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Phytochemical Screening and Antimicrobial Activity of *Piper betle* L. and *Nicotiana tabacum* L. across Dharan, Nepal

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Abstract

Piper betle L. and Nicotiana tabacum L., were studied to find their antibacterial properties. Water, ethanol, methanol, acetone, and petroleum ether were used as solvents for plant crude extraction for phytochemical testings. Total phenolic content and total flavonoid content. Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922) were used as test organisms to determine the antibacterial activity of the plant extracts using agar well diffusion method.

This study showed a significant antibacterial activity against both bacterial strains with a zone of inhibition ranging from 7.16 to 17.83 mm for Piper betle L. and 7.16 to 12.5 mm for Nicotiana tabacum L. against Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922). The study reveals that Nicotiana tabacum L. and Piper betle L. can be a promising source against different pathogenic bacteria if thoroughly explored.

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1. Introduction

Medicinal plants are any plants with medicinal benefits that are employed in conventional medicine or contemporary medicines. Due to the country's great biodiversity and long-standing understanding of herbal remedies, medicinal herbs are important in Nepal's healthcare system. (Adhikari et al., 2019). 60–90% of population living in underdeveloped nations still rely on herbal plants as a primary source of healthcare (Uprety et al., 2010). 56% of high-altitude plants have reportedly been utilized for medicinal value in earlier times in Nepal (Kunwar & Bussmann, 2008). Beyond these several plants like *Nicotiana tabacum* L. and *Piper betle* L., have been found having a long history of medicinal value.

A preliminary test to determine the presence of different secondary metabolites in plants is called phytochemical screening. This includes the detection of bioactive components such as tannins, alkaloids, polysaccharides, Steroids, flavonoids, terpenoids etc. (Edeoga et al., 2005; Mann, 1978)..

Piper betle L. belongs to family Piperaceae. This plant can be found across Malaysia, India, Sri Lanka, Bangladesh, Burma, and Nepal. It is believed that this plant is rich in nutrients, minerals, vitamins, antioxidants, and phytochemicals. Further, it has high antibacterial compounds (Patil et al., 2015; Perumal & Saravanabhavan, 2018; Sarma et al., 2018; Syahidah et al., 2017)

Nicotiana tabacum L. belongs to Solanaceae family and is grown as a cash crop all over the world. It is native to tropical and subtropical America. There has been a thorough evaluation of *N. tabacum* L. medicinal uses elsewhere (Charlton, 2004). *N. tabacum* L. is a common remedy in Ethiopian folklore medicine for treating both human and animal illnesses such cancer, ulcers, coughs, snakebites, and respiratory tract infections. Additionally, it's employed as a Vermifuge (Eshetu et al., 2015; Giday et al., 2010; Yigezu et al., 2014). Extracts from *Nicotiana tabacum* L. too had a promising antibacterial properties (Ameya et al., 2018; HAJJAR et al., 2022; Sharma et al., 2016)

