxide (CaO) is a chemical with a high volume that is used in many different industries. Moreover, calcium oxide is cheap, accessible, and abundant



Fig. 1. Shows the AACaN Zeta potential, SEM and EDX as well as FTIR peak.



Fig. 2. The pH and weight loss (mg) of AACaN and 2 % calcium chloride treated as well as control of fresh-cut apple, blackberries and blue berries. T1: 10 µg/mL of AACaN samples, T2:20 µ



Fig. 3. Firmness and TSS (° brix) (b) of AACaN and 2 % calcium chloride treated as well as control of fresh-cut apple, blackberries and blue berries. T1: 10 µg/mL of AACaN samples, T2:20 µg/mL of AACaN samples, TS1: 2 % calcium chloride samples, C: Untreated Control samples.

in nature. Before further refining, chemicals and substances, including citric acid, glucose, and certain colours, are typically refined using calcium oxide (Imtiaz et al., 2013). More recently, the green synthesis approach has been valued for its reliability, cost-effectiveness, safety, and environmental friendliness (Veeramani et al., 2022).

Plant extracts are plentiful in antibacterial and antioxidant chemicals, which are also suitable for use as capping and reducing mediators for creating NPs in a green manner. Aqueous leaf extracts from *A. arabica* were used to make ZnO-NPs, which were then tested for their ability to kill pathogens in food that are resistant to multiple drugs (Pallavicini et al., 2017). Similar to that, calcium oxide nanoparticles made from aqueous *Acacia arabica* leaf extracts were created in the present study, and they were characterised using UV-visible spectroscopy, Zeta potential, scanning electron microscopy (SEM), and FTIR analysis. These bio-synthesised AACaN were evaluated for enhancing shelf life attributes such as pH, stiffness, weight loss, 2,2-diphenylpicrylhydrazyl



Fig. 4. DPPH and visual appearance of AACaN and 2 % calcium chloride treated as well as control of fresh-cut apple, blackberries and blue berries. T1: 10 µg/mL of AACaN samples, T2:20 µg/mL of AACaN samples, TS1: 2 % calcium chloride samples, C: Untreated Control samples.



Fig. 5. Aroma, texture and decay of AACaN and 2 % calcium chloride treated as well as control of fresh-cut apple, blackberries and blue berries. T1: 10 µg/mL of AACaN samples, T2:20 µg/mL of AACaN samples, TS1: 2 % calcium chloride samples, C: Untreated Control samples.

(DPPH), and sensory evaluation of fresh-cut fruits, which are all related to total soluble solid content (TSS).

4.1. Biosynthesis and characterization of AACaN

A colour shift from green to pale yellow to brown in the *Acacia arabica* leaf extract, which was used to validate the synthesis of CaO-NPs when added to CaO and the appearance of the solution's turbidity. This



Fig. 6. Decay of AACaN and 2 % calcium chloride treated as well as control of fresh-cut apple, blackberries and blue berries. T1: 10 µg/mL of AACaN samples, T2:20 µg/mL of AACaN samples, TS1: 2 % calcium chloride samples, C: Untreated Control samples.

turbidity suggested the emergence of precipitate, which was further calcinated in the muffle furnace at 400 °C and made into a dried powder. The colour changed as a result of the Ca2 + ions' interactions with the secondary compounds' OH sides, creating a stable complex of Ca ions that showed the existence of hydroxyl in the system through the appearance of a brown colour (Ramli et al., 2019). In the present study, the finally obtained AACaN was characterised using analytical techniques to confirm nanoparticle formation.

Zeta potential, a measurement of the actual electric charge on the particle surface, can be used to gauge the stability of the particles. The solution's ionic strength, ionic composition, and pH, as well as its distance from the particle surface, all affect how much electrical potential is measured (Nakatuka et al., 2015). Zeta potential was used in our investigation to test the stability of AACaN. The calcium phosphate nanoparticles' –20.25 mV Zeta potential showed that the calcium phosphate colloid is stable and that the particles do not agglomerate (Kilic et al., 2016). The particle surface is negatively charged, as shown by the negative zeta potential, because of OH, carboxylic groups, and other molecules.

In a second physical research study, scanning electron microscopyenergy dispersive X-ray spectroscopy (SEM-EDX) was used to look at the surface morphology, size, porosity, and shape of the CaO that was made. The SEM-EDX pictures show that the CaO particles produced in this experiment were produced through biological processes, as shown by the EDX diffractogram and SEM image analysis above. Their sizes were uniformly distributed, allowing them to be classified as nanoparticle material (less than 100 nm), and this correlates with other previously described studies (Marquis et al. 2016). In accordance with the EDX diffractogram, the synthesised CaO material mostly consists of 41 (w/w), C-52.13 %, Ca-29 %, O-13.22 %, and 3.44 % of Sn-L. The outcomes more closely resembled those of Qin et al., (2016).

In this study, FTIR analysis was also used to show that Ca ions interacted with the OH functional group of the secondary chemical made from AA leaf extracts. It exhibited O–H, C–H, and C–C vibrations with peaks at 3694.44, 3427.02, 1629.56, 14.77.16, 1415.44, 1069.48, and 869.85 cm-1. As was expected, this might be projected to result from a unique Ca-O vibration caused by the establishment of a chemical

connection between the Ca2 + ion and the CO-functional group (Anantharaman et al., 2016).

