Antibacterial Activity Test Turmeric(*Curcuma longa* L.) Extract Herbal Oil in Extra Virgin Olive Oil Against *Staphylococcus aureus* and *Propionibacterium acnes*

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Abstract. Turmeric and olive oil can be combined to produce herbal oil extracts. The purpose of this study was to determine the content of active compounds and to determine the effect of variations in the concentration of extracts on antibacterial activity. Turmeric is combined with extra virgin olive oil using the hot maceration method. The combination is formulated into five turmeric concentration namely 10%, 20%, 30%, and 40% in 100 mL of solution with variations in oven temperature of 30 °C, 40 °C, 50 °C, and 60 °C. The phytochemical test of secondary metabolite compounds, triterpenoid, flavonoid, and tannin. The herbal oil extract positive for triterpenoid and flavonoid compounds. The results of the antibacterial test against *Staphylococcus aureus* at concentrations of 10%, 20%, 30%, and 40% is 1.7 mm, 3.3 mm, 3.6 mm, and 3.9 mm, while in *Propionibacterium acne* is 1.6 mm, 2.6 mm, 2.9 mm, and 3.6 mm. Total Plate Count (TPC) test on the herbal oil extract against *Staphylococcus aureu* produces living microbes for $5{,}2x10^8$ CFU/ mL, $4{,}8x10^8$ CFU/mL, $3{,}7x10^8$ CFU/mL and $3{,}4x10^8$ CFU/mL, while in *Propionibacterium acne* for $3{,}0x10^8$ CFU/ mL, $2{,}0x10^8$ CFU/ mL, $1{,}6x10^8$ CFU/ mL, and $1{,}3x10^8$ CFU/ mL.

Keywords: *Curcuma longa* L. · *Olive Oil* · *Antibacteria* · *Disc Diffusion* · *Staphylococcus aureus* · *Propionibacterium acnes*

1 Background

Herbal oil is a type of oil that comes from herbal plants and can be used as traditional medicine to cure various types of diseases [1]. The potential of herbal oil as a natural treatment has grown rapidly in the ethnomedicine world because it has a higher level of safety and does not cause harmful effects to the body [2]. One example is the use of turmeric and olive oil as natural remedies for therapy in overcoming skin problems such as acne due to infection accompanied by inflammation.

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1.1 Turmeric *(Curcuma Longa* L.*)*

Turmeric is a type of tropical plant that belongs to the ginger group, has a distinctive color, namely bright yellow with yellowish green flowers (Fig. 1.), single leaf with a pointed tip and base, flat edges, oval in shape and able to live in rainfall of 1000–4000 mm/year with optimum temperature between 19-30ºC (Fig. 2.). Turmeric produces the main tuber in the form of a dark yellow rhizome in the form of a length of up to 20 cm with a thickness of 1.5–4 cm, yellow to reddish orange [3].

1.1.1 Active Compound Content

The active compounds in turmeric is 94% curcumin and essential oils [5]. The volatile oil components is terpenoid compounds consisting of aryl-turmerone (20.20%), α-turmerone (1.84%) and β-turmerone (2.58%), sesquiterpenes (curcumene, turmerone, curlone)., β -elemene) and triterpenoid compounds which is known to have biological activity of 21.22% and have potential as antibacterial [6].

Curcumin as an active polyphenolic substance is able to inhibit the activity of gram-positive and gram-negative bacteria because there are phenolic groups and diketone groups in its structure [9]. The content of curcumin and essential oil in turmeric rhizome acts as an antibacterial and anti-inflammatory so that it can inhibit bacterial growth and cure inflammation due to bacterial infection [10]. The active compounds in turmeric also have great potential to inhibit *Staphylococcus aureus* and *Propionibacterium acnes* bacteria which causes redness of the facial skin accompanied by the formation of abscesses and pus [11].

Fig. 1. Turmeric Plant [4].

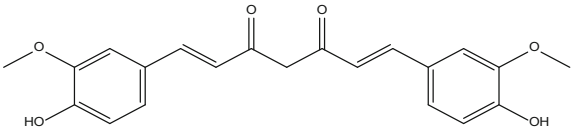

Fig. 2. Curcumin Structure [7].