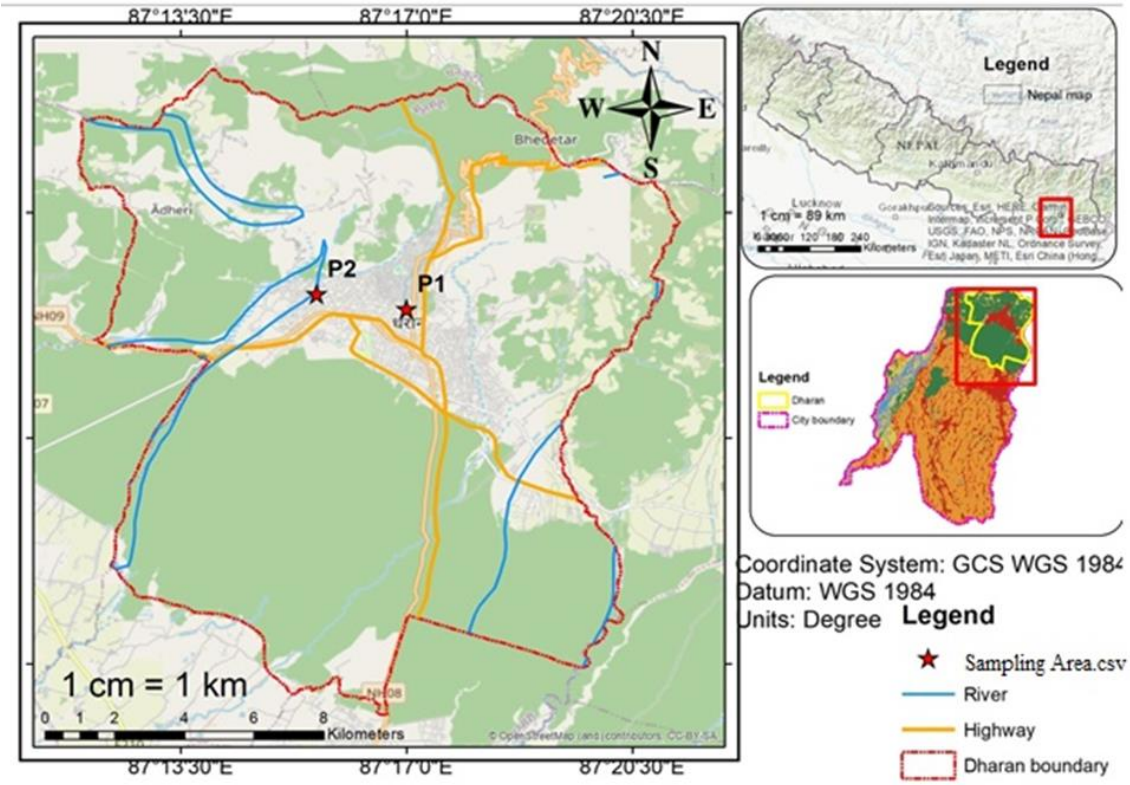


Figure 1: Map showing Study area

A worldwide hunt for new drug sources has been prompted by the rising incidence of microorganisms being resistant to antibiotics. In such scenario, *Piper betle* L. and *Nicotiana tabacum* L., which are frequently utilized in Nepalese traditional medicine can be a promising source to different pathogenic strains if their phytochemicals are explored. Thus, the rationale of the study lies in excavating different phytochemicals compounds of *Piper betle* L. and *Nicotiana tabacum* L., and screening the antibacterial potential of these plants against different bacterial strains.

2. Materials And Method

2.1 Study Area

Dharan sub-metropolitan city lies in Sunsari District, Nepal. A diverse range of flora and fauna can be found across Dharan. The major vegetation is dominated by tropical and sub-tropical mixed forest. The forest area is mainly covered by tree species like *Shorea robusta*, *Dalbergia sissau*, *and Bombax ceiba*. The climate is mild, and generally warm and temperate having an average temperature of around 21.6°C with annual precipitation of nearly 1796mm.

2.2 Collection of Plant samples

Fresh and healthy *Piper betle L.* and *Nicotiana tabacum L.* plant were plucked from the forest of study area. It was then transported to laboratory using plastic bags. The leaf were then plucked and shade dried for 10-15 days. Further, processing's and necessary laboratory work was carried out in the laboratory of Department of Microbiology and Department of Biology, Central Campus of Technology, Dharan.

2.3 Preparation of Sample Extracts (Methanol, Ethanol, Water, Acetone and Petroleum ether)

The Soxhlet equipment was used to carry out the organic extraction. Water and 70% ethanol were utilized for solvent extraction using the cold percolation technique, whereas methanol, acetone, and petroleum ether were used for solvent extraction using the solvent extraction procedure (Ogundiya et al., 2006). In order to do this extraction, 20 g of dried plant powder were placed in a glass thimble, and 200 ml of various solvents were extracted with each

individually. The extraction procedure was continued until the Soxhlet apparatus's siphon tube's solution lost all color. The extracts were concentrated with a Rota-evaporator and then transferred to brown colored bottles and sealed (Nkere & Iroegbu, 2005). The dried plant crude extracts shall be kept in the refrigerator at 4±2ºC for future use.

2.4 Phytochemical test

The extracts were subjected to qualitative phytochemical screening to detect the major phytochemicals present in them. The plant aqueous extract was screened out to identify the constituents (Leucoanthocyanin, emodins, Coumarin, carbohydrates, phenols, tannins, Saponins, alkaloids, flavonoids, steroids, glycosides, terpenoids) using the following standard procedures (Jaradat et al., 2015). The experimental approach was carried out three times for each sample to assure precision. In each replication, readings were taken in three fixed directions, and the average readings were recorded.

2.5 Estimation of Total Phenolic Content (TPC)

The Folin-Ciocalteau reagent was used to determine the total phenolic content of the plant samples. The Folin-Ciocalteau reagent, which is made up of phosphomolybdic and phosphotungstic acids and contains tungsten and molybdenum in the 6+ oxidation state, is used in this approach to measure the reducing capacity of the sample. Molybdenum blue and tungsten blue, as well as perhaps (Phenol-MoW11O40), are produced via reversible one- or two-electron reductions of heteropoly acids produced using the Folin-Ciocalteau phenol reagent. The metals in the reagent have an average oxidation state between 5 and 6 (Agbor et al., 2014; Lallianrawna et al., 2013) .

In accordance with the protocol, 1 mL of the sample was mixed with 1.5 mL of 10% FC-reagent and 2 mL of 2% Na2CO3 solution. The final result was made by mixing the combination with 4 ml of distilled water. It was then left an hour to react in the dark. The absorbance of the solution at 765 nm was measured using a spectrophotometer. For each sample, the experimental procedure was carried out three times. During each replication, readings were obtained in three distinct fixed directions, and the average results were recorded.

