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DETERMINATION OF ANTIBACTERIAL, ANTIOXIDANTS, TOTAL FLAVONOIDS, PHENOLIC AND CARBOHYDRATE CONTENT OF LEAF, LATEX, AND FLOWER EXTRACTS OF *CASCABELA THEVETIA* L.

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ABSTRACT: The aim of this study was the quantitative phytochemical screening of extracts obtained from the leaf, flower, and latex with antibacterial activity and antioxidant activity of *Cascabela thevetia (C. thevetia)*. The Quantitative determinations of total flavonoid, carbohydrate, and polyphenol contents were accomplished by Folin-Ciocalteau, phenol-sulfuric acid, and $AlCl_3$ methods. The in-vitro antioxidant activity was evaluated by the DPPH free radical scavenging activity method. All the samples were screened for antimicrobial activity against gram-positive *(S. aureus, S. pneumonia*) and gram-negative (*E. coli and K. pneumoniae*) bacterial strains by using the disc diffusion and well method followed by calculation of minimum bactericidal concentration (MBC). Phytoconstituents of different samples of *C. thevetia* in different solvents contain total polyphenol and carbohydrate content were highest at methanol flower extract 80.26 ± 0.86 and 178. 15 ± 3.15 µg GAC and GLU equivalent/mg of dry extracts weight respectively. But flavonoid content was methanol leaf extract 654.53 ± 2.08µg QAR equivalent/mg of dry extracts weight. Among them the lowest IC_{50} value was 82.84 performed by aqueous flower extracts compared with standard ascorbic acid. In the antibacterial assay, aqueous flower extracts were shown the highest zone of inhibition (ZOI) 18mm and 12.6 mm against *E. coli* in a well and disc method respectively. The flower and leaves extract of *C. thevetia* revealed prominent antibacterial phenolic and flavonoid properties against various pathogenic bacterial strains, recommending its significant utilization for the mitigation of diverse microbial diseases like diarrhea, Urinary tract infection (UTI), skin infection, etc.

INTRODUCTION: The use of plant-based natural products in the treatment and prevention of diseases and health enhancement has led to the significant attention of the scientific community and the public nowadays. The availability of these medicinal plants provides a cost-effective source with lesser side effects to develop new drugs has drawn much attention among the researchers.

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Plant-based traditional medicine has a long history since ancient civilizations and uses plant materials as a major ingredient in synthesizing drugs [1]. Due to its distinctive geographical variances, Nepal is home to an enormous variety of medicinal plants.

Nepal is located in the portion of Central Himalayas and has contributed about 10% of medicinal plants of the expected 7000 species of flowering plants. Medicinal plants are potent sources of medicine to treat various diseases. Despite the widespread use of medicinal plants in Nepal, there are limited studies on phytoconstituents and their antioxidant activity. Synthetic drugs are used to cure various diseases,

but they are expensive & if used in the long run, they show harmful side effects. Hence, drug development from natural products is a promising field. So, it is necessary to identify them, analyze and explore the antioxidant flavonoid and phenolic contents in the natural resources of Nepal [2].

According to WHO, medicinal plants are the best sources to obtain a variety of new herbal drugs. About 80% of individuals from developing countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated for a better understanding of their properties, safety, and efficacy [3]. Due to the rapid development of resistance to currently used drugs for infectious diseases and the side effects of synthetic drugs, the use of conventional herbal medicine has become a good source for the treatment of common ailments and is also considered to be safe, effective, and inexpensive. Most of the plants and herb species used traditionally have potential antimicrobial, anti-diabetic, antiviral, and anti-inflammatory properties [4].

Medicinal plants play a very important role in the development of alternative drugs without the adverse effects of synthetic drugs. Plants and natural products form the basis of both modern and traditional medicines and are currently widely used in the production of commercially produced drugs. Scientific and reliable reports indicated that about 25% of prescribed medicines worldwide are taken from herbs [5].

Phenolic compounds stop the initiation or propagation of oxidizing chain reactions with free radical species to prevent oxidative damage to the tissues. Such oxidative damages may be significant causative factors of chronic diseases such as cancer, cardiovascular diseases, and inflammatory diseases and have a major role in aging.

The phenolic compounds scavenge the reactive free radical species and exhibit antitumor, antiviral, antimicrobial, and antibacterial activities, and prevent AIDS, mutagenesis, and ulcers. The flavonoids exhibit antitumor, anti-inflammatory, anti-allergic, anti-carcinogenic, antibacterial, and antiviral activities due to their capacity to scavenge reactive free radicals [6].

Carbohydrates are the most widely distributed organic compounds on Earth and are one of the most important ingredients in foods of raw materials. They make up much of our food and are vital to national economies.

Dietary carbohydrates comprise a diverse group of substances including a range of physical, chemical, and physiological properties. Principally carbohydrates are substrates for energy metabolism. They may occur inherently or be added to food products to provide nutrients and are mostly added to improve the texture and overall quality of food products. They may also be immunomodulatory and affect Calcium absorption. These properties influence overall health by contributing to the control of body weight, diabetes, aging, cardiovascular disease, bone mineral density, *etc.* [7].

Polyphenols are the secondary metabolites of plants like tea, coffee, cocoa, olive oil, and red wine contributing to bitterness, astringency, color, flavor, odor, and oxidative stability. Their consumption inhibits hypertension, diabetes, hyperlipidemia development, and obesity. It also has a defense against ultraviolet radiation or pathogen aggression [8].

Cascabela thevetia is a multipurpose small evergreen plant widely distributed in the tropical and subtropical regions of Nepal and India. It belongs to the family Apocynaceae and is commonly known as yellow oleander. It is also locally known as "Kanar or Bitti" in Nepal and India It is mostly found in Asia, Central America, and tropical Africa. It is an evergreen tropical shrub or small flowering tree that bears yellow flowers and deep green or black color fruits [9].

All parts of this plant are highly toxic including leaves, bark, fruits, and flowers. The whole parts of the plants include the different medicinal properties such as leaf decoction used to loosen the bowels and also effective to cure intermittent fevers. The seeds are used as purgative and also seed oil is used to treat jaundice and infections and as insecticides. The roots of *C. thevetia* are applied to tumors [10]. The roots and leaves of *C. thevetia*are used in vata, pitta, cough, bronchitis, renal, and skin diseases [11].

FIG. 1: PLANT *C. THEVETIA*

MATERIAL AND METHOD:

Collection and Identification of Plant Materials: Leaf, flower, and Latex of the *C. thevetia* were collected from Dang, Nawalparasi, and Rupandehi in March 2022. The collected plant material was identified and authenticated from the national herbarium and plant laboratory in Godabhari, Kathmandu Nepal. (Letter no- 079/80) - 121.

