ANTIMICROBIAL ACTIVITY OF HONEY, CINNAMON, TURMERIC AND THEIR COMBINE EFFECT AGAINST ACNE INDUCING BACTERIA

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ABSTRACT

Acne vulgaris is a common skin disease that mostly affects adolescents during puberty and young adults of any age. The available anti-acne products and antibiotics are causing microbial resistance and undesirable side effects, shifting attention toward the crude extracts of medicinal plants and food like honey. The present study emphasizes on screening of antimicrobial activities of honey, cinnamon, turmeric, and their combination against acne bacteria. Altogether 20 acne samples were collected conveniently from grade 10 and 11 students at Xavier International college. Out of 20 participants, 13 were male and 7 were female. The collected acne sample was cultured using a standard bacteriological culture technique in which, *Staphylococcus aureus* was the dominant bacteria (30.76%). Similarly, the ethanolic extracts of honey, cinnamon and turmeric were prepared for phytochemical analysis and their antibacterial activities were evaluated using agar well diffusion technique. Different concentrations of extracts were used on the bacterium and their zone of inhibition was measured in millimeters (mm). Results indicate that single extracts of cinnamon and local turmeric had the highest Zone of Inhibition of 22mm against *Propionibacterium acne.* Our study also revealed that the honey used in this study couldn't be able to inhibit bacterial growth at 25% 50% and 75% concentrations. The antibacterial analysis of multi extracts exhibited different activities for each bacterial strain. A comparison of all result obtained from single extracts and multiple extracts follows the trend: Himalaya honey+ Local Turmeric+ Cinnamon > Patanjali honey + Local Turmeric + Cinnamon > Cinnamon > Local turmeric > Cinnamon + Local Turmeric > Commercial turmeric > Cinnamon + Commercial Turmeric > Himalaya honey + commercial Turmeric + Cinnamon> Patanjali honey+ Commercial turmeric + Cinnamon > Dabur honey + Local Turmeric + Cinnamon >Dabur honey + Commercial Turmeric + Cinnamon > Honey + cinnamon > Honey. Therefore, our study highlights the potential use of Himalaya honey + local turmeric + cinnamon as an alternative treatment for acne vulgaris.

***Keywords**:* Acne vulgaris, honey, cinnamon, turmeric, antimicrobial activity, phytochemical constituent, antibiotic-resistant

1.Introduction

Acne vulgaris is a common skin disease that mostly affects adolescents during puberty and young adults of any age (Syal et al 2020). It is a multifactorial chronic inflammatory disease of pilosebaceous units (Rathi 2011). The pilosebaceous unit consists of hair, hair follicles, and the sebaceous gland (oil gland) of the skin which moistens and balances the pH of the skin by producing sebum. But when the oil gland is too active or overactive, it produces excess oil or sebum which blocks hair follicles and pores present in our skin along with dead skin cells and dirt. In the follicle, a bacterium that primarily thrives on the sebum are *Propionibacterium acne, Staphylococcus aureus, and Corynebacterium* which plays major role to develop acne condition. (Al-Nabati et al 2019). The formation of comedones, erythematous papules, pustules, nodules, and cysts are characteristics of acne vulgaris which mainly attacks sebum-rich areas: face, forehead, chest and shoulders (Sutaria et al 2021, Ghovvati et al 2019).Though multiple anti-acne products, antibiotics, anti-inflammatory drugs and modern systematic cure like laser treatments are available for acne treatment; excessive use and misuse of these drugs can lead to the rising resistance of bacteria and many undesirable side effects (Vora et al 2018).

Since the effective life span of antibiotics is limited and misuse of traditional antibiotics is causing microbial resistance; the attention has been shifted toward the crude extracts of medicinal plants and food like honey. Medicinal properties of plant-like antimicrobial activity, antibacterial, antioxidant, and anti-inflammatory activity mainly depend on the secondary metabolites and the most important bioactive compounds of plants: alkaloids, flavonoids, tannins, sterols, saponins, and phenolic

compounds. These antimicrobial compounds may inhibit the growth of bacteria including antimicrobial-resistant bacteria to treat chronic and infectious diseases. However, the bacteria can show its resistance if only one active ingredient with a specific target is involved, which again became a problem. So, many researchers have done their research on the synergistic effect of different plant extracts (Vaou et al 2021).