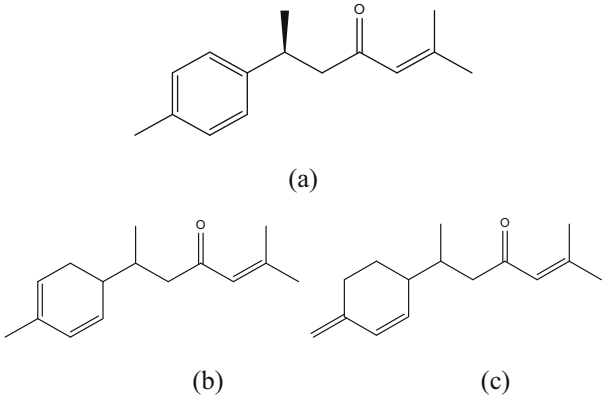

Fig. 3. Essential Oil Structure [8].

1.2 Olive Plant *(Olea Ouropea* L.*)*

Olive plant (*Olea Europea* L.) is a type of woody plant that has a long period of growth of leaves, stems, roots and is able to produce seeds or olives. Ripe olives that are purple-black in color are often extracted for the oil known as olive oil [12]. Olive plants have trees reaching 3–15 m high and single elliptical leaves, small white flowers with a length of 6–10 mm [13].

1.2.1 Active Compound Content

Extra virgin olive oil (EVOO) contains bioactive compounds such as the main flavonoid, namely luteolin (3,84%) and essential oil of the sesquiterpene group, namely eremophilene (5,20%) as an active compound of phenol derivatives [15, 16]. Antibacterial activity in EVOO is caused by the presence of polyphenolic compounds in the form of flavonoids (Fig. 3.), oleuropein and essential oils that have pharmacological effects on microbial activity [17]. Generally, the phenolic compounds in EVOO can be used as an inflammatory or anti-inflammatory [18].

Fig. 4. Olive Plant (*Olea Europea* L.) [14].

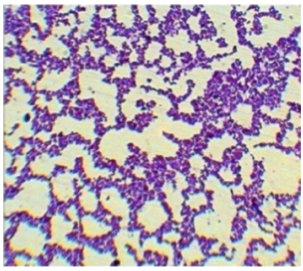

Fig. 5. *Staphylococcus aureus* Bacteria [25].

1.3 Hot Method Maceration

Maceration extraction is an extraction method that aims to attract bioactive compounds such as antibacterials from natural ingredients by utilizing the "like dissolve like" principle where the solvent will tend to dissolve the active compound with the same polarity [19]. One of the modified extraction methods is hot maceration or often called digestion maceration which involves heating at a low temperature of 40-50ºC for 1–2 h depending on the characteristics of the plants used [20, 21]. The hot maceration method is a suitable method for the extraction of active compounds that are heat resistant and easily soluble in certain solvents [22].

1.4 Bacteria

1.4.1 Staphylococcus Aureus

Staphylococcus aureus is a gram-positive bacteria that can live in colonies in short chains at a temperature of 6.5–46 C on the mucosa and skin within 24 h with a diameter of up to 4 mm and a size of about 0.5–1.0 m [23]. *S. aureus* bacteria have one cell wall and do not form spores (Fig. 4.), forming pigments that cause golden yellow colonies, while in nutrient media (Fig. 5.), *S. aureus* bacteria will form pigments that cause milky white colony growth [24].

Staphylococcus aureus is pathogenic bacteria that easily grow on the surface of the facial skin and cause clinical diseases such as the appearance of wounds marked by inflammation on the skin surface [26], the appearance of pus and the formation of abscesses due to the rapid excretion of bacteria [27].

1.4.2 Propionibacterium Acnes

Propionibacterium acnes is a gram-positive bacteria that can cause inflammation of the skin [28]. These bacteria are among the main organisms that play a role in the formation of acne. The characteristics of this bacterium are pointed, with uneven coloring and beads (Fig. 6.), not spores, and sometimes coccoid or round in shape [29].

Propionicbacterium acnes growth is relatively slow and has very pleomorphic characteristics. These bacteria play an important role in producing inflammation through their ability to break down triglycerides into fatty acids.

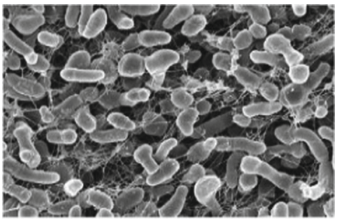

Fig. 6. *Propionicbacterium acnes* [30].

