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Phytochemical Analysis and Synergistic Effects of *Solanum Surattense* And *Piper Longum*

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ABSTRACT

Medicinal plants have become more popular in the recent world pharmaceutical field in the last twenty years. Among them, the species of the genus Piper and species of the genus Solanum play an important role in treating respiratory, and digestive diseases and possess general tonic properties. In our investigation, the synergistic effect of chloroform extract of Solanum surattense and Piper longum has been evaluated by preliminary phytochemical analysis, and the functional groups of bioactive compounds are analyzed by FTIR spectroscopy. The antimicrobial activity was evaluated by the agar well diffusion method. The phytochemicals such as Resins, Carbohydrates, Flavonoids, glycosides, and Proteins are present Whereas the plant extracts Solanum surattense and Piper longum showed good antibacterial activity, and better activity was noted against the antifungal species. The study concludes that the synergistic effects of the plant Solanum surattense and Piper longum have high therapeutic efficiency towards fungal species.

Keywords:Antibacterial activity, Antifungal activity, *Piper longum,* Phytochemical study, *Solanum surattense.*

INTRODUCTION

Folk medicine additionally called Traditional medicine (also known as indigenous medicine) includes health aspects of lore that emerged over periods within the folk beliefs of varied societies before the time of current medicine. According to WHO, traditional medicine is defined as "the total of the knowledge, skills, and practices supported the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, employed in the upkeep of health yet as within the prevention, diagnosis, improvement or treatment of physical and mental illness" (WHO). It's been used throughout the planet in many developed countries. Today, in healthcare industries this medicine plays an important role.

The biological activity of phytochemicalsand their medicinal properties are reported by many scientists. Many of the plants are employed in traditional medicine to exhibit common ailments and to promote a healthy life. According to WHO reports, most people (80% of the planet's population) mainly rely on traditional medicine for treating their common diseases[1].Because traditional medicines are easily affordable, accessible, and low-cost.*Solanum surattense* (Solanaceae) is a perennial widely used traditional and folklore medicine. It's distributed in Australia, Ceylon, India, Malaysia, Polynesia, and other geographical areas [2].*Solanum virginianum*, also called *Surattense* nightshade, yellow-fruit nightshade, yellow-berried nightshade, Thai green eggplant, Thai striped eggplant (from the unripe fruit), is additionally referred to as Indian nightshade or yellow berried nightshade plant, the common name is Kantakari, *Solanum surattense* Brum. f. and *Solanum xanthocarpum*Schrad and Wendl are synonyms of *Solanum virginianum L*. [3]. Dashmularishta, which is employed as a syrup for lactating mothers, an important constituent of Ayurvedic preparation was taken from the roots of *S.surattense*[3]. The seeds are used for conditions like cough and asthma. For the treatment of a cavity, teeth pain, pus formation, and associated swelling of gums, seeds together with mustard oil were used as an excellent treatment [4]. Root poultice accustomed to treating piles is practiced as conventional medicine in many villages of South India. The varied parts of the plants like stem flowers and fruits of *S.surattense* are used to treat the burning sensation of the feet [5]. In ancient times fruits are used for the treatment of inflammatory disease as well as leprosy and cough.

Piper longum is a climber, usually cultivated for its fruit, which is sometimes dried and used as a spice and seasoning. The origin of plant is in south India, which is close kind of like the black pepper plant *(Piper nigrum*). It belongs to the Piperaceae family. Many medicinal properties of dried fruits and roots of the plant *Piper longum* are reported.They are widely used as a stomachic, thermogenic, aphrodisiac, expectorant, laxative, digestive, emollient, and amoebic, anti-asthmatic, since the older days and even have antibacterial activity [6,7]. The root of the plant is employed as a carminative, used for liver diseases, and acts as a stomachic, abortifacient, and aphrodisiac. Fruits of the plant possess

diuretic and tonic properties, and various biological properties like pain reliever in joints, used for snakebite, scorpion stings, and visual disorders. *Piper longum* has been widely used as a drug for leprosy and tuberculosis (TB) [8].