Honey has been studied as an anti-acne because of its phytochemical, anti-inflammatory, antimicrobial, and antioxidant properties. The antioxidant property of honey: polyphenol helps to prevent oxidative stress which plays a significant role in the pathogenesis of acne vulgaris. The anti-inflammatory properties of honey decrease local inflammation caused by bacteria like *P. acne* thus, reduce the redness of acne. The osmotic effect of the high sugar content and low moisture content of honey is its antibacterial properties (Angie et al 2020).

Similarly, turmeric and cinnamon have a long history in traditional and modern science. The most important application of turmeric against acne is to control sebum production by the sebaceous glands, which is one of the factors to cause acne pathogenesis. Curcumin, the main bio-active compound in turmeric, has anti-inflammatory, antioxidant, and antibacterial properties that help reduce swelling, itching, and redness associated with acne (Sanghvi 2021). Cinnamon also shows its anti-inflammatory activity by inhibiting the production of nitric oxide- an inflammatory agent and CoX-2 – a pro-inflammatory agent which is responsible for inflammatory conditions in the human body (Julianti et al 2017).

This paper investigates the sensitivity or resistance of acne-causing bacteria to the ethanolic extracts of honey, turmeric, and cinnamon and compares the antimicrobial property of each material. This may lead to the discovery of an alternative form of treatment other than antibiotics being used at present, to which many of the bacteria are developing resistance.

2. Materials and Methods

The research method was quantitative and primary data were collected from March 2022 to June 2022. The variables of the study were the growth pattern of bacteria isolated from acne samples, the presence or absence of phytochemical constituents, the antibacterial activity of plant extracts , honey, and their combined effect on bacterial species.

Commercial honey, turmeric, and cinnamon bark were purchased from Bhatbhateni - chuchhepati, Kathmandu. Local turmeric was collected from Gokarna, Suntakhan, Kathmandu. All the samples were collected in a sterile bag and were brought to the microbiology lab of Xavier International College for further processing.

2.1 Preparation of Extract

2.1.1 Ethanolic Extract of turmeric and cinnamon

cinnamon's bark was washed, dried, and ground in a grinder. To prepare the ethanolic extract, 25gm dried powder of each sample (commercial turmeric, local turmeric, cinnamon) was loaded into Soxhlet apparatus with 150ml of 96% ethanol for 48 hours, followed by filtration using Whatmann no.1 filter paper. Then, it was allowed to evaporate using evaporating dish at 50℃ for the removal of ethanol from the extract. Finally, the dense extract was diluted in 10% Dimethyl Sulphoxide (DMSO) to obtain a standard working solution (Maharjan et al 2019, Adhikari et al 2020).

2.1.2 Processing and Preparation of honey extract

Each collected honey sample was first filtered with sterile mesh and allowed to heat at 30C for 30 minutes to reduce viscosity. For purity check, each sample was inoculated on a blood agar plate incubating overnight. Then, samples free from contamination were stored in a refrigerator maintaining around 4℃ temperature.

For ethanolic extract,10g of honey with 25ml of solvent (ethanol) was taken in a centrifuge tube to centrifuge it at 3000rpm at 25℃ for 10 minutes. Then, resulted extract was filtered and diluted in 10% Dimethyl Sulphoxide (DMSO) to obtain a working solution (Joshna k et al 2019, Asokan et al 2017).

2.2 Phytochemical analysis of extracts

2.2.1 Phytochemical analysis of Turmeric and Cinnamon

Some portion of individual extracts was subjected to phytochemical analysis for evaluation of various phytochemical constituents.