1.5 Active Compound Phytochemical Test

Identification of active compounds in natural ingredients can be known through phytochemical tests [31]. Phytochemical test is the stage of testing the content of secondary metabolites such as triterpenoid, flavonoid, and tannin in plants that have high bioactivity capabilities, one of which is antibacterial [32]. Phytochemical test results can be observed through visual color changes [33]. The more concentrated the resulting color change, the higher the secondary metabolite content [34].

2 Research Methods

This research is a laboratory experimental study to prove the antibacterial ability of turmeric extract in olive oil against grambacteria. Positive bacteria are *Staphylococcus aureus* and *Propionobacterium acnes.* This research includes sample extraction, phytochemical test, antibacterial activity test and sample identification using *fourier transform infra red* (FTIR).

The test materials used were turmeric rhizome (*Curcuma longa* L.) and pure olive oil (Extra Virgin Olive Oil). Coarsely chopped turmeric rhizome was added with extra virgin olive oil with variations in turmeric concentration of 10%, 20%, 30% and 40% (gr/ml) to obtain a solution volume of 100 mL. Samples were heated for 2 h at oven heating temperatures of 30°C, 40°C, 50°C, and 60°C, then stirred using a stir bar for 10 min. The thick extract of a mixture of turmeric and olive oil was filtered using Whatmann no 1. The filtered filtrate was stored at 4 °C before further testing.

Phytochemical tests were carried out on each variation of the concentration of herbal oil extract and heating temperature. The triterpenoid test was carried out by taking 1 ml of the sample and then adding 3 drops of anhydrous acetic acid and 1 drop of concentrated H_2SO_4. The flavonoid test was carried out by taking 1 ml into a test tube, then adding 2 drops of concentrated HCl and shaking vigorously. The next step is to add 1 g of magnesium powder (Mg) then shake it vigorously and let it separate. The tannin test was carried out by taking 1 ml of the sample and putting it in a test tube, then adding 2–3 drops of 1% $FeCl_3$.

The antibacterial activity test used disc diffusion method at the best temperature of 60 °C. Bacterial isolates were rejuvenated by taking 2–3 colonies aseptically. The rejuvenating bacteria were inoculated in sterile nutrient broth liquid medium and incubated at 37ºC. The turbidity of the bacterial suspension was uniformized by measuring the Optical Density (OD) of 0.5 at a wavelength of 600 nm. The disc paper was soaked in

herbal oil and single extracts for 30 min. Bacterial inoculum was put in a petri dish as much as 100 l, then 8 ml of nutrient media was added to make it sterile and allowed to solidify. The inhibition zone formed around the paper disc is calculated by the formula in Eq. (1) as follows:

$$Lz = Lav - Ld \tag{1}$$

Calculation of the number of bacterial cells by taking herbal oil extract with concentrations of 10%, 20%, 30% and 40% into each test tube containing nutrient broth media, vortexed and incubated for 24 h and diluted in stages starting from 10^{-1}-10^{-10}. The sample dilution stage was carried out by preparing 10 sterile test tubes and filled with 9 mL of sterile 0.85% NaCl. Bacterial control and extract solutions at concentrations of 10%, 20%, 30% and 40% were pipetted 1 mL each into the first test tube and calculated as the first dilution rate (10^{-1}). These steps were repeated until the dilution level was 10^{-10}. Each dilution sample solution was taken as much as 0.1 mL and then put into sterile petri dishes aseptically. Petri dishes were incubated for 24. Colony growth was observed and counted manually.

Based on the American Standard Technic and Method (ACSM), the number of live microbes can be calculated visually in the range of 30–300 colonies then it is calculated using the formula in Eq. (2) as follows:

$$\Sigma \text{ mikroba hidup} = \Sigma \text{ koloni x Fp (CFU/mL)} \tag{2}$$

$$\Sigma \text{ mikroba hidup} = \text{a, b x Fp (Faktor Pengenceran)}$$

3 Result

Extraction the sample for 2 h aims to increase the contact between the sample and the solvent. Storage of the filtrate at a temperature of ± 4 °C aims to avoid bacterial growth.