Firstly, the collected leaves, flowers, and latex were cleaned and arranged properly. The leaves and flowers were left for shade drying for two weeks. The latex was put into freeze at 4°C. The drying process was accomplished in the pharmacognosy laboratory of Crimson College of Technology at room temperature with natural airflow. The room was monitored from time to time. After complete drying, the dried leaves and flowers were hand-crushed to reduce their size. Completely dried plant materials were used for the extraction process.

Comminution of Dried Samples: The completely dried leaves and root bark were comminuted with mortar and pestle and grinder to make small particles.

Extraction Procedure: A triple cold maceration extraction procedure was performed with 200 g of leaves and flowers of *C. thevetia* soaked with 1000 mL of methanol and water in different conical flasks. Dried latex was crushed, and 15 grams of latex was also soaked in 200 ml of methanol, and water in different conical flasks with occasional shaking for 72 hours. Each plant sample (Leaf, Latex, and flower) was extracted separately in two different solvents to obtain six different extraction samples. After each extraction liquids were strained and filtered. The extraction process was performed in a triplicate manner and the filtrate was mixed by using some modification [2].

The methanol and water macerate of *C. thevetia* was concentrated to dry in a rotary evaporator under reduced pressure and controlled temperature (45 °C) for final extracts. Then the obtained crude drug was kept in a petri dish, covered with aluminum foil, and preserved in a desiccator for a few days. All the extracts were then refrigerated at 4 °C until use. The yield value was calculated in percentages.

Phytochemical Screening: The confirmatory qualitative phytochemical screening of plant extracts was performed to identify the main classes of compounds (tannins, saponins, flavonoids, alkaloids, phenols, glycosides, steroids, and terpenoids) present in the extracts following standard protocols [12].

Determination of Total Phenolic Content (TPC): The total phenolic contents of the extracts of different plant parts samples were determined using the Folin-Ciocalteu method with trivial modification of gallicacid using as a standard the method [13, 14] with slight modifications. In the study, the extract solution of 1mg/mL concentration was made from the ethanolic stock solution. 1 mL of ethanolic stock solution was mixed with 1 mL FC reagent followed by 5 mL distilled water and shaken for 5 minutes subsequently, 1 mL of 10% Na_2CO_3 was added, and the mixture was then allowed to stand for 30 minutes at 40 °C. Then the absorbance was measured utilizing a UV spectrophotometer at 765 nm against a blank without extract. All the measurements were evaluated in triplicate.

The phenolic content was calculated as mg of gallic acid equivalent per gram of the dry extract by using a standard gallic acid calibration curve.

Total Flavonoid Content (TFC): The total flavonoid content was determined using Aluminium chloride method [13, 6] quercetin was taken as a reference standard. Different concentrations of quercetin were prepared from the stock solution (1mg/mL) using ethanol as a solvent. 1mg/ml concentration of the leaf, flower, and latex extract was prepared. 1 mL of plant extract dissolved in 4 mL of distilled water and 0.3 mL of 5% $NaNO_2$. After 5 minutes 0.3 mL of 10% $AlCl_3$ was added and incubated for 5 minutes. 2mL of 1M

NaOH was added to the solution. Immediately the test solution was filled with distilled water to make the final volume 10 ml. Similarly, a blank was prepared without a sample. The mixture was incubated for 30 minutes at room temperature, followed by the absorbance of the sample was measured against the blank using a UV-Vis spectrophotometer at 510 nm. All the measurements were examined in triplicate.

Values were expressed in terms of flavonoid content, mg QE (Quercetin equivalent) per g of dry weight (DW basis) of sample extract based on the calibration curve of standard Quercetin.

Total Carbohydrate Content (TCC): The total carbohydrate content in different extracts of *C. thevetia*was determined by the Phenol-Sulphuric acid method adopted by [14, 15] with slight modification. In this test, the standard compound was glucose. Firstly, 1 mg/mL of the stock solution was prepared. the different concentrations of glucose standards (15.625 µg/mL, 31.25µg/mL 62.5 µg/mL, 125 µg/mL, 250 µg/mL, and 500 µg/mL,) were prepared by serial dilution technique. In 10 mL of the test tube, 2ml of the sample (1 mg/ml), 1Ml of the 5 % phenol solution, and 5 mL of the concentrated Sulphuric acid were mixed properly and kept for 10 minutes then the tube contents were mixed and placed in a water bath at 25-30 °C for 20 minutes. The absorbance reading of the blank and the sample got respectively at 490 nm. All the measurements were examined in triplicate.

Determination of Antioxidant Activity by DPPH Method: The antioxidant effect of Leaf, Flower, and Latex of *C. thevetia* was examined by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity method according to established method [12, 15] with slight modification. The DPPH solution of 0.1mM was prepared by dissolving 59.148 mg of DPPH (MW: 394.32 g) in methanol sand made up to 1000 mL volume. The solution was sonicated for a few minutes and stored in the refrigerator until use. The volumetric flask was completely wrapped with aluminum foil throughout the experiment. DPPH stock solution using methanol yielded a usable mixture with an absorbance of around 0.973 at 517 nm. 100 mg of ascorbic acid was dissolved in 100 mL of Methanol to make the concentration of 1 mg/ml solution. The ascorbic acid solution thus prepared was diluted into different concentrations (10 µg/mL, 5 µg/mL, 2.5 µg/mL, and 1 µg/mL) to determine the IC_{50} as standard. In a test tube, 3 mL DPPH workable solutions were combined with 100 µL of leaf extract. Three milliliters of solution containing DPPH in 100 µL of methanol is often given as a standard. After that, the tubes were kept in complete darkness for 30 min. The absorbance was therefore determined at 517 nm.

Measurement of free Radical Scavenging Percentage: In brief, 4ml of different extract solutions (31.25 µg/mL, 62.5 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL, and1000 µg/mL of the sample was mixed with 4 ml of DPPH solution (0.1mM)and incubated in a dark place. After 30 minutes, the absorbance of the sample mixture was monitored at 517 nm with the help of a UV spectrophotometer. Methanol and ascorbic acid were chosen as negative and positive controls, respectively. Each test was accomplished in triplicate. The free radical inhibition percentage was determined and calculated using the following formula.

Percentage radical scavenged (% RSA) = $[(A_o-A_1) / A_o] \times 100\%$ ……. (1)

Where A_o was the absorbance of the DPPH Solution and A_1 was the absorbance of the sample.