1. Alkaloid test (Wagner's test)

To the 2ml of each test solution, a few drops of Mayer's and Wagner's reagents were added. The formation of brown-reddish precipitation indicates a positive test for alkaloids.

2. Flavonoid test (Alkaline reagent test)

About 3ml of each extract was treated with 10% NaOH solution or 10% NH4OH solution for the development of intense yellow color which indicates the presence of flavonoid.

3. Terpenoid test (chloroform test)

To the mixture of 2ml of the test solution and 2ml chloroform, 2ml of H2SO4 was added and heated at 65℃ in a water bath for 120s to give reddish-brown color.

4. Phenols (FeCl3 test)

Phenols were tested by the Ferric Chloride test in which about 2ml of each test solution was treated with 3-4 drops of 5% FeCl3 solution to give a deep blue or black color.

5. Tannin

To the 4ml of each test solution, 4ml of FeCl3 was added. The formation of blue-green or a blue-black coloration shows positive for tannin.

6. Anthocyanins

2ml of each extract solution was added to 2ml of the mixture containing HCl (2M, 1ml) and ammonia (4M, 1ml). The pink-red color turns to a blue-violet color indicating the presence of anthocyanin.

7. Glycoside test

2ml of each test solution was taken in a test tube to dissolve with 4ml of glacial acetic acid with a few drops of 5% Ferric Chloride followed by 1ml of concentrated H2SO4. The formation of a brown ring at the interface is evidence of glycoside.

8. Saponin test

About 2ml of the test solution and 2ml of distilled water were taken in a test tube to shake vigorously for 15 seconds. After 15 minutes, persistent frothing appears as a positive indicator for saponin.

(Sawant et al 2013, Ahmed et al 2020)

2.2.2 phytochemical analysis of honey samples

The active phytochemical compound present in the extracted honey sample was detected by following Official Methods of Analysis (AOAC) (Joshna k et al 2019, Asokan and Jayanthi 2017).

1. Carbohydrate test

To 2ml of test solution, 2ml of Benedict's reagent was added and heated in a boiling water bath for 2 minutes. Red precipitation indicates the presence of sugar.

2. Amino acid test

1ml of 5% Ninhydrin solution was added to 2ml of test solution. The formation of purple color indicates the presence of amino acid.

An alkaloid, Flavonoid, Terpenoid, Phenol, Tannin, and Saponin tests were performed as like in **3.6.1**

2.3 Isolation of Bacteria from acne samples

Only mature pimples that appeared around the face were chosen. First, it was cleansed gently with 70% alcohol and was popped with a lancet needle. Then, the pimple pus was swabbed with a sterile cotton swab dipped in normal saline. Collected pus specimens were directly cultured into a Blood agar incubating at 37℃ for 24 hours. Colonies that appeared in blood agar were further sub-cultured on the Nutrient agar and incubated at 37℃. The isolated bacteria were identified through Gram staining and different biochemical test (catalase test, oxidase test, Methyl Red-Voges-Proskauer (MR-VP) test, Triple Sugar Ion test (TSI), and coagulase test (slide test).

2.4 Screening for Antimicrobial Activity

2.4.1 Standardization of bacterial suspension

The bacterial suspensions were standardized following the CLSI guidelines. The bacteria were grown in Muller Hinton Broth for 18-24h, followed by the matching of bacterial suspension to the turbidity equivalent to 0.5 McFarland solutions (1-2*10^8 cfu/ml).

2.4.2 Agar well Diffusion Method

The antimicrobial activity of ethanolic extracts of the selected sample was determined by the Agar Well Diffusion technique, following CLSI guidelines (2011). Agar Well Diffusion technique was performed in Muller Hinton Agar. 20ml of sterilized MHA was poured into a sterile Petri plate. After solidification, the plates were inoculated by spreading a volume of the microbial inoculum over the entire agar surface (using a sterile cotton swab). Then, 4 wells were made in the MHA agar plate, using a sterile cork-borer of 6mm diameter to load four different concentrations of 25%, 50%, 75%, and 100% of selected extracts. (Maharjan et al

2019). The plates were allowed to settle at refrigerator temperature at 4℃ for about 2 hours. After incubation at 37℃ for 24 hours, all plates were examined for any zone of inhibition, and the diameter of these zones was measured with a scale (Khadgi 2016).