2.6 Estimation of Total Flavonoid Content (TFC)

The flavan nucleus distinguishes flavonoids, a class of secondary plant phenolics having diphenylpropanes (C6-C3-C6) skeletons. With over 4,000 flavonoids discovered too far, they are extensively distributed in plants and can be found in leaves, seeds, bark, and flowers. These substances offer defense against harmful UV rays, viruses, and herbivores. In addition to a lower incidence of heart disease, flavonoids are thought to have antioxidant and chelating properties that contribute to their positive health benefits (N. Alam & Sharma, 2020; Aryal et al., 2019; Khalid et al., 2016; Miean & Mohamed, 2001) Chalcones, flavones, flavonols, flavandiols, anthocyanins, condensed tannins (or proanthocyanidins), and aurones are the six main subgroups of flavonoids that are present in higher plants (Oksana et al., 2012).

Typically, 1 ml of the sample was mixed with 10% AlCl3 and 1 ml of 1M sodium acetate. The combination was then given 4 ml of distilled water, and it was then kept in the dark for an hour. The absorbance was eventually determined at 415nm. The experimental technique was run three times on each sample. Readings were taken three different fixed orientations throughout each replication, and the average results were noted.

2.7 Antimicrobial Assay of Plant Extracts

Mueller Hinton Agar (MHA) plates were used for the agar well diffusion technique antimicrobial testing of extracts from various plants (Monica, 2000; Okeke et al., 2001). In order to adjust the turbidity to 0.5 McFarland standards, the test organisms were inoculated in Nutrient broth and cultured overnight at 37°C, yielding a final inoculum of 1.5 108 CFU/ml. With standardized microbial culture broth, the MHA plate was lawn cultured. Dimethyl sulfoxide (DMSO) was used to create 100 mg/ml plant extracts. With the

aid of a sterile cork-borer (6 mm), five 6 mm wells need to be bored into the infected medium. Each well was filled with 10 μl, 20 μl, 30 μl, 40 μl extracts from different plants: positive control (antibiotics discs) for bacteria and negative/solvent control (DMSO),

Table 1: Results of the Phytochemical Screening of Methanol Extract (ME); Petroleum ether Extract (PE) and Aqueous extract (WE) extract of *Piper betle L.*.

Detection	Reference	Results				
		Leaf Extracts			Stem Extracts	
		ME	WE	PE	ME	PE
Phenols	White/Pale yellow ppt.	+	+	+	+	-
Flavonoids	Yellow ppt	+	+	+	-	+
Tannins	Bluish Black/ Greenish Yellow colour	-	+	+	+	-
Saponins	Frothing	+	+	+	-	-
Steroid	Red colour	+	+	+	+	+
Terpenoids	Grey colour	-	-	-	-	-
Alkaloids	Dull white ppt	-	+	-	+	-
Carbohydrates	Reddish brown / Brick red colour	-	+	-	+	-
Emodins	Red colour	-	-	-	-	-
Coumarin	Yellow colour	-	+	-	+	+
Leucoanthocyanin	Red colour upper layer	-	-	-	-	-
Glycosides	Golden yellow / yellow colour	+	+	+	+	+

"+" indicates Presence and "-" indicates Absence, Note: AE= Acetone Extract, EE= Ethanolic Extract, ME= Methanolic Extract, PE= Petroleum ether Extract and WE= Aqueous extract

respectively. The experimental procedure was carried out in triplicates for each sample. Readings were taken three different fixed orientations throughout each replication, and the average results were noted. It was incubated for 18 to 24 hours at 37°C after being given about 30 minutes to diffuse at room temperature (Aneja et al., 2009; Khokra et al., 2008; Rios et al., 1988). After incubation, the test compounds' antimicrobial activity was determined by looking at the plates for the development of a clear zone around the well. Inhibitory zones (ZOI) were detected and quantified in millimeters.

2.8 Minimum Inhibitory Concentration (MIC) of plant Extracts

The efficiency of antimicrobial medicines against certain pathogens is frequently assessed using the minimum inhibitory concentration (MIC). The 96-well titer plate method was used in this investigation to evaluate the MIC values of *Piper betle L.* and *Nicotiana tabacum L.* extracts.

In order to reach various concentrations, the test extracts are prepared by a series of dilutions in a sterile medium for the 96-well titer plate method. The plates are then incubated at a temperature that is ideal for the bacterial strain being tested, with 5µl of bacterial suspension added to each well containing the diluted extract of 50 µl with 45 µl of nutrient broth. After incubation, the MIC is calculated by looking at the extract's lowest concentration at which bacterial growth is inhibited. The MIC values discovered using this technique offer a numerical evaluation of the extracts' potency against the test bacteria. The antibacterial activity of the extract against the microorganism under test is stronger

the lower the MIC value. In this work, the MIC values of *Piper betle* and *Nicotiana tabacum* extracts against the bacterial strain *Escherichia coli* (ATCC 25922) were determined using the 96-well titer plate method.

Table 2: Results of the Phytochemical Screening of Methanol Extract (ME); Petroleum ether Extract (PE) and Aqueous extract (AE) extract of *Nicotiana tabacum L.*.