The minimum concentration of sample that can completely scavenge 50 % of DPPH free radicals is known as inhibitory concentration 50 (IC_{50}). The trend method in Microsoft Excel was used to determine IC_{50} in all *C. thevetia* extract samples.

Antimicrobial Activity Test: The Agar Disc diffusion method was used to screen the antibacterial activities of different solvent extracts as displayed by methods [6, 16, 17] with slight modification.

Organism Collection: For the research project, the organisms of both gram-positive (*S. aureus, S. pneumonia*) and gram-negative (*E. coli, K. pneumonia*) were utilized. All the strains were obtained as a gift from National Path Laboratories, Butwal Pvt. Ltd. Rupandehi, Nepal. Commercially available Gentamycin (as a gram-positive standard)

and Ciprofloxacin (as a gram-negative standard) were used.

Sample Preparation and Serial Dilution: For each plant extract, 100 mg was taken accurately in a closed small tube and dissolved thoroughly in 1 ml DMSO with the help of sonication. Then finally dissolved samples were stored in a safe place until use. Each 10 µL of sample solution contained 1 mg of plant extract.

Preparation of Media: Muller Hinton Agar (MHA) media preparation.

Activation of Culture Plates: The media plates made previously and frozen at 5 °C were incubated to dry enough. Then the media plates were cooled in a sterilized laminar airflow hood.

Preparation of Bacterial Suspension: Initially, nutrition broth media was prepared and sterilized. After that, 5mL nutrient broth was poured into four different sterilized test tubes. Bacterial suspensions of *S. epidermidis, S. aureus, E. coli, and K. pneumonia* were prepared to suspend bacteria (from subculture media) with the inoculating loop to each respective test tube and incubated at 37 °C for 23 hours. The turbidity of the inoculum's suspension was compared with 0.5 McFarland solutions.

Preparation of Filter Paper Disc: Approximately 5mm diameter of filter paper disc (from Whatman's No. 1 filter paper) was prepared and sterilized for 15 min at 115 °C.

Inoculation of Bacteria into the Media: A sterile cotton swab stick was dipped into the turbidity-adjusted bacterial suspension. The sterile cotton swab sticks later rotated firmly inside the wall of the tube above the fluid level. This step helped to maintain the microbial uniformity of microorganisms in the cotton swab. After that, the dried surface of the media plate was inoculated by rubbing the cotton swab stick (loaded with microorganisms) over the entire sterile media surface. This process was repeated two more times to maintain the uniform distribution of microorganisms. The same technique was repeated for each microorganism. The same technique was repeated for each microorganism *S. aureus, S epidermidis, K. pneumonia, and E. coli.* Finally, media plates were divided into four equal parts to insert the filter disc, containing sample extracts, blank control, and standard antibiotic disc at an equal distance.

Insertion of the Antibiotic Disc and Extract Disc into the Culture Media: After the complete inoculation of microorganisms, the position of the antibiotic and paper disc to the petri dish. 10 µL of each extract (1 mg of extract per disc) was poured into two paper discs (doublet manner) and, the third paper disc was used for negative control (10 µL DMSO). All the plates were inoculated at 37 °C for 24 hours. All the measurements were examined in triplicate.

Measurement of ZOI: The zone of inhibition was determined after 24 hours of incubation, the culture media was taken out from the incubator, and the inhibited area (ZOI) by the different extracts and antibiotics was measured in mm, with the help of the Vernier scale [5].

Determination of MIC and MBC: The broth dilution method was used to determine the minimum inhibitory concentration (MIC) and Minimum Bacterial concentration (MBC) of plant extracts by method [17] with slight modification. MIC was taken as the lowest concentration that prevented the visible growth of the bacterial culture. The easy technique to observe the inhibition of growth is the absence of turbidity in the examined tubes. However, it was very challenging to ensure whether the turbidity was due to the nature of the plant extract or due to the growth of the bacteria.

Thus, MBC was investigated to determine the minimum concentration of the plant extract that can completely kill the tested microorganisms, i.e., MBC. For determination, the refrigerated MHA petri plates were incubated at 37 °C for 45 minutes and transferred into a laminar airflow (LAF) hood. After those samples from each diluted test tube (obtained after MIC examination) were sub-cultured on MHA plates followed by incubation for the next 24 hours at 37 °C. Finally, the minimum concentration of plant extract that completely prohibited the microorganism growth over the media surface was noted as the MBC.

Statistical Analysis: Data were recorded as a mean of three determinations of absorbance for each concentration, from which the linear correlation coefficient (R^2) value was calculated. The regression equation is given as Y = mx + c, where *Y* is the absorbance of extract, *m* is the slope from the calibration curve, *x* is the concentration of extract, and *c* is the intercept. The regression equation was used to calculate the concentration of extracts. All the experiment was conducted at least 3 times (n = 3); the statistical mean was calculated with ± standard deviation (SD) using Microsoft Excel 2007.

RESULT AND DISCUSSION:

Quantitative Analysis of Phytochemical Constituents:

Total Phenolic, Total Flavonoid and Total Carbohydrate Content: Polyphenols are abundantly present phytochemical constituents in plants. The hydroxyl group present in the molecule can scavenge free radicals. Thus, there is a strong correlation between antioxidant potency and the total phenol content of many plant species. It has been proven that phenolic compounds are efficient hydrogen donors and serve as very good antioxidants [14]. Flavonoids are a highly diversified and widespread group of natural phenolic compounds. The hydroxyl position present in the flavonoid compounds governs antioxidant properties and it depends on the electron or hydrogen donation capacity of flavonoids to a free radical [17]. The abundant organic molecules produced during photosynthetic activity and major structural components of plant call are known as carbohydrates. They are the vital energy source that regulates the metabolic process, stimulates insulin secretion powerful neurotransmitters, and alters serotonin concentration [19].

In our study, the quantitative estimation of total phenol was accomplished by using the Folin-Ciocalteu reagent in terms of gallic acid as standard with equivalent to (GAE). The total flavonoid content was accomplished by using the Aluminium chloride method in terms of quercetin as standard with equivalent to (QE) and total carbohydrate was carried out by using phenol-sulphuric acid method in terms of glucose as standard. All the results of *C. thevetia* plant methanolic and aqueous extracts of leaf, flower, and latex are represented in **Table 1** and the calibration curve for standard is represented the **Fig. 2** for gallic acid, **Fig. 3** for quercetin and **Fig. 4** for glucose respectively. Also bar graphs **Fig. 5**, **Fig. 6**, and **Fig. 7** represents the Total phenol, Total flavonoid, and Total carbohydrate content individually.