MATERIALS AND METHODS

Collection of the plant:
The fruits of the plant *Solanum suranttense*were collected from the K.K. Nagar area in between the first week of February and also the fruits were separated by knife and the leaves and thorns were removed with help of the blade. After that, they were shade dried at room temperature for several days and then milled into coarse powder by a mixer grinder.

The coarse powder of fruits of *Piper longum* was brought from the market of the K.K. Nagar area. An equal proportion (100g) of coarse powder from those two plants was taken and evenly mixed and they were used as a sample for further investigation.

Preparation of chloroform extract:
The combinations of those two extracts were prepared by mixing 1g of dried Powdered sample into 100 ml of chloroform. They are stirred well with the help of a glass rod and filtered using Whatman filter paper No 1. The extract was collected within the Eppendorf tube for further analysis.

Preliminary phytochemical screening:
To identify the various chemical compounds, present within the plant extract the preliminary phytochemical screening was performed. The qualitative determination of the groups of organic compounds was administered within the extract of plant material. (Evans, 2002).

Detection of the resins:
To 0.5ml of plant extract, 3ml of $CuSO_4$ solution is added. Shake for about 1-2 min. formation of a green color precipitate indicates the presence of resins.

Detection of Tannins:
To 2ml of plant extract, 2-3ml of 10% HCL is added and boiled for 5-6 min. The formation of red color indicates the presence of tannins.

Detection of Carbohydrates:
To 0.5ml of extract, 0.5ml of Benedict reagent is added ad boiled for 2 min. Color changes and the precipitate is formed. It indicates the presence of carbohydrates.

Detection of Glycosides (Born Trageru's Test):
Take 2ml of hydrolysate, add 3ml of chloroform, shake vigorously, then the chloroform layer gets separated. To that add a 10% ammonia solution. The ammonium layer turns pink color indicating the presence of glycosides.

Detection of Proteins Estimation (Brad Ford Method):
To 1ml of extract, add 5ml of brad ford reagent, Take OD at 575nm.

Biuret Test:
To 2 ml of extract, add 1 drop of 2% $CuSO_4$ solution and add 1 ml of 95% ethanol, then add 2 to 3 sodium hydroxide pellets. The formation of pink color indicates the test is positive.

Test for Saponins:
To 50 mg of extract add 20 ml of distilled water. Shake vigorously for 15 min, and at 2 cm layer of foam formation indicates the presence of saponins.

Test for Gums: Take 100 mg extract and dissolve it in 2 ml of distilled water. Add 2 ml of absolute alcohol with constant stirring. White color cloudy precipitate indicates gums & mucilage.

Detection of Flavonoids To 0.5 ml extract, add 4 ml of 1% ammonia and to this add 1 ml of conc H_2SO_4. The formation of yellow color indicates the presence of flavonoids.

Detection of Phenol: To 50 mg of extract, add 5 ml of water and to this add a few drops of 5% ferric chloride. The formation of dark green color indicates the presence of Phenol.

ANTIMICROBIAL ACTIVITY: (AGAR WELL DIFFUSION METHOD, 1940):

ANTIBACTERIAL ACTIVITY:

Principle:
The antimicrobials present within the given sample were allowed to diffuse out into the medium and interact during a plate freshly seeded with the test organisms. The resulting zones of inhibition are going to be uniformly circular as there'll be a confluent lawn of growth. The diameter of the zone of inhibition is often measured in millimeters.

AGAR- WELL DIFFUSION METHOD:

Nutrient Agar Medium:
The medium was ready by dissolving 2.8 g of the commercially out agar Medium (Hi-Media) in 100ml of water. The dissolved medium was autoclaved at 15 LBS pressure at 121 °C for a quarter-hour. The autoclaved medium was mixed well and poured onto 100 mm Petri plates (25-30ml/plate) while still liquefied.

Nutrient broth:
The nutrient broth was ready by dissolving a pair of .8 g of commercially accessible nutrient medium (Hi-Media) in 100 ml H2O and cooked to dissolve the medium fully. The medium was distributed as desired and sterilized by autoclaving at 15 LBS pressure (121ºC) for a quarter-hour.