Detection	Reference	Results of *Nicotiana tabacum*			
		ME	WE	EE	AE
Phenols	White/Pale yellow ppt.	+	+	-	+
Flavonoids	Yellow ppt	+	+	+	-
Tannins	Bluish Black/ Greenish Yellow colour	-	-	+	-
Saponins	Frothing	+	+	+	-
Steroid	Red colour	+	+	+	+
Terpenoids	Grey colour	-	-	-	-
Alkaloids	Dull white ppt	+	-	+	-
Carbohydrates	Reddish brown / Brick red colour	-	-	-	-
Emodins	Red colour	-	-	-	-
Coumarin	Yellow colour	+	+	+	-
Leucoanthocyanin	Red colour upper layer	-	-	-	-
Glycosides	Golden yellow / yellow colour	+	+	+	-

"+"indicates Presence and "-"indicates Absence, Note: AE= Acetone Extract, EE= Ethanolic Extract, ME= Methanolic Extract, PE= Petroleum ether Extract and WE= Aqueous extract

3. Results And Discussion

3.1 Phytochemical screening of different Plant Extracts

The results of the phytochemical screening have been summarized in Table 1 and Table 2 Both *Piper betle* L. and *Nicotiana tabacum* were found to have similar sorts of phytochemicals, but in different amounts, based on the results of the detection of numerous chemical constituents in the leaf and stem extracts of both plants as summarized in Table 1 and Table 2.

Based on earlier phytochemical studies, *P. betle* L. has a wide range of physiologically active substances, the quantities of which differ across different plant species. We confirmed the results of previous investigations by utilizing ethanolic extracts to indicate the presence of alkaloids, flavonoids, tannins, phenols, steroids, saponins, and quinones (Perumal & Saravanabhavan, 2018) In support of the present findings, a qualitative phytochemical examination of methanolic extracts from P. betle leaves revealed the presence of steroids, alkaloids, phenols, flavonoids, tannins, saponins, glycosides, and terpenoids (Syahidah et al., 2017). Additionally, the ability of various extraction solvents to extract particular phyto-constituents varied. In the work of Saini et al., 2016 , aqueous extracts contained a wide variety of compounds, such as steroids, diterpenes, tannins, cardiac glycosides, flavonoids, saponins, phenols, coumarin, and alkaloids, whereas petroleum ether and chloroform extracts showed limitations in extracting more than two tested phyto-constituents (Patil et al., 2015). It's important to remember, though, that the presence and variety of phytochemicals are greatly influenced by the extraction method and solvent selection. Kaushik et al.(2010) reported that a preliminary phytochemical screening of *Nicotiana tabacum* leaves revealed similar results, including the identification of alkaloids, phenols, flavonoids,

phytosterols, triterpenoids, tannins, and carbohydrates. These findings are consistent with our research, indicating a convergence of findings from other investigations.

3.2 Total Phenolic Content

Phenolic compounds are a complex class of naturally occurring secondary metabolites formed from

Table 3: Total Phenolic Content in different extracts of *Piper betle L.* and *Nicotiana tabacum L.*.

Plant extract (100mg/ml)	Absorbance	TPC as GCE = $\frac{C\ X\ V}{m}$ mg/g
Water Leaf *Piper betle*	1.386	210.194± 0.29
Petroleum Ether leaf *Piper betle*	1.347	200.074 ± 6.15
Methanol leaf *Piper betle*	1.681	286.176± 1.10
Methanol stem *Piper betle*	1.688	287.892±1.10
Water *Nicotiana tabacum*	1.438	223.65 ± 0.49
Methanol *Nicotiana tabacum*	1.316	192.27 ± 0.05

Table 4: Total flavonoid content in different extracts of *Piper betle L.* and *Nicotiana tabacum L.*.

Plant extract (100mg/ml)	Absorbance	TFC as QE = $\frac{C\ X\ V}{m}$ mg/g
Water *Piper betle* leaf	0.892	51.24 ± 0.05
Petroleum Ether *Piper betle* leaf	0.064	11.30 ± 0.01
Methanol *Piper betle* leaf	1.201	66.15 ± 0.06
Methanol *Piper betle* Stem	1.527	81.86 ± 0.31
Acetone *Nicotiana tabacum*	1.847	97.306 ±0.15
Water *Nicotiana tabacum*	0.580	36.182 ± 0.03
Ethanol *Nicotiana tabacum*	1.251	68.578 ± 0.08
Methanol *Nicotiana tabacum*	1.348	73.258 ± 0.11

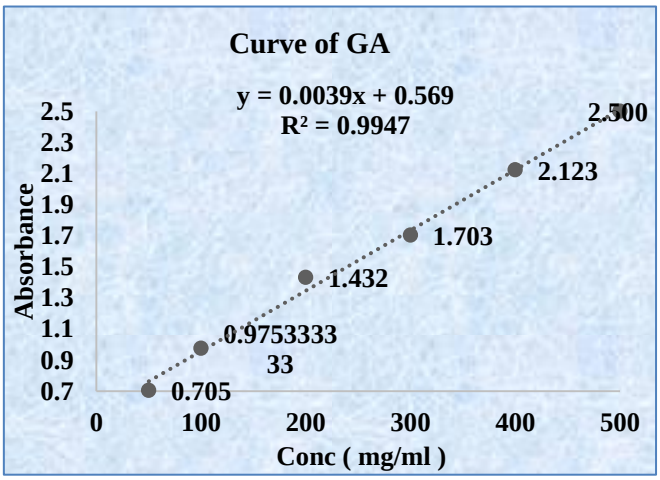
plants. They have a wide range of biological and pharmacological properties, including anti-inflammatory, anti-microbial, anti-cancer, and antioxidant properties. Using the Gallic acid calibration curve, the total phenol concentration was calculated as a milligram of Gallic acid equivalent. The absorbance was measured, and for each solution, the following values were recorded.