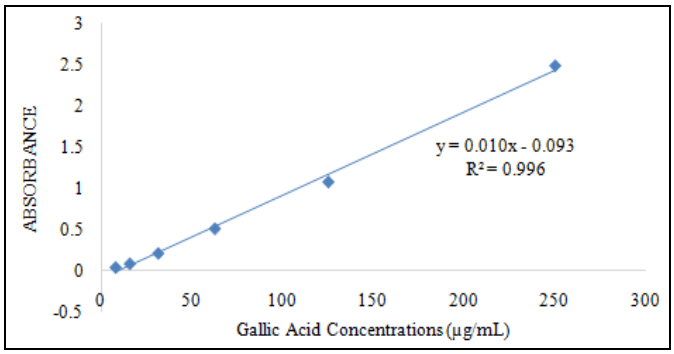

FIG. 2: CALIBRATION CURVE OF GALLIC ACID FOR TOTAL PHENOLIC CONTENT DETERMINATION

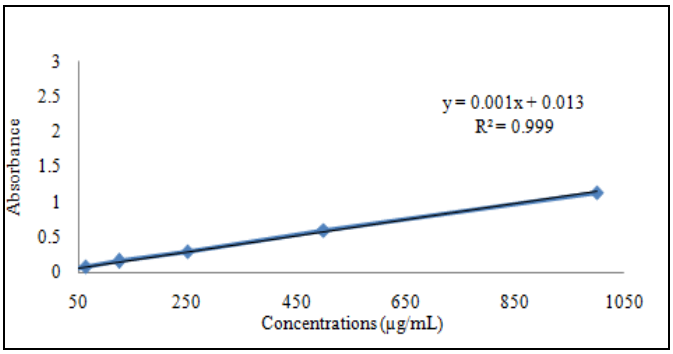

FIG. 3: CALIBRATION CURVE OF QUERCETIN FOR TOTAL FLAVONOID CONTENT DETERMINATION

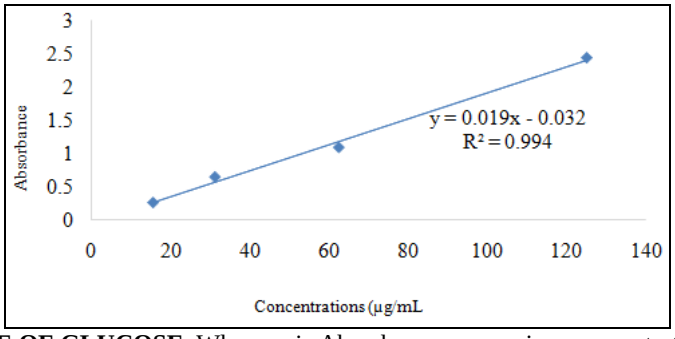

FIG. 4: CALIBRATION CURVE OF GLUCOSE. Where, y is Absorbance, x examines concentration, R^2 is the regression value

TABLE 1: RESULTS FOR THE TOTAL PHENOL CONTENT, TOTAL FLAVONOID CONTENT, AND TOTAL CARBOHYDRATE CONTENT OF *C. THEVETIA* LEAF, FLOWER AND LATEX EXTRACT IN DIFFERENT SOLVENTS

Extract	Total Phenol Content (µg GAE/mg)	Total Flavonoid Content (µg QURE/mg)	Total Carbohydrate Content (µg GLUE/mg)
MLE	48.37 ± 0.39	654.33 ± 2.08	143.47 ± 4.38
ALE	34.32 ± 0.89	12.0 ± 5.29	35.84± 0.71
MFE	80.26 ± 0.86	177.66 ± 5.50	178.15± 3.25
AFE	33.22 ± 3.12	9.0 ± 1.0	47.84 ± 1.07
MLAE	35.24 ± 0.95	8.66 ± 0.57	6.94 ± 0.26
ALAE	28.64 ± 0.94	18.66 ± 3.05	54.94 ± 0.59

Where, MLE= Methanol extract of the leaf; ALE= Aqueous extract of the leaf; MFE= Methanol extract of flower; AFE= Aqueous extract of flower, MLAE= Methanol extract of latex; ALAE= Aqueous extract of latex respectively.

The *C. thevetia* plant of methanolic and aqueous extracts of leaf, flower, and latex of the total phenolic content of the extracts was calculated from the regression equation of the calibration curve (y = 0.010x – 0.093; R^2 = 0.996), and expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). The total flavonoid content of the extracts was calculated from the regression equation of the calibration curve (Y= 0.001x + 0.013; R^2 = 0.999) and expressed as mg Quercetin equivalents (QE) per gram of sample (mg/g).Similarly, the carbohydrate content of the extracts was calculated from the regression equation of the calibration curve (Y= 0.019x - 0.032; R^2 = 0.994) and expressed as mg Glucose equivalents per gram of sample (mg/g) were represented in **Table 1** and the calibration curve for standard were represented as **Fig. 2** for gallic acid, **Fig. 3** for quercetin and **Fig. 4** for glucose respectively. Also bar graphs as **Fig. 5**, **Fig. 6**, and **Fig. 7** represents the total phenol, total flavonoid, and total carbohydrate content respectively.

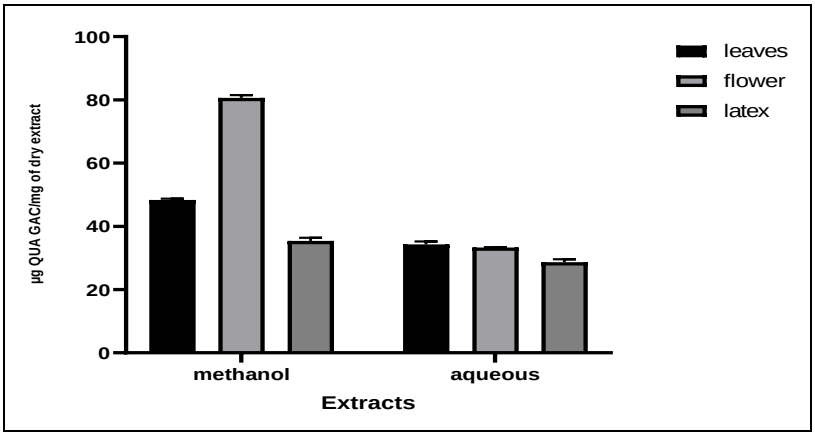

FIG. 5: BAR DIAGRAM REPRESENTING TOTAL PHENOLIC CONTENT OF *C. THEVETIA* LEAF, FLOWER, AND LATEX EXTRACTED IN DIFFERENT SOLVENTS