2.4.3 Determination of combine activity

For synergistic activity between honey & turmeric, honey & cinnamon, turmeric & cinnamon, and turmeric & cinnamon & honey, 2ml of each extract were mixed and the agar well diffusion technique was carried out in MHA agar plates as mentioned in 3.8.2 (Maharjan et al 2019).

3. RESULT

3.1 Phytochemical Test of Turmeric and Cinnamon

The phytochemical screening test results revealed that the ethanolic extract of local turmeric, commercial turmeric, and cinnamon contain at least four secondary metabolites. Phenols and tannin were present in both turmeric and cinnamon. Glycoside and Anthocyanin were positive for turmeric only. Alkaloid and Terpenoid were present only in cinnamon, whereas Saponin and Flavonoid were absent in all extracts (Table 1)

Table 1: Phytochemical screening test of plant extracts

Phytochemicals	Tests	Plant extracts		
		Turmeric		Cinnamon
		Local Turmeric	Commercial turmeric	
Alkaloid	Wagner's test	-	-	+
Flavonoid	NaOH test	-	-	-
Terpenoid	Salkowski's test	-	-	+
Phenols	Ferric chloride test	+	+	+
Tannin	Ferric chloride test	+	+	+
Glycoside		+	+	-
Saponin	Foam test	-	-	-
Anthocyanin		+	+	-

Notes: Present = +, Absent = -

3.2 Phytochemical Test of Honey

Honey contains various phytochemical compounds that have a function as bioactive compounds. In a phytochemical test of honey samples, the presence of carbohydrate, terpenoid, and saponin was detected in all samples. However, Amino acid, Alkaloid, Flavonoid, Phenols, and Tannin were negative for all Dabur, Himalaya, and Patanjali honey.

Table 2: The phytochemical test of selected honey samples

Phytochemicals	Tests	Ethanolic extracts of Honey samples		
		Dabur honey	Himalaya honey	Patanjali honey
Carbohydrate	Benedict's test	+ +	+ +	+ +
Amino acid	Ninhydrin test	-	-	-
Alkaloid	Wagner's test	-	-	-
Flavonoid	NaOH test	-	-	-
Terpenoid	Salkowski's test	+	+	+
Phenols and Tannin	Ferric chloride Test	-	-	-
Saponin	Foam test	+	+	+

Notes: Highly present = ++, Present = +, Absent = -

3.3 Growth pattern of Acne bacteria

All acne samples (inoculated into blood agar) showed bacterial growth after 24h of incubation. Based on Gram staining, morphological features, cultural characteristics, and biochemical test, the bacterial isolates were assigned to five bacterial species in which 60% was Gram-positive and 40% was Gram-negative bacteria. *S. aureus* was the most common isolated bacteria which was 30.76%, followed by *P. acne* (26.92%), *S. epidermidis* (23.07%), *Pseudomonas* (15.38%) and *E. coli* (3.87%).