Procedure:
Petri plates containing 20 ml medium were seeded with the 24-hour culture of microorganism strains (*Staphylococcus aureus and E. Coli*). Wells were cut and completely different concentrations of sample America (100µg/ml,50µg/ml,20 µg/ml and 10 µg/ml) was another. The plates were then incubated at 37 °C for 24 hours. The antibacterial drug activity was assayed by the activity of the diameter of the inhibition zone shaped around the wells. Antibiotic drug antibiotic was used as positive management. The values were calculated by mistreatment Graph Pad Prism 6.0 code (USA).

ANTIFUNGAL ACTIVITY:

Principle:
The anti-fungal agent gift within the given sample was allowed to diffuse out into the medium and move freshly seeded with the take-a-look-at organisms. The ensuing zones of inhibition are going to be uniformly circular as there's awfully plate are going to be a merging field of growth. The diameter of the zone of inhibition may be measured in millimeters.

AGAR- WELL DIFFUSION METHOD:

Potato Dextrose Agar Medium:
The potato dextrose agar medium was ready by dissolving 20 g of potato infusion, 2g of dextrose, and 1.5 g of agar in 100 ml of H2O. The dissolved medium was autoclaved at 15 LBS pressure at 121 °C for a quarter-hour. The autoclaved medium was mixed well and poured onto 100 mm Petri plates (25-30 ml/plate) while still liquefied.

Procedure:
Petri plates containing 20ml potato dextrose agar medium was seeded with a 24-hour culture of fungal strain *Aspergillus fumigates and Aspergillus niger*,wells were cut and different concentration of sample US (100 µg/ml, 50 µg/ml, 20 µg/ml and 10 µg/ml) was added. The plates were then incubated at 37°C for 24 hours. The anti-fungal activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Amphotericin B was used as a positive control. The values were calculated using Graph Pad Prism 6.0 software (USA).

Fourier Transform Infrared Spectroscopy (FTIR) Spectroscopic Analysis for the Identification of Functional Groups of Combined Extract of *Solanum surattense* and *Piper longum.*

Perkin Elmer Spectrophotometer system was used to perform FTIR analysis, which was used to detect the characteristic peaks ranging from 400-4000 cm-1 and their functional groups. FTIR peak values were recorded. For the spectrum confirmation, every analysis was repeated twice.

RESULT AND DISCUSSION

PRELIMINARY PHYTOCHEMICAL ANALYSIS:

Table 1: Qualitative phytochemical analysis of chloroform extracts of *Solanum surattense* and *Piper longum*

S.NO	Phytochemical constituents	Concentrations
1.	Resin	+++
2.	Carbohydrates	++

3.	Tannins	-
4.	Saponins test	-
5.	Flavonoids	+++
6.	Phenol	-
7.	Glycosides	+
8.	Protein	+++
9	Gum	-

+ Present , - Absent ,++ medium, +++ high concentration

The phytochemical characteristics of Solanum surattense and Piper longum investigated are summarized in Table-1. The outcome of the qualitative phytochemical analysis in the chloroform extract revealed the presence of Resins, Carbohydrates, Flavonoids, and Proteins (Table 1) which could make the plant extract useful for treating different ailments and having the capacity of providing applicable drugs for human use. Whereas the fruit extract of Solanum surattense was investigated individually [9] it showed the presence of bioactive compounds such as Terpenoids, Alkaloids, Flavonoids, Proteins, Saponins, and Coumarins. In another study, the fruit extract of Piper longum was prepared with chloroform and analyzed for phytochemical screening and it showed the presence of Alkaloids, Proteins, Volatile oils, Tannins, and traces of Hexose sugars [10].