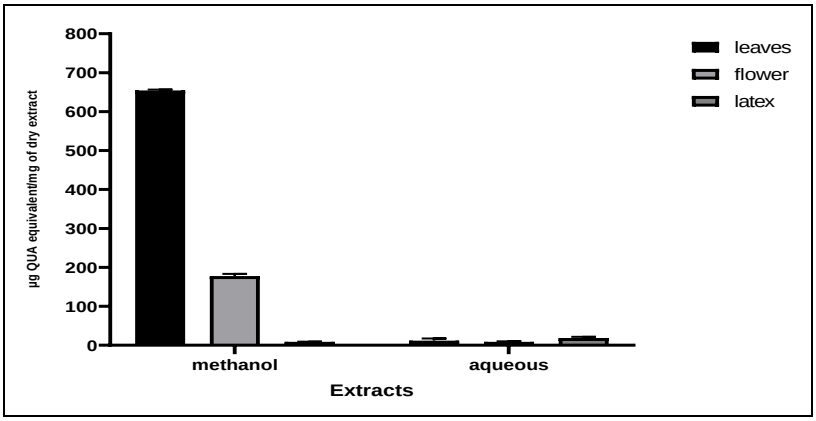

FIG. 6: BAR DIAGRAM REPRESENTING TOTAL FLAVONOID CONTENT OF C. THEVETIA LEAF, FLOWER, AND LATEX EXTRACTED IN DIFFERENT SOLVENTS

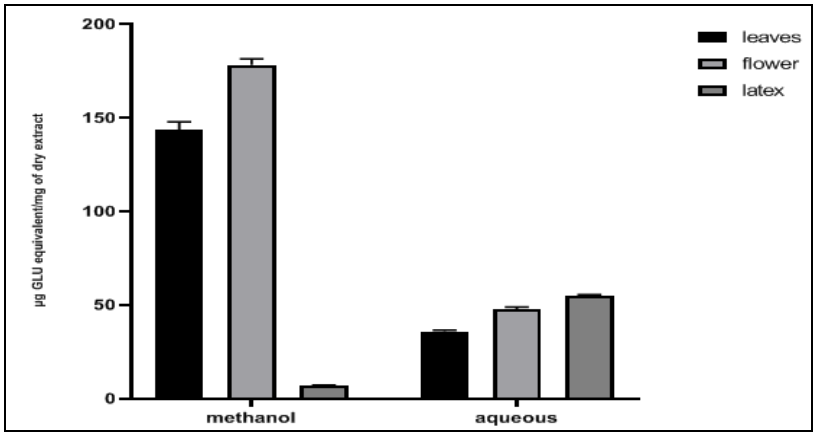

FIG. 7: BAR DIAGRAM REPRESENTING TOTAL CARBOHYDRATE CONTENT OF *C. THEVETIA* LEAF, FLOWER, AND LATEX EXTRACTED IN DIFFERENT SOLVENTS

There is variation in total phenolic content ranging from methanolic and aqueous extracts of leaf, flower, and latex. It is to be noted that the highest phenolic content was recorded in methanol flower extract (MFE 80.26 ± 0.86) and the lowest phenolic content was recorded in aqueous latex extract (ALAE 28.64 ± 0.94) as shown in above **Table 1**.

But in a previous study, the phenolic content in ethyl acetate and acetone extract of the flower is reported as 164 µg/mL and 176 µg/mL respectively [10] results show our report was somewhat lesser content also the highest phenol content was found in fruit (33.59 ± 0.38 mg GAE/gm dry weight) and lowest was found in root parts (21.47 ± 0.16 mg GAE/gm dry weight) of *Thevetia peruviana* species respectively.

In the study, the highest flavonoid content was recorded in methanol leaf extract (MLE 654.33 ± 2.08), and the lowest flavonoid content was recorded in methanol latex extract (MLAE 8.66 ± 0.57) as shown in **Table 1**. However, in a previous study, the flavonoid content in ethyl acetate and acetone extract of flower only is reported as 185µg/mL and 198 µg/mL respectively [12].

Similarly, it is noted that the highest carbohydrate content was recorded in methanol flower extract (MFE 178.15 ± 3.25) and the lowest carbohydrate content was recorded in methanol latex extract (MLAE 6.94 ± 0.26) as shown in above **Table 1**. The carbohydrate content of leaf, latex, and flower extract of *C. thevetia* has not been previously reported yet in methanol and aqueous solvents. However qualitative phytochemical analysis shows there is a presence of carbohydrate content in aqueous, chloroform, and methanol extracts of whole plant extracts [21].

Antioxidant Potency Determination by DPPH Radical Scavenging Activity: Natural products are a tremendous and consistent resource for the development of new drugs. Sometimes plant-derived natural compounds have gained attention because of their potential to act as cytotoxic and chemopreventive activity. Various plants have already been proven to possess high antioxidant properties containing high amounts of phenolics and flavonoid [18].

The hydrogen atom or electron donation ability of each plant extract against DPPH free radical was measured from the bleaching of violet-colored ethanol solution of DPPH. The DPPH radical absorbs at 517 nm. The radical scavenging activity was determined by monitoring the decrease in absorbance [21, 17]. The percentage radical scavenging activity of different samples with different concentrations is shown in **Table 9**.

Among the six extracts of *C. thevetia* shows the highest capacity to reduce the DPPH free radical 88.84 ± 0.18 at the concentration of 1000 µg/ml and the lowest scavenging capacity was shown by methanol latex extract (1.15±0.18) at a concentration of 7.8125 µg/ml. The IC_{50} value of aqueous flower extract was 87.19µg/ml whereas the standard value of ascorbic acid was 6.41 as shown in **Table 2.** The result obtained in our study is shown in **Fig. 8** and **Fig. 9** in the bar diagram.