The phytochemical test of herbal oil extracts and single extracts showed positive results for triterpenoid and flavonoid compounds at concentrations of 10%, 20%, 30% and 40% and variations heating temperature of 30 °C, 40 °C, 50 °C dan 60 °C can be seen in Table 1.

Herbal oil has non-polar properties so that when the phytochemical test for triterpenoid and flavonoid compounds was carried out (Fig. 7.), it gave positive results. When observed from the color change of the extract, the higher the concentration and extraction temperature, the more concentrated the color produced, so it is assumed that the content of triterpenoid and flavonoid compounds is relatively higher. The color density of the extract produced during the phytochemical test indicated that the higher the content of secondary metabolites in the plant [35].

The phytochemical test of herbal oil extracts showed positive results for triterpenoid and flavonoid compounds. Positive triterpenoid test of herbal oil extract was indicated by a change in color to green accompanied by the formation of a brownish ring. Positive flavonoid test was indicated by a color change to orange. The higher the concentration of the extract and the heating temperature, the more concentrated the color of the

Table 1. Phytochemical test results for secondary metabolite compounds

Extraction Temperature	Turmeric Extract Concentration	Group of Secondary Metabolic Compounds		
		Triterpenoids	Flavonoids	Tannins
30 °C	10%	+	+	-
	20%	+	+	-
	30%	+	+	-
	40%	+	+	-
40 °C	10%	+	+	-
	20%	+	+	-
	30%	+	+	-
	40%	+	+	-
50 °C	10%	++	++	-
	20%	++	++	-
	30%	++	++	-
	40%	++	++	-
60 °C	10%	+++	+++	-
	20%	+++	+++	-
	30%	+++	+++	-
	40%	+++	+++	-
Turmeric		+	+++	-
Olive oil		+	+++	-

Fig. 7. Herbal Oil Extract

extract produced. The color density of the extract indicated that the higher the content of triterpenoid and flavonoid compounds in the herbal oil extract (Fig. 8.). The results of the phytochemical test of herbal oil extracts at concentration of 40% with variations in extraction temperature can be seen in Fig. 8.

Phytochemical test of single extract of turmeric and olive oil showed positive triterpenoid and flavonoid compounds which can be seen in Fig. 9.

The results of the phytochemical test of the single extract in this study showed conformity with the results of previous studies. Triterpenoid test on turmeric produces

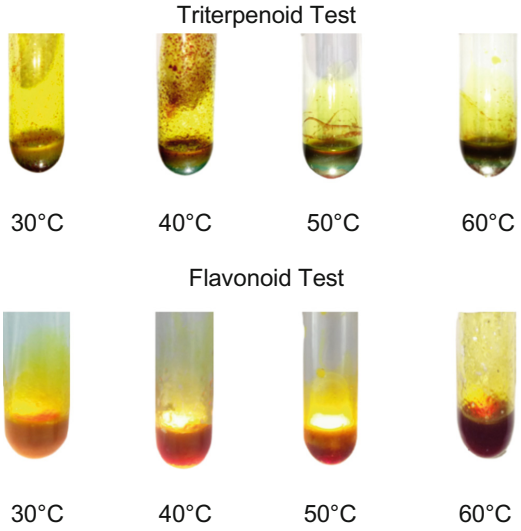

Fig. 8. Phytochemical Test Results of Herbal Oil Extracts at 40% Concentration with Variation of Extraction Temperature.

Table 2. The results of antibacterial activity tests on herbal oil extracts against *S.aureus* and *P.acnes*

Turmeric Extract Concentration	Average Diameter of Inhibition Zone (mm)	
	Staphylococcus aureus	*Propionibacterium acnes*
10%	1.7	1.6
20%	3.3	2.6
30%	3.6	2.9
40%	3.9	3.6
Turmeric	6.3	9.7
Olive Oil	4.4	9.1
Amoxcilin 1%	11	24.2
DMSO 5%	-	-

an orange color [36]. Triterpenoid test on olive oil produces a brownish green color [37]. Turmeric contains flavonoid compounds which are indicated by a color change to orange-red when phytochemical tests are carried out [38]. The flavonoid test on extra virgin olive oil produced a light yellow color [39].