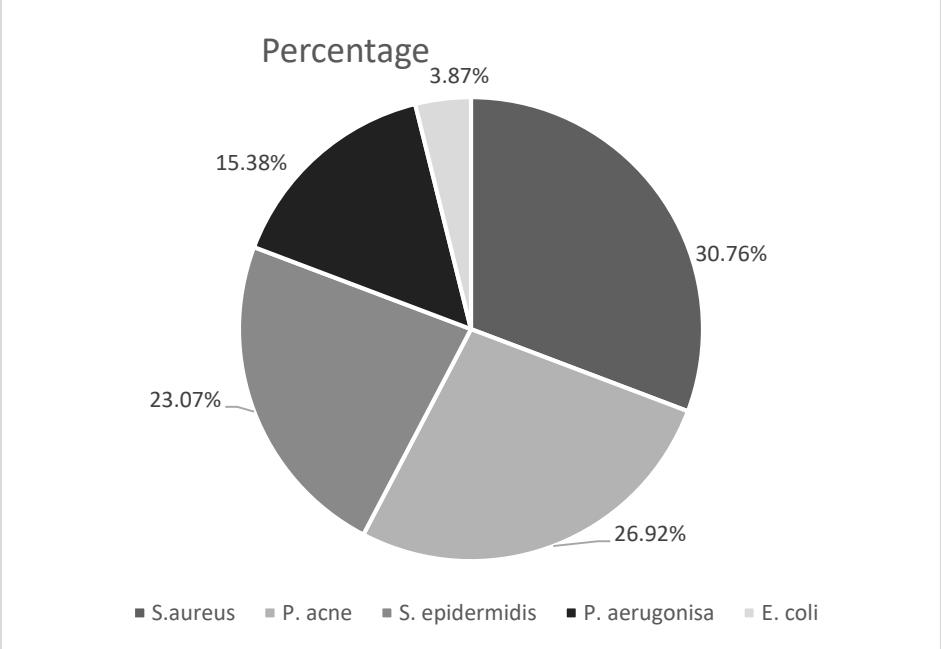

Figure 5: Growth pattern of Acne bacteria in Blood Agar

3.4 Antibacterial Activity of Turmeric and Cinnamon against bacterial strains

Selected plant extracts exhibited different efficiency depending on their concentration and bacterial strains. On 100% concentration, local turmeric and Cinnamon showed the highest antibacterial effect against *P. acne* with a zone of inhibition of 22mm and commercial turmeric with 20mm. However, this effect was decreased with 75%, 50%, and 25% concentration. Cinnamon was more effective *to S. aureus* and *S. epidermidis* than turmeric.

Table 3: Antibacterial activity of plant extracts against five bacterial species

Plant extracts	Concentration (in %)	*P. acne* (mm)	*S. epidermidis* (mm)	*S. aureus* (mm)	*P. aeruginosa* (mm)	*E. coli* (mm)
Local turmeric	25%	15mm	11mm	11mm	12mm	12mm
	50%	17mm	12mm	14mm	14mm	14mm
	75%	19mm	14mm	14mm	15mm	16mm
	100%	22mm	17mm	15mm	16mm	18mm
Commercial turmeric	25%	13mm	10mm	10mm	11mm	11mm
	50%	15mm	11mm	12mm	13mm	13mm
	75%	18mm	13mm	13mm	14mm	14mm
	100%	20mm	16mm	14mm	15mm	17mm
Cinnamon	25%	14mm	13mm	11mm	12mm	11mm
	50%	17mm	15mm	13mm	13mm	15mm
	75%	20mm	16mm	14mm	14mm	16mm
	100%	22mm	18mm	18mm	15mm	18mm

Here, the zone of inhibition was measured in mm

3.5Antibacterial activity of honey against five bacterial species

Each microorganism exhibited different sensitivity to the different concentrations of tested honey. At high concentration (100%), all honey tested exhibited antibacterial activity against *S. aureus, P. acne, S. epidermidis*, *Pseudomonas*, and *E. coli* Patanjali honey was more effective than the other tested honey.

Table 4: Antibacterial Activity of Honey against five bacterial species

Honey samples	Concentration (in %)	*P. acne* (mm)	*S. epidermidis* (mm)	*S. aureus* (mm)	*P. aeruginosa* (mm)	*E. coli* (mm)
Dabur Honey	25%	-	-	-	-	-
	50%	-	-	-	-	-
	75%	-	-	-	-	-
	100%	11mm	8mm	7mm	11mm	10mm
Himalaya Honey	25%	-	-	-	-	-
	50%	-	-	-	-	-
	75%	-	-	-	-	-
	100%	11mm	7mm	8mm	12mm	11mm
Patanjali Honey	25%	-	-	-	-	
	50%	-	-	-	-	
	75%	-	-	-	-	
	100%	12mm	9mm	9mm	12mm	12mm