ANTIBACTERIAL ACTIVITY:

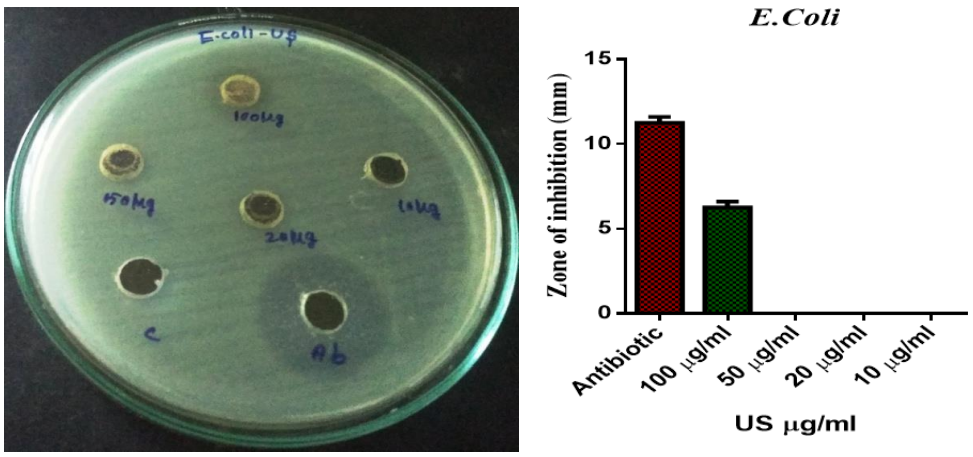

Fig 1:Synergistic effect of *Solanum surattense* and *Piper longum* against *E.coli*

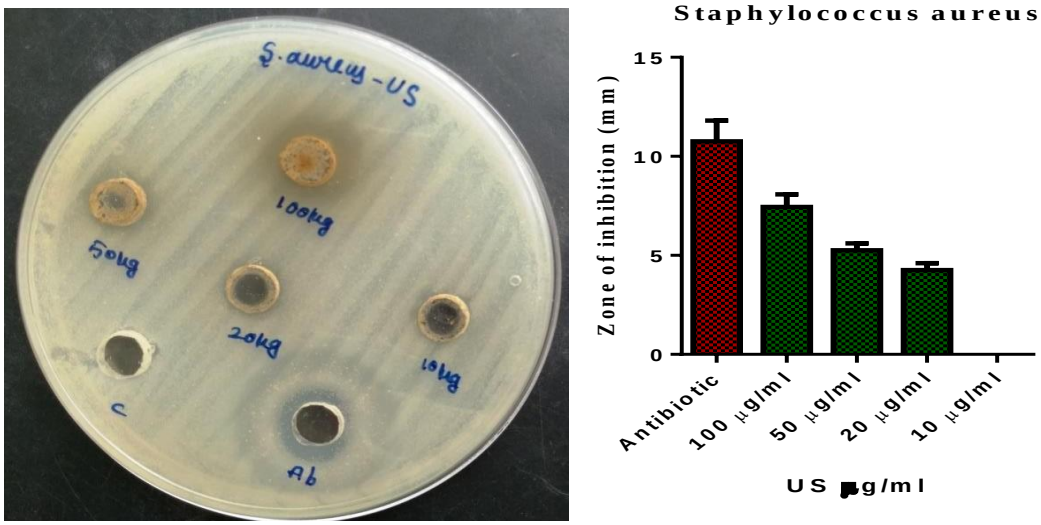

Fig 2: Synergistic effects of *Solanumsurattense* and *Piper longum* against *Staphylococcus aureus*

Table 2. Antibacterial activity of *Solanum surattense* and *Piper longum* against *Staphylococcus aureus and E.Coli*

S.NO	Name of the test organism	Zone of inhibition (mm) SD ± Mean				
		100µg/ ml	50µg/ml	20 µg/ml	10µg/ml	GEN
1.	*Staphylococcus aureus*	6±0.5	0	0	0	11.5±0.5
2.	*E.Coli*	7.9±0.9	5.5±0.5	4.5±0.5	0	10.5±1.5

GEN- Gentamicin

The Antibacterial potential of chloroform extract was evaluated against Gram-positive Bacteria, *Staphylococcus aureus*, and Gram-negative bacteria, *E.coli*. Compared to Gram-positive bacteria the extract showed marked efficiency towards Gram-positive bacteria. The antibacterial activity of the synergistic effect of *Solanum surattense* and *Piper longum* was shown in Table 2. The *Staphylococcus aureus* showed a zone of inhibition of about 6mm towards 100µ/ml of the plant extract, whereas *E.coli* shows 7.9mm towards 100µg/ml of the plant extract. But somehow it is lesser than the antibiotic Gentamicin.