TABLE 2: RESULTS FOR THE DPPH FREE RADICAL SCAVENGING EFFECT OF *C. THEVETIA* LEAF, FLOWER, AND LATEX IN DIFFERENT SOLVENTS

Concentration (µg/ml)	%DPPH Radical Scavenging Activity								IC50
	7.8125	15.625	31.25	62.5	125	250	500	1000	
ALE	4.23±0.49	20.16±0.43	28.39±0.24	34.69±0.34	43.01±0.7	47.36±0.37	50.37±0.49	53.74±0.18	137.06
MLE	2.57±0.45	25.10±0.62	30.39±0.31	34.69±0.32	39.83±0.6	47.57±0.46	51.48±0.56	69.95±0.37	151.22
AFE	14.19±0.3	27.94±0.84	50.32±0.55	55.08±2.47	70.5±0.18	82.01±1.84	84.48±0.63	88.84±0.18	87.19
MFE	2.26±0.94	24.36±1.35	27.61±0.74	35.06±0.65	40±0.44	42.63±1.86	47.44±0.63	52.55±0.46	148.63
ALAE	3.29±0.75	10.86±0.49	20.57±0.75	33.90±1.54	40.94±0.4	47.53±0.37	53.20±0.8	64.48±0.25	566.6
MLAE	1.15±0.18	7.61±0.67	15.47±0.31	24.93±0.37	32.59±0.7	43.45±0.49	56.62±0.62	64.73±0.18	580.68
MLE	2.57±0.45	25.10±0.62	30.39±0.31	34.69±0.32	39.83±0.6	47.57±0.46	51.48±0.56	69.95±0.37	151.22
AA									6.41

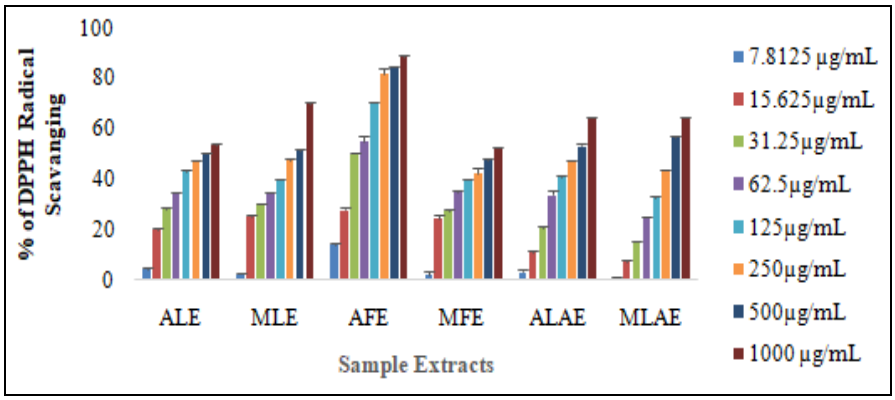

FIG. 8: BAR DIAGRAM SHOWING DPPH FREE RADICAL SCAVENGING CAPACITY OF *C. THEVETIA*. µg GAE equivant /mg of dry extract, µg QE equivalent /mg of dry extract.

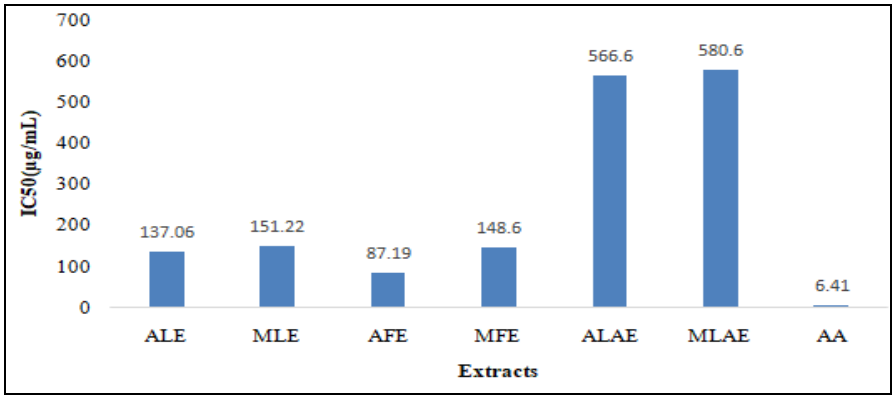

FIG. 9: BAR DIAGRAM OF IC50 VALUES OF DIFFERENT EXTRACTS ALONG WITH ASCORBIC ACID

FIG. 10: ANTIOXIDANT ACTIVITY

In comparison with article [21] the IC_{50} value of aqueous extracts of whole plants was 60.25 µg/mL which was approximately similar to our study with aqueous flower extracts i.e., 87.19 µg/mL.

Also in the previous study [11], the IC_{50} value of *C. thevetia* plant with ethyl acetate and acetone extract was found to be 90.52 and 59.6 µg/mL respectively.

Antibacterial Analysis: A total of six different extracts obtained from the leaves, flowers, and latex of *C. thevetia* were screened for their antibacterial activity against four different bacterial strains in the good diffusion method. Their antibacterial potency was quantitatively confirmed by inhibition zone absence or presence all over the disc loaded with the extract. It is confirmed that the result of the extract is more sensitive to gram-negative bacteria than gram-positive bacteria. The results of the antibacterial activity of aqueous and methanol extract of leaf, flower, and latex of *C. thevetia* against various bacteria by using well diffusion and disc diffusion methods were tabulated in **Table 3** and **Table 4.** In this study, the flower extract of both aqueous (AFE) and methanol (MFE) extracts shows maximum antibacterial activity against the *E. coli* with ZOI values of 18 ± 0.28 and 14.85 ± 0.63 mm respectively by using well diffusion method as compared to another bacterial strain K. *pneumonia, S. aureus* and *S. pneumonia*. The bacterial activity was compared to the ZOI of standard antibiotic disc Ciprofloxacin. Also by disc diffusion method, it was found the same result with a slight variation of ZOI the flower extract of both aqueous (AFE) and methanol (MFE) extracts shows maximum antibacterial activity against the *E. coli* with ZOI value of 12.60 ± 0.28 and 10.55 ± 0.35 mm respectively. However, MIC could not quantify the uncertainty of whether turbidity was due to bacterial growth due to the plant extract nature. Thus, MBC was calculated and expressed as mg/ml. The MBC values of different investigated samples were in the range of 0.19 mg/ml to 50 mg/ml. The maximum MBC activity of MFE was shown at 25mg/ml in *S. aureus and* the maximum activity of AFE was shown at 25 mg/ml concentration. The MFE was sensitive to *S. aureus and K. pneumonia* whereas, AFE was sensitive to *S. pneumonia* and *E. coli* which is mentioned in **Table 3.** The results obtained in our study are shown in **Fig. 9, 10**, and **11** respectively. The MBC value of methanol flower extract and aqueous flower extract is highly potent than other examined sample extracts. Some studies have reported the presence of different phytochemicals had an inhibitory effect against microorganisms. The result of this study suggests that these extracts have good potential for developing bio-inspired antibacterial drugs. The results obtained in our study are shown in **Fig. 11** and **12** respectively.