3.6 Combine Effect between Turmeric and Cinnamon

Combination of Cinnamon and local turmeric showed synergistic effect against *S. epidermidis* and *P. aeruginosa*, additive effect to *E. coli* and antagonistic effect to *P. acne* and *S. aureus*. Similarly, combination of commercial turmeric and cinnamon demonstrated additive effect against *P. aeruginosa* whereas antagonistic effect to remaining bacterial strains.

Table 5: Combine effect between turmeric and cinnamon

Combine extracts	Concentration (in%)	*P. acne* (mm)	S. epidermidis (mm)	*S. aureus* (mm)	*P. aeruginosa* (mm)	*E. coli* (mm)
C+ L.T	25%	14mm	13mm	11mm	12mm	14mm
	50%	15mm	15mm	12mm	13mm	15mm
	75%	17mm	16mm	14mm	14mm	16mm
	100%	20mm	20mm	16mm	17mm	18mm
C+ C. T	25%	13mm	12mm	10mm	11mm	12mm
	50%	15mm	13mm	12mm	12mm	14mm
	75%	16mm	15mm	13mm	13mm	15mm
	100%	18mm	18 mm	14mm	16mm	17mm

3.7 Combine Effect between Honey and Turmeric

Combining turmeric with honey, showed an antagonistic effect on all bacterial strains.

Table 6: Combine Effect Between Honey and Turmeric

Combine extracts	Concentration (in %)	*P. acne* (mm)	*S. epidermidis* (mm)	*S. aureus* (mm)	*P. aeruginosa* (mm)	*E. coli* (mm)
Dabur+ L.T	75%	15mm	15mm	14mm	15mm	15mm
	100%	17mm	16mm	16mm	16mm	17mm
Himalaya + L.T	75%	16mm	15mm	15mm	15mm	16mm
	100%	18mm	17mm	16mm	17mm	17mm
Patanjali + L.T	75%	16mm	16mm	15mm	16mm	16mm
	100%	18mm	17mm	17mm	17mm	18mm
Dabur+ C.T	75%	14mm	13mm	13mm	14mm	14mm
	100%	16mm	14mm	14mm	15mm	16mm
Himalaya + C.T	75%	15mm	14mm	13mm	14mm	15mm
	100%	17mm	15mm	15mm	16mm	16mm
Patanjali + C.T	75%	16mm	14mm	13mm	14mm	15mm
	100%	17mm	15mm	15mm	16mm	16mm

Here, L.T= Local turmeric, C.T= Commercial turmeric

3.8 Combine Effect between Honey and Cinnamon

The combination of all honey and cinnamon showed an antagonistic effect against all bacterial strains. Only a combination of Patanjali honey and cinnamon showed a synergistic effect against *P. aeruginosa* with 18mm ZOI.

Table 7: Combine Effect Between Honey and Cinnamon

Combine extracts	Concentration (in %)	*P. acne* (mm)	*S. epidermidis* (mm)	*S. aureus* (mm)	*P. aeruginosa* (mm)	*E. coli* (mm)
D+ C	75%	13mm	14mm	13mm	16mm	14mm
	100%	14mm	15mm	15mm	17mm	15mm
H + C	75%	13mm	14mm	14mm	15mm	15mm
	100%	15mm	16mm	15mm	16mm	16mm
P+ C	75%	14mm	16mm	15mm	17mm	16mm
	100%	16mm	17mm	16mm	18mm	17mm

3.9 Combine Effect between Honey, Cinnamon, and Turmeric

The combination of Himalaya honey+ local turmeric + cinnamon and Patanjali honey + cinnamon + local turmeric showed synergistic effect against *S. epidermidis*, *P. aeruginosa*, and *E. coli* in the range of 18-20mm ZOI whereas Dabur honey + cinnamon + Turmeric showed synergistic effect *to P. aeruginosa* and antagonistic effect to remaining bacterial strains.