A study [11] revealed that the fruit extract of the plant *Solanum surattense* showed marked antibacterial activity against species *E.coli* of about 17.8mm toward 100µg/ml of the extract. It showed a zone of inhibition of 14.0mm against the bacteria Staphylococcus aureus towards 100µg/ml of the plant extract. When compared to the antibiotic activity, the plant extract showed a much lesser amount of zone of inhibition. Hence the Antibacterial potential of the combined extract of *Solanum surattense* and *Piper longum* showed a marked efficiency towards *Staphylococcus aureus* and *E.coli* than the individual analysis of *Solanum surattense* and *Piper longum*.

Hence the Antibacterial potential of the combined extract of *Solanum surattense* and *Piper longum* showed a marked efficiency towards *Staphylococcus aureus* and *E.coli* than the individual analysis of *Solanum surattense* and *Piper longum*.

ANTIFUNGAL ACTIVITY:

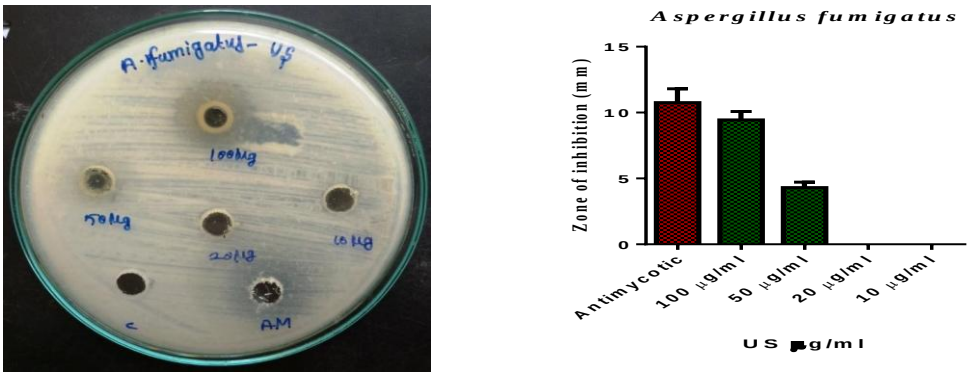

Fig 3: Synergistic effect of *Solanum surattense* and *Piper longum* against *Aspergillus fumigates*

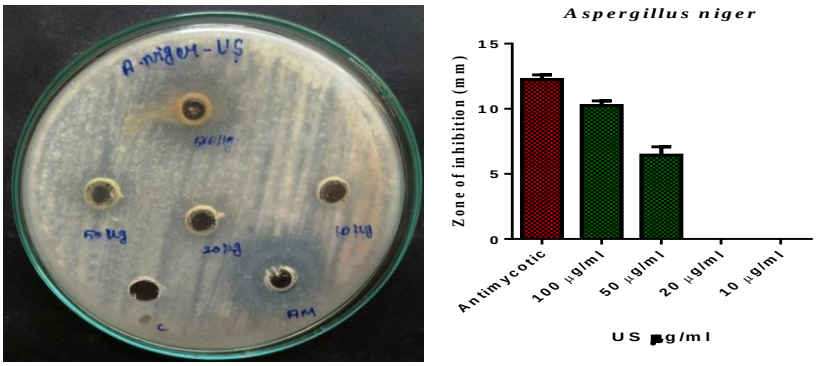

Fig 4: Synergistic effect of *Solanum surattense* and *Piper longum* against *Aspergillus niger*

Table 3. Antifungal activity of *Solanum surattense* and *Piperlongum* against*Aspergillus fumigates* and *Aspergillus niger*.