The results of the test of the inhibitory power of the non-polar extract of turmeric rhizome (*Curcuma longa* L.) in extra virgin olive oil against the growth of Staphylococcus aureus bacteria resulted in a positive value. The average diameter of the inhibition zone at extract concentrations of 10%, 20%, 30% and 40% against *S.aureus* respectively are

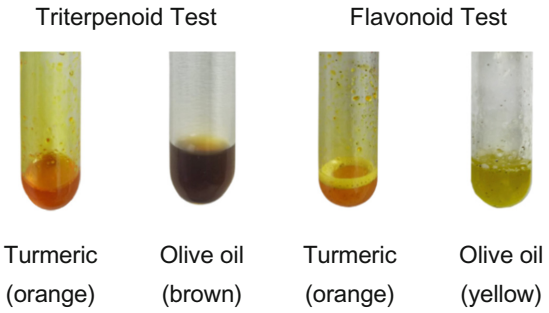

Fig. 9. Triterpenoid and Flavonoid Test Results in Single Extract

1.7 mm, 3.3 mm, 3.6 mm and 3.9 mm while for *P.acnes* respectively are 1.6 mm, 2.6 mm, 2.9 mm, and 3.6 mm. The results of the antibacterial activity test can be seen in Table 2.

The sensitivity of *S. aureus* bacteria to the addition of herbal oil extracts varied as indicated by the increase in the inhibition zone along with the increase in sample concentration (Fig. 9.). The results of this study are in accordance with the results of research on the antibacterial activity test of the combination of black cumin oil and olive oil at concentrations of 0.25%, 0.5% and 1% resulting in inhibition zones of 0 mm, 12.47 mm and 16.23 mm so that it can It was known that an increase in the concentration of the extract had an effect on the antibacterial activity produced [40].

The combination test in this study showed a discrepancy between the results with previous studies where the herbal oil extract produced an average diameter of the inhibition zone that was smaller than the single extract. The antibacterial activity test of single extract of turmeric and single extract of olive oil in this study resulted in inhibition zones respectively are 4.80 mm and 3.33 mm. When compared with the results of a study on the antibacterial activity test of a single extract of olive oil, lime cream and a combination of lime cream and olive oil, a 1:1 ratio of *S. aureus* bacteria resulted in inhibition zones of 8 mm, 9 mm and 9 mm, respectively [41].

The decreased antibacterial activity in the combined extract was possible because the percentage of active compounds in the herbal oil extract was low due to the less than optimal extraction process. Extraction involving the use of essential oils from plants is more suitable to be carried out using steam distillation [42]. Extraction of herbal plants can use the soxhletation method at a temperature of 60–65 °C and involves a concentration process using a rotary evaporator at controlled pressure and temperature [43].

The weak antibacterial activity of the herbal oil extract is thought to be due to the combination of the two ingredients producing antagonistic properties. Antagonistic effects (opposite) can be caused by the two materials used having the same mechanism of action with the same binding site from the same target [44]. Flavonoids can inhibit bacteria by causing damage to the permeability of the bacterial cell wall and microsomes as a result of the interaction of flavonoids and bacterial DNA [45]. Triterpenoids are able to inhibit bacteria through their interaction with porins (transmembrane proteins) and form strong polymers so that the formation of bacterial cell walls is disrupted [46]. The similarity of the mechanism of action between flavonoid compounds and triterpenoids

Table 3. The results of the average number of live microbes in herbal oil extracts against *S.aureus* and *P.acnes*

Turmeric Extract Concentration	Total Plate Count (TPC) (CFU/mL)	
	Staphylococcus aureus	*Propionibacterium acnes*
10%	$5{,}2x10^8$	$3{,}0\ x10^8$
20%	$4{,}8x10^8$	$2{,}0\ x10^8$
30%	$3{,}7x10^8$	$1{,}6\ x10^8$
40%	$3{,}4x10^8$	$1{,}3\ x10^8$
Bacteria control	$7{,}1x10^8$	$4{,}3\ x10^8$

affects the destruction of bacterial cell walls so that the combined extract produces an antagonistic effect that causes the antibacterial activity to decrease and the combined agent produces lower inhibitory power than the effect of the single agent [47]. The herbal oil extract produced is classified as a partial antagonist, namely an agonist that has low effectiveness, resulting in a weak maximal effect [48].