The total phenolic content (TPC) was evaluated for six different extracts of *Piper betle* L. and *Nicotiana tabacum* L. Shown in Table 3. Water leaf extract of *Piper betle* L. (210.194± 0.29mg GAE/g), aqueous extract of *Nicotiana tabacum* (223.65 ± 0.49 mg GAE/g), petroleum ether leaf extract of *Piper betle* L. (200.074 ± 6.15 mg GAE/g), and methanol extract of *Nicotiana tabacum* (192.27 ± 0.05 mg GAE/g) were the respective TPC. These findings show that the phenolic content of *Piper betle* L. is higher than that of *Nicotiana tabacum*, and that the stem extract of *Piper betle* L. has the highest phenolic content of all the extracts examined. Previous investigation of *Piper betle* L. showed the methanolic extract's total phenol content was determined to be 124.42 ± 0.14 mg of GAE/g of the extract (Akter et al., 2014). Other study of *Piper betle* L. the total phenolic content of ethanol, methanol, ethyl acetate, and hexane extracts was determined to be 249.96 ± 6.42, 115.04±3.60, 124.54±3.84, and 55.30±1.13, respectively, in distinct research carried out by Nguyen et al. (2020). Nacoulma et al. (2012) found that the TPC of

Nicotiana tabacum L. in aqueous/methanol, as well as chloroform extracts, was 86.0 ± 1.7 and 3.0 ± 0.53, respectively.

3.3 Total Flavonoid Content

The measurement of the flavonoid content in plant extracts is done using a crucial metric known as total flavonoid content (TFC). Secondary metabolites known as flavonoids are found in abundance in the plant world and have been linked to a number of pharmacological effects, including anti-inflammatory,



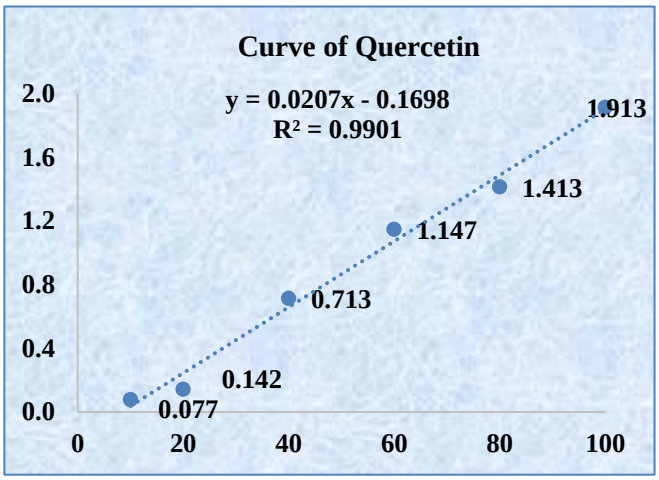


Figure 2 (Left): Calibration curve of Gallic acid for Determination of TPC. **Figure 3 (Right)**: Calibration curve of quercetin for TFC determination

anti-cancer, and anti-microbial capabilities. TFC was evaluated using $AlCl_3$ Calorimetric method (Kim et al., 2020)

Different extracts of *Nicotiana tabacum* L. and *Piper betle* L. were examined to assess their Total flavonoid content (TFC) (Table 4). The TFC of the extracts were given in mg/g and represented as quercetin equivalents (QE). The findings demonstrated that the stem extract of *Piper betle* L. had the greatest TFC value among the extracts (81.86 ± 0.31mg/g), followed by the leaf extract in methanol (66.15 ± 0.06 mg/g), and the leaf extract in water (51.24 ± 0.05 mg/g). The lowest TFC value was found in the petroleum ether extract of the leaf (11.30 ± 0.01 mg/g). The acetone extract from *Nicotiana tabacum* had the greatest TFC value (97.306 ±0.15 mg/g), followed by the methanol extract (73.258 ± 0.11mg/g), the ethanol extract (68.578 ± 0.08 mg/g), and the aqueous extract (36.182 ± 0.03mg/g). Overall, the findings imply that the TFC value of the extracts might be greatly impacted by the various extraction solvents used. The high TFC value of the *Nicotiana tabacum* acetone extract suggests that acetone is a more effective solvent than the other solvents tested in this investigation for extracting flavonoids from this plant. In a similar vein, the methanol extract of *Piper betle* L. stem had the greatest TFC value of all the plant's extracts, indicating that methanol is a superior solvent for extracting flavonoids from this plant's stem.