S.NO	Nameofthe test organism	Zone of inhibition(mm)				
		100µg/ml	50µg/ml	20µg/ml	10µg/ml	AM
1.	*Aspergillusniger*	10.5±0.5	6.9 ±0.9	0	0	12.5±0.5
2.	*Aspergillusfumigatus*	9.9±0.9	4.6 ±0.6	0	0	10.5±1.5

AM_ Amphotericin

The antifungal activity showed promising effects against plants and plant-based products [12,13,14]. The antifungal potential was tested against species *Aspergillus niger* and *Aspergillus fumigates*.100µg/ml of the plant extract showed a zone of inhibition of about 10.5mm against Aspergillus niger and showed 9.9mm against species Aspergillus fumigates. Whereas the Antimycotic (Amphotericin) showed a zone of inhibition of about 12.5mm against *Aspergillus niger* and 10.5mm against *Aspergillus fumigates* shown in Table3.

The Antifungal activity of different extracts of various parts of the species *Piper longum* was investigated. The Antifungal activity of roots of *Piper longum* in chloroform extract showed a zone of inhibition of 9mm for *Aspergillus niger* and 7mm for *Aspergillus fumigates*. The zone of inhibition of chloroform extract of leaves of *Piper longum* showed 13mm for Aspergillus niger and 10mm for *Aspergillus fumigates*. And the chloroform extract of the stem of *Piper longum* showed 9mm for the species *Aspergillus niger* and 7mm for *Aspergillus fumigates* [15].

The antifungal activity of methanol extract of *Solanum virgianum*. It showed a zone of inhibition of 17mm against food poisoning fungi *Curvularia sp*. And zone of inhibition of 19mm against fungal species *Alternaria sp*[16].

Hence the synergistic effect of *Solanum surattense* and *Piper longum* showed greater potential toward the fungal species than the individual extract.

Therefore, from the above investigation of the synergistic effect of *Solanum surattense* and *Piper longum*, it is confirmed that the plant's extract was more effective against fungal species than the bacterial species and also it showed lesser difference with the Antimycotic (Amphotericin). Hence the combined form of plant extract can be used against various fungal diseases in a form of drugs.

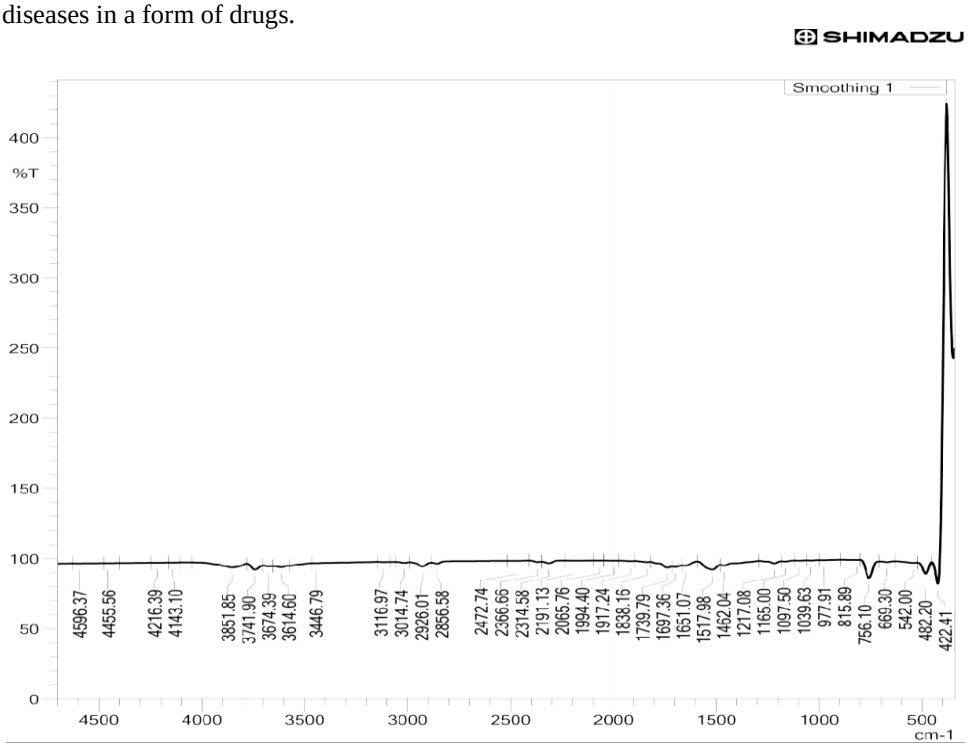

Fig 5: FTIR Spectroscopic Analysis of Functional Groups in the synergistic effect of *Solanum surattense* and *Piper longum.*