TABLE 3: ANTIBACTERIAL ACTIVITY OF LEAF, FLOWER, AND LATEX EXTRACT OF *C. THEVETIA* IN DIFFERENT SOLVENTS AGAINST DIFFERENT BACTERIA USING THE WELL DIFFUSION METHOD

	Zone of inhibition in mm (Mean±SD)			
Different samples	***E. coli***	***K. pneumoniae***	***S. aureus***	***S. pneumoniae***
ALE	7±0.70	8.55±1.06	8.8±0.56	9.1±0.70
MLE	9±0.70	9.4±1.13	8.2±0.56	12.05±0.49
AFE	18±0.28	12.15±0.49	16.1±0.42	11.1±0.42
MFE	14.85±0.63	10.15±0.63	14.05±0.49	12.95±1.20
ALAE	8.5±0.98	–	–	7.05±0.21
MLAE	8.05±0.77	7.2±0.56	7.75±0.63	–
Ciprofloxacin	35.25±1.76	13.65±0.21	33.25±1.06	35.5±1.41

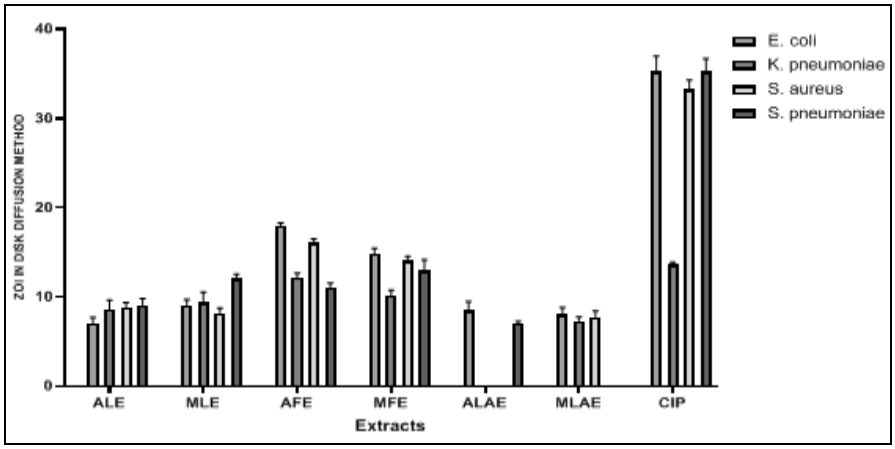

FIG. 11: THE BAR DIAGRAM REPRESENTING ZOI OF SENSITIVE EXTRACTS OF *C. THEVETIA* AGAINST DIFFERENT BACTERIAL STRAINS BY WELL DIFFUSION METHOD

TABLE 4: ANTIBACTERIAL ACTIVITY OF LEAF, FLOWER, AND LATEX EXTRACT OF *C. THEVETIA* IN DIFFERENT SOLVENTS AGAINST DIFFERENT BACTERIA USING THE DISC DIFFUSION METHOD

	Zone of inhibition in mm (Mean±SD)			
Different samples	***E. coli***	***K. pneumoniae***	***S. aureus***	***S. pneumonia***
ALE	8.5±0.42	–	7.6±0.28	7.6±0.28
MLE	7.55±0.35	7.1±0.42	9.5±0.28	9±0.28
AFE	12.6±0.28	10.1±0.42	9.7±0.14	8.95±0.21
MFE	10.55±0.35	7.8±0.28	10.6±0.28	9.6±0.28
ALAE	8.55±0.21	7.1±0.42	–	8.35±0.21
MLAE	7.1±0.42	–	–	–
Ciprofloxacin	28.55±1.34	14.75±0.64	–	–
Gentamicin	–	–	15.1±0.42	15.65±0.21

Note: − indicates inactive in the evaluated concentrations.

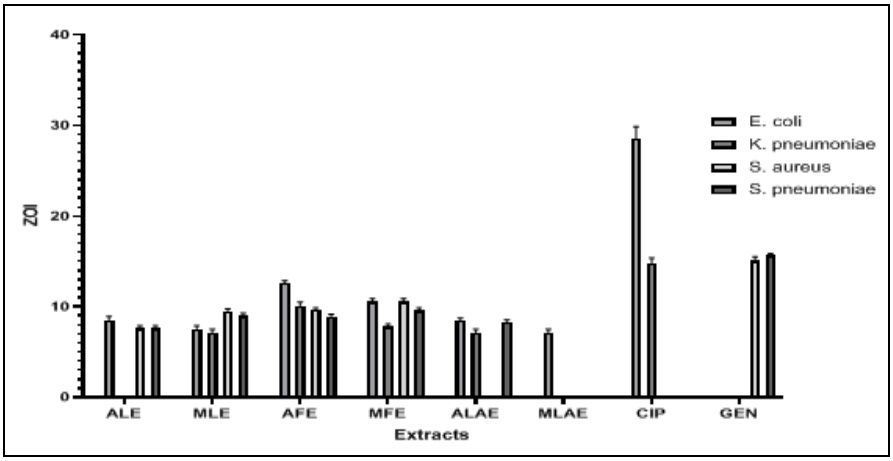

FIG. 12: THE BAR DIAGRAM REPRESENTING ZOI OF SENSITIVE EXTRACTS OF *C. THEVETIA* AGAINST DIFFERENT BACTERIAL STRAINS BY DISC DIFFUSION METHOD

However, MIC could not quantify the uncertainty of whether turbidity was due to bacterial growth due to the plant extract nature. Thus, MBC was calculated and expressed as mg/ml. The MBC values of different investigated samples were in the range of 0.19 mg/ml to 50 mg/ml. The maximum MBC activity of MFE was shown at 25mg/ml in *S. aureus and* the maximum activity of AFE was shown at 25 mg/ml concentration. The MFE was sensitive to *S. aureus and K. pneumoniae* whereas, AFE was sensitive to *S. pneumonia* and *E. coli* which is mentioned in **Table 3.** The MBC value of methanol flower extract and aqueous flower extract is highly potent than other examined sample extracts. Some studies have reported the presence of different phytochemicals had an inhibitory effect against microorganisms. The result of this study suggests that these extracts have good potential for developing bio-inspired antibacterial drugs.