Table 8: Combine effects between Honey, Cinnamon, and Turmeric

Combine extracts	Concentration (in %)	*P. acne* (mm)	*S. epidermidis* (mm)	*S. aureus* (mm)	*P. aeruginosa* (mm)	*E. coli* (mm)
D+C+ L.T	75%	16mm	15mm	14mm	16mm	17mm
	100%	18mm	17mm	16mm	18mm	18mm
H+C+ L.T	75%	18mm	17mm	15mm	16mm	16mm
	100%	22mm	20mm	16mm	18mm	19mm
P+C+ L.T	75%	17mm	17mm	15mm	16mm	17mm
	100%	20mm	19mm	17mm	19mm	20mm
D+C+ C.T	75%	16mm	14mm	13mm	15mm	16mm
	100%	17mm	16mm	15mm	16mm	17mm
H+C+ C.T	75%	17mm	15mm	14mm	15mm	15mm
	100%	20mm	18mm	15mm	17mm	17mm
P+C+ C.T	75%	17mm	16mm	14mm	17mm	16mm
	100%	18mm	18mm	15mm	18mm	18mm

Here, D = Dabur honey, H = Himalaya honey, P = Patanjali Honey

L.T = Local Turmeric, C.T = Commercial Turmeric C = Cinnamon

4. Discussion

Many plants and food used in traditional medicine represent rich sources of natural bioactive substances with health-promoting effects and no side effects compared to antibiotics (Nabavi et al 2015). They produce secondary metabolites (phytochemicals), which have shown remarkable antimicrobial efficacy against different bacterial pathogens, when used alone or as a synergist. The result of the phytochemical analysis showed that each plant extracts contain at least four secondary metabolites. The phytochemicals- terpenoid and alkaloid present in cinnamon are known to have antibacterial properties (Mohamed et al 2020, Abiya et al 2018). Honey contains various phytochemical compounds that have a function as bioactive compounds. In this study, all the tested honey contains carbohydrates, terpenoids, and saponin.

In this study, the representative organism isolated from 20 acne lesions were *P. acne, S. aureus S. epidermidis, Pseudomonas, and E. coli,* which were cultured in an anaerobic condition. Among the bacteria isolated, *S. aureus* was the most dominant with 30.76% followed by *P. acne* (26.92%) and S. epidermidis with 23.07%. A similar study was conducted in India, the result showed that *S. aureus* was the dominant bacteria with 39%, followed by *Propionibacterium acne* (33%) and *S. epidermidis* with 21%. Since the most frequent bacteria isolated from acne patients were *Staphylococcus aureus*, acne vulgaris may be mainly caused by *Staphylococcus aureus* than *Propionibacterium acne* (Hassanzadeh et al 2008). However, most of the researchers concluded that two bacteria are mainly associated with acne pathogenesis: *Propionibacterium acne* and *S. epidermidis* (Julianti et al 2017).

The interesting finding of this study was the isolation of Gram-negative bacteria (*E. coli* and *Pseudomonas aeruginosa*) from acne lesions. This is associated with Gram-negative folliculitis, an acne-like disorder caused by bacterial infection. Other Gram bacteria include *Klebsiella*, *Serratia*, and *Proteus* species. Gram-negative folliculitis (GNF) may result from long-term treatment of acne with tetracycline or topical antibiotics. GNF is often mistaken as a worsening of acne as it usually occurs in patients with existing acne, around the area of the upper lip under the nose, to the chin, and cheeks. Superficial pustules with relatively few papules and comedones are caused by *Klebsiella, E. coli,* and Serratia species; whereas there is another term for the infection of Pseudomonas aeruginosa known as Spa pool folliculitis (Oakley 2014).