Table 4: Characteristic Infrared Absorption Frequencies

Bond	Compound type	Frequency range, cm- 1
O-H	Alcohol (free)	3674.39
O-H	Alcohol (intermolecular bonded)	3614.60
O-H	Alcohol (intermolecular bonding)	3446.79
O-H	Alcohol (intramolecular bonded).	3116.97
O-H	Alcohol (intramolecular bonded).	3014.74
N-H	Amine salts	2926.01
C-H	Alkane	2856.58
C≡C	Alkyne	2191.13
N=C=S	isothiocyanate	2065.76
C=C=C	Allene	1994.40
C=C=C	Allene	1917.24
C-H	Aromatic compound	1838.16
C=O	Esters	1739.79
C=O	Conjugated aldehyde	1697.36
N-H	Amine	1651.07
N-O	Nitro compound	1517.98
C-H	Alkane	1462.04
C-O	Alkyl aryl ether	1217.08
C-O	Esters	1165.00
C-N	Amine	1097.50
S=O	Sulfoxide	1039.63
C=C	Alkene	977.91
C-H	Phenyl Ring Substitution Bands	815.89
C-H	Phenyl Ring Substitution Bands	756.10
C-H	Phenyl Ring Substitution Bands	669.30
C-Br	Halo compound	542.00

CONCLUSION

Solanum surattense and *Piper longum* plant species are non-deadly and widely grown-up plants throughout tropical areas. It's safe for the preparation of herbal medicines likewise as synthetic drugs for the treatment of varied diseases. For preliminary phytochemical screening of the synergistic effect of *Solanum surattense* and *Piper longum* the plant extract was prepared in chloroform solvents and was found to contain resins, Flavonoids, carbohydrates, glycosides, and proteins. Hence, the above plant extract could be explored for its highest therapeutic efficacy by pharmaceutical companies to develop safe drugs for various ailments. Andtherefore, the plant extract was also tested for its antimicrobial potency. The Antibacterial potency was tested against *Staphylococcus aureus* and *E. coli*. The Anti-fungal potency was tested against *Aspergillus niger* and *Aspergillus fumigates*. Due to the intrinsic tolerance of microorganisms and also the difference in chemical nature and the structure of the constituents the test organisms showed a varying degree of sensitivity. Compared to the bacterial species the plant extract showed marked efficacy towards fungal species *Aspergillus niger* and *Aspergillus fumigates*. Interestingly, the results on the synergistic effects

within the well diffusion test also showed a direct correlation with the inhibitory effect, so the zone of inhibition within the combination test was greater than either alone test.

The results of the current study, which examined the inhibitory effects of the extracts and their active ingredients on bacterial species *S.aureus* and *E.coli* and fungal species *Aspergillus niger* and *Aspergillus fumigates* demonstrated that the synergistic effects of both combinations on the studied pathogen strengthened the antimicrobial effects on this pathogen. This suggests that, in the future, this combination could be used as a polyherbal antimycotic compound to control fungal infections and also it could be further studied for the treatment of Eosinophilia. We also examined the possibilities of synergistic effects of *Solanum surattense* and *Piper longum* with commercial antimicrobial agents such as Gentamicin and Amphotericin. They possess a synergistic activity with commercial antibiotics, which could have several beneficial effects for patients. This synergistic activity could enable the reduction of doses of commercial antibiotics, which successively would scale back their toxic effects. Furthermore, infection is often associated with oxidative stress. Therefore, these plants with their antioxidant properties may reduce the injuries associated with these infections.

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