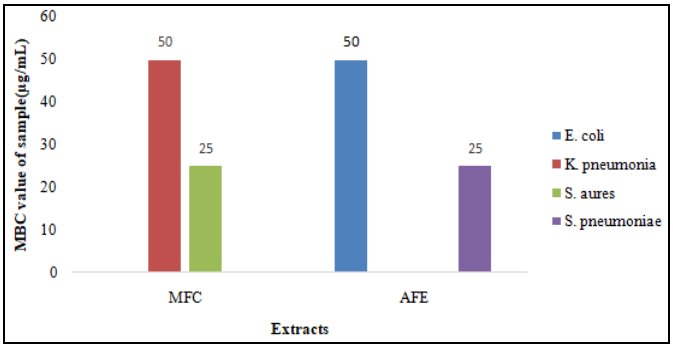

FIG. 13: THE BAR DIAGRAM REPRESENTING MBC OF FLOWER

TABLE 5: MBC VALUE OF FLOWER EXTRACT OF *C. THEVETIA* EXTRACT IN DIFFERENT SOLVENTS AGAINST DIFFERENT BACTERIA

Bacterial Strains	**MBC value of samples (mg/mL)**	
	MFE	**AFE**
E. coli	–	50
K. pneumoniae	50	–
S. aureus	25	–
S. pneumoniae	–	25

Note: '–' indicates Inactive in the evaluated concentrations.

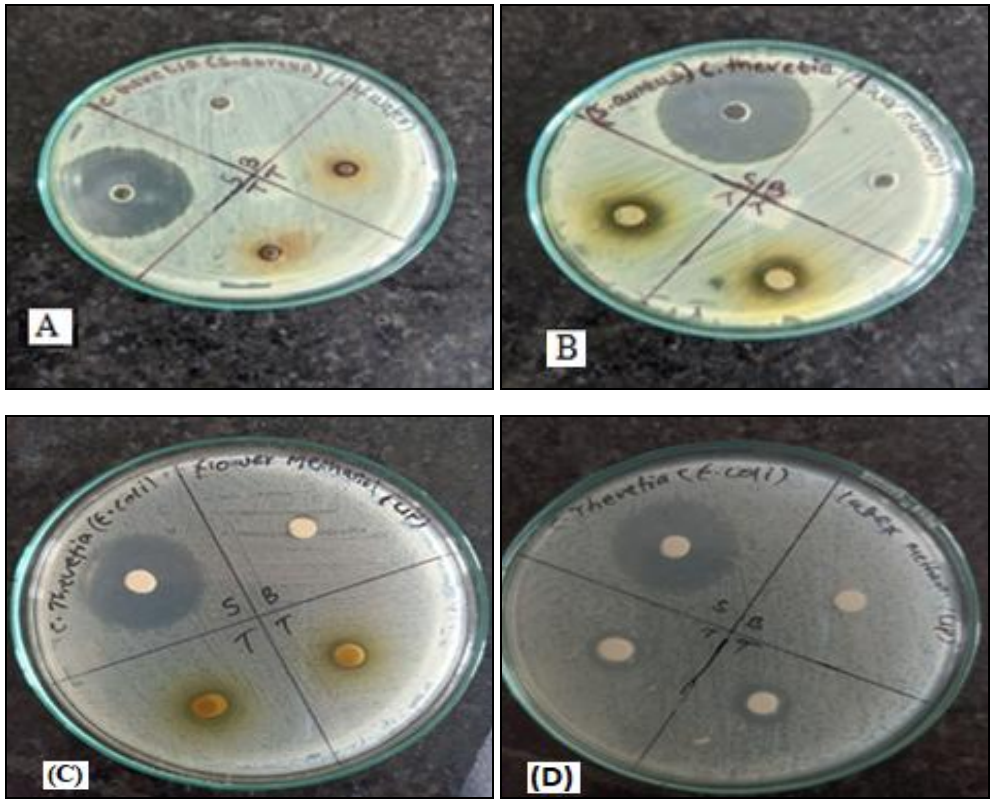

FIG. 14: (A) C. THEVETIA FLOWER METHANOL IN *S. AUREUS* BY WELL DIFFUSION METHOD (B) FLOWER METHANOL IN *E. COLI* BY WELL DIFFUSION METHOD (C) FLOWER METHANOL IN *E. COLI* BY DISC DIFFUSION METHOD (D) LATEX METHANOL IN *E. COLI* BY DISC DIFFUSION METHOD

In a previous study [17] the flowers of *C. thevetia* aqueous and ethanolic extract showed maximum antibacterial activity against *E. coli* with a zone of inhibition of 16 mm and 17 mm respectively. For the fruits, aqueous and ethanolic extracts showed maximum antibacterial activity against *E. coli* with zones of inhibition 13 mm and 15 mm respectively. Methanol whole plant extract of *C. thevetia* showed high antibacterial activity and was found almost similar to this study [19]. In study [20] the results (Bacterial) maximum zone of inhibition was observed in them ethanol whole plant extract of *C. thevetia* when compared to the other extracts of chloroform and aqueous. Results indicated that naturally occurring alkaloids and their synthetic derivatives have analgesic, anti-spasmodic, and bactericidal activities. Each of this group of compounds has been reported to possess antimicrobial activity and reported to exert its effects by affecting the cell membrane integrity of the bacteria. This suggested that the previous report was found almost similar to this study.

CONCLUSION: The present study shows that the methanolic and aqueous extract of the leaf, flower, and latex has potent anti-bacterial activity and phytoconstituents. The flower extract of *C. thevetia* evinced prominent antibacterial properties against various pathogenic bacterial strains, recommending significant utilization in the mitigation of diverse microbial diseases like diarrhea, dysentery, skin infection, urinary tract infection, dental infection, *etc*. It may be due to the polyphenol and flavonoid components.

Recommendation: The antifungal activity, anti-inflammatory activity, antidiabetic, and isolation of phytoconstituents of *C. thevetia* were still unknown. So, it is highly recommended for research for such activities. The flower extracts of *C. thevetia* show potent phytoconstituents. So, it is highly recommended to test the anti-inflammatory, antifungal, and antidiabetic activity of flower extracts of *C. thevetia.*

Data Availability: The data used to support this study available from the corresponding author upon request.

Author Contributions: The study designed by Arjun B, Nabin P, Manisha P, Nageswar NG, Sumit DC, and Gautam PC. The manuscript was

written by Arjun B and Nabin P. The experiments were conducted by Arjun B, Nabin P, Manisha P, Nageswar NG, Sumit DC, and Gautam PC and the data were analyzed by Arjun B, Nabin P, and Sumit DC. All the authors helped with the final draft. All the authors have perused the work and given their approval.

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CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest regarding the publication in this paper.

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