Combination is better to do on extracts that have gone through a separation or purification process to get pure compounds than using crude extracts because in crude extracts it is suspected that there are still many compounds that are possible to react with one another so that it can affect the activity involved. Generated. This study used crude extract of herbal oil so that the resulting combination did not produce synergistic antibacterial activity [49]. Separation of compounds contained in herbal oil extracts needs to be separated by several separation techniques such as *thin layer chromatography* (TLC) to find out more about the compounds contained in it. In addition, other separation techniques that can be used for herbal oil extracts are through fractionation and isolation of targeted compounds.

Calculation of the number of bacteria using TPC (Total Plate Count) which involves the level of dilution aims to get a culture that is not too dense so that cells or bacterial colonies that grow can be counted. The higher the dilution rate, the less the number of bacteria contained in the sample [50]. Bacterial colonies that grow can be observed directly and counted in the range of 30–300 colonies [51]. The results of the average number of live bacteria in herbal oil extracts against *S. aureus* bacteria can be seen in Table 3.

The addition of turmeric extract concentration showed a decrease in the number of bacteria which was not too significant but was able to reduce the number of live bacteria from the control. Bacterial control resulted in an average number of live microbes $7.1x10^{10}$ higher than turmeric extract. The higher the concentration of turmeric extract, the greater the decrease in live bacteria, respectively, by $5.2\ x\ 10^8$, $4.8x10^8$, $3.7\ x\ 10^8$ and $3.4\ x\ 10^8$.

The results of the herbal oil extract test in olive oil using TLC-densitometry and identification by FTIR showed the solubility of curcumin was $2.31 \pm 0.02\%$ at a temperature of 60 °C. The spectral pattern produced from FTIR showed the same peak as the standard (curcumin) but there was a shift in the wave number of 1747.803 cm^{-1}

shows the absorption band of C = O *Stretching*, absorption wave number 1514,544 cm^{-1} shows absorption of C = C *Streching* and absorption of C-H *bending* is shown at wave number 1377.570 cm^{-1}, absorption band C-O-C *Stretching* is shown at wavelength 1162.859 cm^{-1} and the wave number of 966,966 cm^{-1} is the absorption of the C-H *deformation* band. The higher the temperature, the more the solubility of the substance will increase. The higher the temperature, the faster the movement of the particles to the solvent and the weaker the cell permeability, this will make it easier for the solvent to extract the active substances contained in the sample so as to produce higher levels [52].

For the results of the analysis of herbal oil extracts using FTIR compared to the single extract, namely turmeric and pure olive oil, the results show that herbal oil extracts tend to follow the spectral pattern of pure olive oil. This is possible because of the comparison between the turmeric sample powder and olive oil. Where more solvent than the sample powder is dissolved.

As for the analysis, the wave number of 3466 cm^{-1} shows the presence of *sterching* vibrations of the O-H group. The wave number 3005 cm^{-1} shows the vibration of the *cis olefinic* (C = CH) double bond, then there is asymmetric CH_2- (methylene) *streching* vibration at wave numbers 2935 cm^{-1} and 2855 cm^{-1}. While at the absorption wave number 1745 cm^{-1} there is an absorption pattern of C = O *streching* from the carbonyl ester triglyceride functional group. At wave number 1625 cm^{-1} is absorption from C = C *streching* and at wave number 1517 cm^{-1} there is a benzene ring structure. The wave number of 1461 cm^{-1} is a moderate to strong absorption pattern due to the vibration of CH_2, at the wave number of 1375 cm^{-1} with a moderate to strong absorption pattern due to the vibration of the bending O-H bond, and in the wave number region of 1164 cm^{-1} it shows the presence of vibrations from C-O *streching*.

Based on the analysis above, it can be concluded that there are several functional groups that have characteristics possessed by curcumin, including O-H, C = O, C = C, C-O and C-H. It is possible that there are turmeric compounds in pure olive oil solvent.

4 Conclusion

Herbal oil extracts contain triterpenoid secondary metabolites and flavonoids in all concentration variations with the best results obtained at a concentration of 40% and at a temperature of 60 °C which indicated a color change the solution becomes more concentrated.

The antibacterial activity test of *Staphylococcus aureus* and *Propionicbacterium acne* produced the largest inhibition zone at a concentration of 40% and a temperature of 60 °C with the inhibition zones respectively are 3.65 mm and 3.6 mm.

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