In the present study, the antibacterial activity of honey, turmeric, and cinnamon was tested against five bacterial strains by using the agar well diffusion method. The ethanolic extracts of local turmeric and cinnamon showed high activity for *P. acne, E. coli,* and *S. epidermidis* at high concentrations and low activity at low concentrations (25%). However, *S. aureus* and *P. aerugonisa* were slightly less sensitive microbes. In a similar study conducted by (Ahmed et al 2020), the cinnamon extract had the highest activity against *S. aureus* at all concentrations; also showed high activity for *E. coli* at high concentrations and low activity at low concentrations (12.5%). For *P. aeruginosa* the extracted oil showed high activity at high concentration only; besides no activity was found at low concentration (12.5%). Similarly, a recent study by (Joshi et al 2021) reported that *E. coli* was found to be the most susceptible bacteria to the turmeric extracts, followed by *S. aureus* and *P. aerugonisa*. In the previous studies, the anti-acne investigation was done on white turmeric rhizomes which showed no zone of inhibition against both *P. acnes* and *S. epidermidis* (Setyani et al 2020). In comparison, yellow turmeric rhizomes used in this study successfully inhibit all the bacterial strains isolated from pimple pus. This could be due to the presence of the active ingredient of yellow turmeric: curcumin, which is confirmed to have strong antimicrobial potential (Adamczak et al 2020).

Honey is a powerful antimicrobial agent. However, its effectiveness and potency against microorganisms depend on its chemical constituent, type of honey produced, processing method, and storage condition. In our present study, the antibacterial properties of artificial honey were evaluated, which conclude all the tested honey lost their ability to inhibit bacterial growth at 25% 50%, and 75% concentrations. Patanjali honey had the highest activity among all the microorganisms tested. *P. acne, Pseudomonas,* and *E. coli* were the most sensitive microbes with 12mm ZOI. This result resembles the study of (Zainol et al 2013). He investigated the antibacterial activity of artificial honey against *S. aureus, E. coli, Bacillus cereus,* and *P. aeruginosa*. The pattern of sensitivity was *S. aureus* > *P. aeruginosa* > *E. coli*. Another study used artificial honey in their study, where at 10% and 5% artificial honey was able to inhibit the growth of *P. aeruginosa* but had no measurable growth of *E. coli.*

Several authors believe that the antibacterial activity of honey may have been attributed to the sugar content rather than other constituents. However, this

activity varies markedly from honey to honey. In this study, though the sugar content was highly present in the tested honey, the antibacterial activity was not effective as it was expected to be. A possible explanation behind this result is that the antibacterial activity of honey is not attributable to sugar content only. Similarly, in one study, artificial honey was incorporated into nutrient agar to prevent the growth of 18 strains of MRSA, whereas natural honey: manuka, and pasture honey at least 10 times more dilute than artificial honey prevented the growth of MRSA. This implies that artificial honey is not effective as natural honey which supports our present study (Cooper et al 2002).

From the foregoing findings, the combination of turmeric with cinnamon led to a synergistic effect against *S. epidermidis* and *P. aeruginosa*, an additive effect against E. coli, and an antagonistic effect against *P. acne* and S. aureus. Similarly, the combination of both honey with turmeric and honey with cinnamon showed an antagonistic effect against all the bacterial strains except *P. aeruginosa* whereas, a synergistic effect was observed on *P. aeruginosa.* This result is highly less than the study of (Julianti et al 2017) which reported that the combination of cinnamon bark extract and honey had an additive effect against *P. acnes* and *S. epidermidis*. In addition, the antibacterial analysis of combined extracts together exhibited different activities for each bacterial strain. Dabur honey + local turmeric + cinnamon showed the same effect as the combination of honey with turmeric and cinnamon. Himalaya honey + local turmeric + cinnamon showed an additive effect against *P. acne*, an antagonistic effect against *S. aureus*, and a synergistic effect against remaining bacteria. On the other hand, Patanjali honey + local turmeric + cinnamon showed an antagonistic effect against *P. acne* and S. aureus and a synergistic effect against remaining bacteria.

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