TFC values for *Nicotiana tabacum* extracts in aqueous/methanol and chloroform were significantly lower in this investigation (8.5 ± 0.15 and 6.3 ± 0.06 QE mg/g, respectively) than in previous research (Nacoulma et al., 2012). On the other hand, Nguyen et al. (2020) reported TFC values of 27.82 ± 1.25, 34.55 ± 1.10, 29.07 ± 0.96, and 10.72 ± 0.17 QE mg/g in *Piper betle* leaf extracts using ethanol, methanol, ethyl acetate, and hexane, respectively. Additionally, the methanol extract of *Piper betle* leaves showed a higher TFC value of 52.16 ± 0.61 QE mg/g (B. Alam et al., 2013) eventually lower than current study.

3.4 Antimicrobial Assay of Plant Extracts

To learn more about medicinal flora and its true usefulness, it is necessary to investigate medicinal plants as antimicrobial agents, but using a standard method of analysis is crucial (Ríos & Recio, 2005). Although different solvents and extraction processes exhibit different antimicrobial activity for plants.

Investigations of the antibacterial effects of *Piper betle L.* and *Nicotiana tabacum* extracts against *E. coli* and *Staphylococcus aureus* were done independently (Figure 4 and Figure 5). *Nicotiana tabacum* and *Piper betle* extracts were found to have greater antibacterial activity against *Staphylococcus aureus* (ATCC 25923) than *E. coli* (ATCC 25922) when compared to the two bacterial strains. Only the *Nicotiana tabacum* aqueous extract and the petroleum ether extract from the leaves of the *Piper betle* showed zones of inhibition for E. coli at a volume of 20 µl, measuring 7.16 mm and 8.26 mm, respectively. The *Piper betle* stem extract in petroleum ether, the ethanol-based *Nicotiana tabacum* extract,

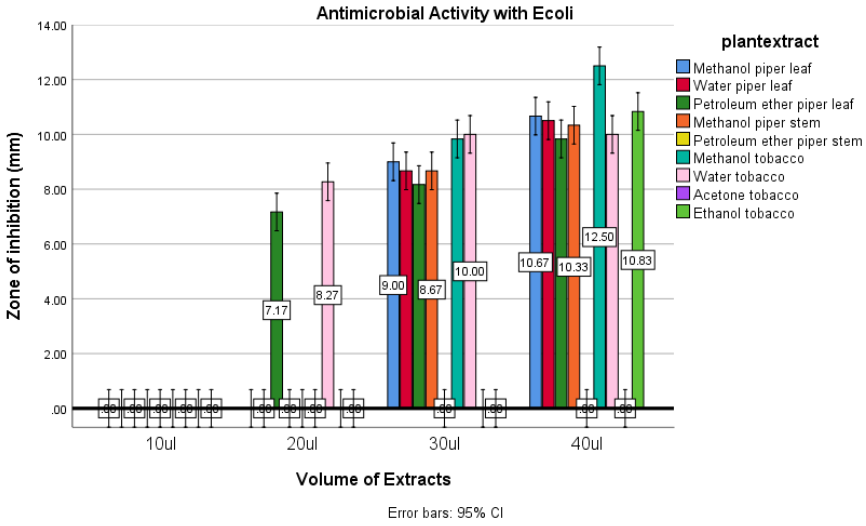


Figure 4: Antimicrobial activity of Volume of Plant Extracts of *Piper betle L.* and *Nicotiana tabacum* with error against *E.col* (ATCC 25922) .

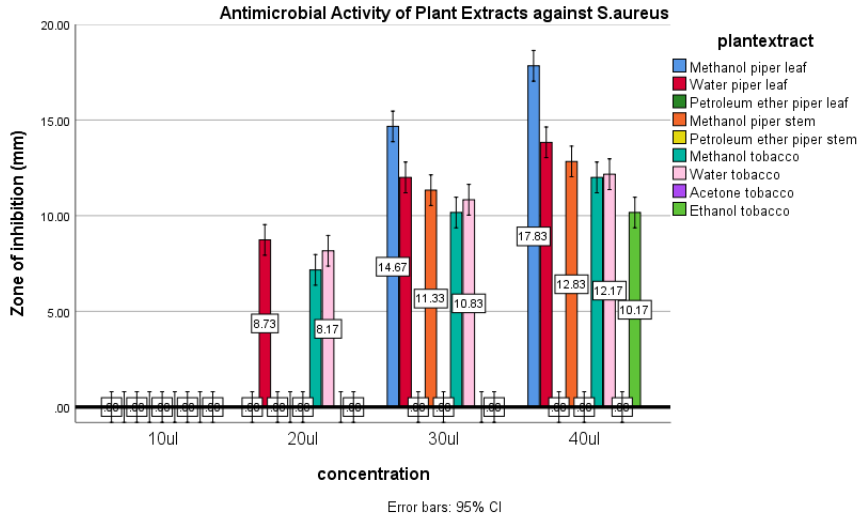


Figure 5: Antimicrobial activity of Concentration of Plant Extracts of *Piper betle L.* and *Nicotiana tabacum* with error against *Staphylococcus aureus*.

and the acetone-based *Nicotiana tabacum* extract all failed to show any antibacterial action against *E. coli* (ATCC 25922). Zones of inhibition against *E. coli* (ATCC 25922) were seen in several extracts, ranging from 8 to 10 mm. With a ZOI of 12.5 mm, *Nicotiana tabacum* methanol extract has the greatest ZOI of all of them. Three extracts, including a *Nicotiana tabacum* water and methanol extract and a *Piper betle* water and leaf extract, showed zones of inhibition for

Staphylococcus aureus (ATCC 25923). Zones of inhibition ranged from 8.17 to 14.67 mm. The aqueous extract of *Piper betle* leaf came in second with a ZOI of 13.83 mm, followed by the methanol extract of *Piper betle* stem with a ZOI of 12.83 mm, and by methanol extract of *Piper betle* leaf with a ZOI of 12.16 mm.