The FTIR analysis of Balashanmugam and Kalaichelvan's, (2015) study showed that the N–H, O-, C = C, and C–H groups were substituted hydroxyl and amino groups for flavonoids. The FTIR results of plant extracts by themselves don't show any peaks for calcium ions. Instead, they only show peaks for calcium nanoparticle formation, which shows that secondary metabolites in plant extracts act as agents for nanoparticle formation.

4.2. Effect of AACaN on the various qualities attributes of freshly cut fruits

Morga et al., (2019) reported that the pH has a direct impact on how stable the nanoparticles are since all nanoparticle types are constant near the isoelectric point. The double layer's characteristics can be changed by a change in pH, and these changes have a direct impact on the zeta potential and increase the possibility of flocculation or coagulation. The pH variations of freshly cut apples, blackberries, and blueberries were stored at 5 °C for up to 20 days. The apples, blackberries, and blueberries are assumed to have a moderately acidic pH of 4 to 5. It turned out that the pH of fresh-cut fruits treated with AACaN (T1-10 μ g/mL and T2-20 μ g/mL) and TS1 (2 % CaCl₂) was different from the control after 10 to 20 days. The breakdown of acids during respiration during storage would result in an increase in pH compared to an untreated control.

AACaN affected the weight loss of stored fresh-cut fruits of apples, blackberries, and blueberries. According to the findings, AACaN (T1–10 μ g/mL and T2–20 μ g/mL), Ts1 (2 % Cacl₂), and fresh-cut fruits avoided weight loss compared to the untreated control. During a 75-day storage period, pear fruit treated with CaCl₂ outperformed untreated fruit in terms of decreasing weight loss, according to Mahajan and Dhatt (2004). In the current study, the control group experienced the most weight reduction, while the fresh-cut fruit groups treated with T2 and TS1 (2 % Cacl₂) for 20 days experienced the least weight loss. The lower weight loss observed in calcium-treated fruits may be due to calcium treatments' effectiveness in maintaining membrane functionality and

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integrity. The results mentioned above are consistent with those of our study (Veeramani et al., 2022), who discovered that dipping fruits in calcium solutions of various concentrations decreased the percentage of weight loss in fruits.

According to previous studies, firmness is an important indicator of fruit quality, and a loss in firmness could be a serious issue (Veeramani et al., 2022; Kov et al., 2005). In this study, the tissue stiffness of freshly cut fruits of fruits of apples, blackberries, and blueberries treated with T2 (20 μ g/mL) (AACaN) and TS1 (2 % CaCl2) was much higher than in the control group (Fig. 7). Moreover, the study found that AACaN (20 μ g/mL) produced firmer results than 2 % calcium chloride. Calcium, which has a negative impact on stiffness and breaks down the cell wall, protects enzymes from the cell wall (White and Broadley, 2003). Additionally, calcium has been found to decrease the ripening and softening processes by lowering the frequency of exhalation and ethylene production during fruit development (Guan and Fan, 2010). The firmness of fruit has also been demonstrated to be improved by calcium application (Fallahi et al., 1988; Neilsen et al., 2005; Benavides et al., 2002).

In comparison to the control, all treatments produced minimal TSS (Fig. 8). Additionally, TSS decreased as the AACaN concentration rose to 20 μ g/mL, then 2 % calcium chloride. TSS often rises when fruits ripen and the starch is converted into soluble sugars. However, it's possible that decreased activity is what caused the lower TSS levels after the treatment of the enzymes that hydrolyze polysaccharides into mono-saccharides (Agar et al., 1999). The fruits wrapped in air showed a declining trend in the antioxidant activity of DPPH. AACaN (20 μ g/mL) and 2 % calcium chloride-treated fruits, however, have greater antioxidant activity than untreated fruits.

The appearance evaluation of apple, blueberry, and blackberry slices treated with AACaN ($20 \mu g/mL$) and 2% calcium chloride was improved in comparison to the control. The apple slices were just below the point at which they could be sold after 25 days, and this outcome was mirrored by Aguayo et al., (2010). The aroma in apple, blueberry, and blackberry slices treated with AACaN and CaCl2 had a noticeable difference when compared to the control. The texture in apple, blueberry, and blackberry slices treated with AACaN and CaCl2 had exhibited good texture during the entire storage time.

The degradation of apple, blueberry, and blackberry slices was reduced when treated with AACaN and CaCl2 after 20 days. According to Aguayo et al., (37), untreated or treated apple slices stored in the air or MA for a short period of time (7 days) exhibited browning, microbiological degradation, and poor sensory quality. Hence, our investigations have revealed that the sliced fruits treated with AACaN did not decay for up to 20 days when compared to the control sliced fruits.

5. Conclusion

In the current investigation, an aqueous leaf extract of Acacia arabica was used to materially characterise calcium oxide nanoparticles and determine their positive effects on fresh-cut apple quality parameters that affect shelf life. AACaN at 20 μ g/mL is beneficial in enhancing sensory, chemical, and visual qualities, according to the study. The shelf life was significantly increased by this nano-application, which also added a large nutritional value while maintaining a storage quality that was acceptable.

CRediT authorship contribution statement

Chinnadurai Veeramani: . Mohammed A. Alsaif: Supervision. Muhammad Ibrar Khan: Methodology. Ahmed S. El Newehy: Methodology. Ali Alshammari: Methodology. Khalid S. Al-Numair: Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

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