Different extracts of *Piper betle* L. and *Nicotiana tabacum* L. have different antibacterial effects on both bacterial strains, according to an analysis of variance (ANOVA) (Table 5). This difference is significant.

Patil et al. (2015) demonstrated, 10% of a 50 µl concentration of butanol from *Piper betle* leaves was found to be efficient against *Proteus vulgaris*, while

Table 5: ANOVA table of Comparison of Plant extracts

Tests of Between-Subjects Effects						
Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	AA against E coli	2507.848[a]	35	71.653	201.314	0.000
	AA against S aureus	3731.929[b]	35	106.627	219.597	0.000
Intercept	AA against E coli	1738.416	1	1738.416	4884.206	0.000
	AA against S aureus	2184.301	1	2184.301	4498.560	0.000
concentration* plant extract	AA against E coli	690.143	24	28.756	80.792	0.000
	AA against S aureus	1097.840	24	45.743	94.208	0.000
concentration	AA against E coli	1183.388	3	394.463	1108.272	0.000
	AA against S aureus	1240.665	3	413.555	851.715	0.000
plant extract	AA against E coli	634.317	8	79.290	222.770	0.000
	AA against S aureus	1393.423	8	174.178	358.719	0.000
Error	AA against E coli	25.627	72	0.356		
	AA against S aureus	34.960	72	0.486		
Total	AA against E coli	4271.890	108			
	AA against S aureus	5951.190	108			
Corrected Total	AA against E coli	2533.474	107			
	AA against S aureus	3766.889	107			
a. R Squared = .990 (Adjusted R Squared = .985)						
b. R Squared = .991 (Adjusted R Squared = .986)						

5% of the same concentration was the most successful in preventing the growth of *Salmonella typhimurium*, *Staphylococcus aureus*, and *Bacillus cereus*. With the exception of the fungus, the study discovered that butanol extract was more successful at suppressing bacterial growth than extracts made using alternative solvent systems. The aqueous extract produced more phytochemicals; however, its antibacterial action was less effective despite this. *Piper betle* leaf extracts in aqueous and acetone were ineffective against *Proteus vulgaris*. However, against *A. niger*, neither of the extracts displayed a zone of inhibition. Based on the findings of Sarma et al. (2018), all leaf extracts from *Piper betle* L., which includes aqueous, hexane, acetone, and ethanol, displayed antibacterial efficacy against the test pathogens. The ethanolic, aqueous, hexane, and acetone extracts showed the strongest antibacterial activity. The best Pharmaco-

logical activity against *Bacillus subtilis* was found in an ethanolic extract of the Banarasi variety, according to Datta et al. (2011). According to Datta et al. (2011), *Piper betle* L. extracts have been shown to have antibacterial action against both gram-positive and gram-negative infections. In addition, Agarwal et al. (2012)showed that *Piper betle* L. extracts in the aqueous, acetonic, methanolic, and ethanolic forms all exhibited antibacterial action against both gram-positive and gram-negative infections, with the ethanolic form of the extract being more potent. A zone of inhibition (mm) of the Banarasi and Meetha leaf ethanolic extracts against different pathogens (*B. subtilis, E. coli,* and *S. cerevisiae*). Methanolic and ethanolic extracts demonstrated strong antibacterial activity against several pathogens. But Kaur & Mondal. (2014) demonstrated that *Piper betle* L. extract is ineffective against *S. aureus* and *E. coli*.

Considering the outcomes of Ameya et al. (2018), the inhibitory spectra of plant extracts from *Nicotiana tabacum* L. against the human-type culture strains ranged from 66.29 mm^2 to 159.9 mm^2. The greatest inhibitory value was 159.9 ± 11.31 mm^2 against *S. aureus*, and the next-highest value was 119.23 ± 18.7 mm^2 against *P. aeruginosa*. But against *K. pneumonia*, the inhibitory potential was only modest (66.29 ± 11.61 mm^2). The greatest inhibitory value of 97.41 ± 19.62 mm^2 was used against *S. aureus* in the case of the tested clinical isolates, however *Salmonella enteric subsp. enteric serotype Typhi* (72.8 ± 12.9 mm^2) was shown to be remarkably resistant. The inhibitory area for the examined biofilm-forming uropathogens was 130.72 ± 12.5 mm^2 for *E. coli* and 147.5 ± 10.82 mm^2 for *Klebsiella species*, respectively. As per the research by Sharma et al. (2016), *S. aureus* and *P. aeruginosa*, both of which have inhibition lengths of 10.667±1.527mm and 5.33±1.154mm, respectively, and gram-positive bacteria, were the targets of the stem of *Nicotiana tabacum* methanolic extract's greatest antibacterial activity. The methanolic extract of *Nicotiana tabacum* leaves did not, contrary to earlier investigations, demonstrate any efficacy against *S. aureus*. In addition, a methanolic extract of *Nicotiana tabacum* stem shown inhibitory activities against *B. amyloliquefaciens*, *E. coli*, and *P. aeruginosa* with inhibition lengths of 4 ± 1.00 mm, 1.667 ± 0.577 mm, and 5.33 ± 1.154mm, respectively.

3.5 Minimum Inhibitory Concentration (MIC) of plant Extracts

The minimum inhibitory concentration is the lowest concentration at which the observable bacterial growth on the culture plates is inhibited. Minimum inhibitory concentrations are essential for diagnostic laboratories because they support determining if bacteria are resistant to an antimicrobial agent and tracking the activity of novel antimicrobial drugs. (Sen & Batra, 2012). According to the study's outcomes (Table 6), ciprofloxacin, a common antibiotic used to treat *E. coli* (ATCC 25922) infections, has the lowest MIC value of all the compounds under investigation at 0.78125.

Table 6: MIC performance of different extracts of *Piper betle L.* and *Nicotiana tabacum* against *E.coli* (ATCC 25922).

Plant Extracts (100 mg/ml)	MIC value
Methanol *Piper betle* Leaf	6.25
Water *Piper betle* Leaf	12.5
Methanol *Piper betle* Stem	3.125
Methanol *Nicotiana tabacum*	6.25
Water *Nicotiana tabacum*	3.125
Ciprofloxacin	0.78125

This shows that among all the examined extracts and compounds, ciprofloxacin had the strongest inhibitory activity against *E. coli* (ATCC 25922) bacteria. Comparatively, the MIC values of *Piper betle* stem and *Nicotiana tabacum* aqueous extracts both came in at 3.125, which is close to the MIC of ciprofloxacin. The MIC values of *Piper betle* leaf and *Nicotiana tabacum*, which were slightly higher than ciprofloxacin but remained equivalent at 6.25, also showed considerable inhibitory activity. However, when compared with ciprofloxacin and the other extracts, the aqueous extract of *Piper betle* leaf showed less inhibitory effectiveness against *E. coli* (ATCC 25922) bacteria with a higher MIC value of 12.5.

The methanol extract had a Minimum Inhibitory Concentration (MIC) of 100 mg/ml during the tube dilution experiment on *Nicotiana*

tabacum leaf, whereas the aqueous extract had a higher MIC of 200 mg/ml. Additionally, in the MIC test, the methanol extract had the lowest MIC of 25 mg/ml against *Candida albicans*, and only the methanol extract at a concentration of 200 mg/ml showed a fungicidal effect (Anumudu et al., 2019). Furthermore, According to the Akinpelu & Obuotor, (2000), the MIC values for *Nicotiana tabacum* leaves are: *Escherichia coli* (NCIB 86) - 0, *Klebsiella pneumoniae* (NCIB 418) - 0, *Proteus vulgaris* (NCIB 67) - 0, *Serratia marcescens* (NCIB 1377) - 11-22, with a MIC of 12.5; *Shigella dysenteriae* (LIO 15) - 1.56; *Staphylococcus aureus* (NCIB 8588) - 3. Results by Hoque et al. (2012) showed that the MIC ranged from 0.625 to 0.75 mg/ml against various test organisms when an ethanol extract of betel leaf was tested. The ethanol extract showed the greatest inhibition against *S. aureus* (0.625mg/ml), *V. cholerae* (0.625mg/ml), and *E. coli* (0.625mg/ml) among the test organisms. However, *E. coli* and *S. dysenteriae* showed the lowest levels of inhibition (0.75 mg/ml).

4. Conclusion

The present investigation used a variety of extraction solvents to examine the antibacterial activity of plant extracts from *Nicotiana tabacum* L. and *Piper betle* L. against *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). The analysis also tried to identify the presence of phytochemicals and any potential antibacterial effects. According to the findings, both plants significantly inhibited both bacterial strains with diverse levels of zone of inhibition. The extracts contained a wide range of bioactive substances, including phenols, tannins, Saponins, alkaloids, flavonoids, steroids, and glycosides, that have been researched for their Pharmacological properties. The total phenolic content of the extracts was also determined and found to be higher in methanol extracts of piper leaf and stem than in nicotine extracts. The study also found that nicotine extracts had a greater amount of TFC than piper extracts. The Study highlights the importance of maintaining traditional knowledge and making sustainable use of natural resources while highlighting the potential of these plant extracts as sources of antibacterial compounds in nature. The findings of this study may contribute to the development of organic antibiotics as a replacement for synthetic ones and provide information on the conventional therapeutic applications of these herbs. The overall results show that more study is required to properly explore the possible uses of such plant extracts as antibacterial agents.

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