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***Research Article** (Open Access)*

ANTIMICROBIAL, ANTIDIABETIC AND ANTICANCER ACTIVITY OF *PIPER BETEL* (BETEL LEAF)

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ABSTRACT
The present investigation was stretched to study the Phytochemical analysis, Antibacterial, Antifungal, Antidiabetic, Anticancer activities of methanol extract of *Piper betel* leaves, It is found to have a good source of phenolic compounds possessing antioxidant, ability to reduce blood sugar, protect against both gram positive- gram negative. The antimicrobial activity was evaluated by using agar well diffusion method. Agar disc diffusion method performed for antifungal activity, antidiabetic activity was carried out through as alpha-amylase inhibition assay and MTT assay for anticancer. The results of phytochemical screening showed the presence of bioactive components such as Carbohydrates, Tannins, Flavonoids, Phenol, Coumarins. The Antibacterial activity against *Escherichia coli* shows the zone of inhibition varying from 6.6 to 11.2 and with *Klebseilla sp* shows the zone of inhibition varying from 9.3 to 17.4 .Zone of inhibition recorded in *Klebseilla sp* was high when compared to *Escherichia coli* .The Antifungal activity reveals significant zone of inhibition against *Candida tropicalis* varying from 0.6 to 3.2 and *Trichoderma sp* varying from 9.3 to 17.4.Zone of inhibition recorded in *Trichoderma sp* was high when compared to *Candida tropicalis.* The Antidiabetic activity showed 20.81 in 1000µl concentration 5.19 in 200 µl. The result of anticancer activity with *Piper betel* working on PC3-Prostate cancer cell-line reveals that the cell viability increases in lower concentrations 3.4µg with 98.4% with high cell viability. At higher concentration 32.4% in 1000µg the viability of cell decreases. From this study it is evident that *Piper betel* has Antimicrobial, Antidiabetic, Anticancer activities with less side effects.

***Keywords*:** *Piper betel, Bacteria like Escherichia coli and Klebseilla sp, Fungi like Candida albicans and Trichoderma sp.*

INTRODUCTION
Infectious diseases are the major cause of death in the developing countries. An enormous number of microorganisms exists in living and its environment with uncontrolled and rapid growth of microbes can lead to some dangerous problems. The situation is worsening due to emerging drug resistance and hence, there is an urgent need to find new molecules to treat infectious diseases. Researchers finding a new method and solution by testing plants used to treat infectious diseases in traditional systems of healing, since medicinal plants have been a source of many pharmaceutical drugs for a range of diseases, including viral, bacterial, fungal, and protozoal infections, as well as for cancer (Mitali *et al.,* 2020). They are comparatively cheaper and safer than the modern formula medicines (Ammara *et al.,* 2009)

Medicinal plants have been playing a vital role throughout human history. Medicines from plant product are used by about 60% of the world population (Grover *et al.,* 2002) and still they are preferred by many people who can't afford pharmaceutical products for their physical and psychological requirements (Hasler and Blumberg 1999). Recently, a lot of attention focused on producing medicines in natural products. The biologically active compounds such as phytochemical contain naturally chemical compound from plants, which provide health advantages for humans further than those attributed to macronutrients and micronutrients (Suchada *et al.,* 2023). Plants containing beneficial phytochemical may supplement the needs of the human body by acting as natural antioxidants, minerals, vitamins A, C, E, and Phenolic compounds such as flavonoids, tannins, and lignins (Ivana *et al.,* 2006).

In recent years, plants are prospective source of antimicrobial agents in different countries (Usman *et al.,* 2013). About 60 to 90% of populations in the developing countries use crude plant extracts as herbal or ayurvedic medicine for the treatment of human infectious diseases (Ganesh *et al.,* 2013). In the present days popping up of antibiotic resistance and related toxicity issue limits the use of antimicrobial agents (Karen *et al.,* 2010) and is quick revival in research of the antimicrobial role of plants against resistant strains due to comparable safety and potency (Alviano and Alviano 2009).

The microbes are becoming more resistant everyday against the present medicines even that many microbes are multidrug resistant. So, these multidrug resistant microbes with several antibiotics influence an enormous threat on public health can causes several side effects can also cause several side effects (Hajipour *et al.*, 2013 and Jhon *et al.,* 2006).A wide variety of fungal genera causes mostly in fruits and vegetables related to quality problems, nutritional value, organoleptic characteristics, and limited shelf life (Agrios 2004) and some are aslant responsible for allergic or toxic disorders among consumers due to the production of mycotoxins or allergens.

Diabetes mellitus is a chronic metabolic disorder, dysfunction with disturbances of carbohydrate, fat and protein metabolism resulting from the defects in insulin secretion, insulin action, or both and failure of various organs in the body. Diabetes mellitus have the characteristic symptoms such as thirst, polyuria, blurred vision and loss of weight (Rakesh *et al.,* 2015). In the last decade researchers were conducted in India have highlighted that not only is the commonness of diabetes high but also that it is increasing rapidly in the urban population (Ramachandran *et al.,* 2002). The Indian adults with diabetes is estimated that approximately 33 million. This number would rapidly increase to 57.2 million by the year 2025. The World Health Organization's expert group on diabetes has advised greater research into traditional therapeutic herbs.

Cancer is a second leading causes of death worldwide public health burden in both developed and developing countries. Many modern therapies are undergone to reduce the multiple of cancer cells but the peoples are fear of side effects in chemotherapy as well other therapies and prefer to use of natural plant products for anticancer (Mahmoud *et al.,* 2022). In India as well in neighboring counties human beings have used the plants for medicinal purposes for centuries of the world (Said *et al.,* 2002). For these reasons, world health organization [WHO] supports the use of traditional medicines which are efficacious and nontoxic. To treat and prevent this fatal condition, there is a continuing need for novel therapeutics (Nighat *et al.,* 2022).

Piper betel (Heart-shaped leaves) is a woody, perennial and climbing vine belonging to Piperaceae Family are magnificent reservoirs of phenolic compounds with antiproliferative, antimutagenic, antibacterial, and antioxidant properties. Betel leaves are good source for cultural, medicinal and economical aspects having a high nutrient which prevents from ingestion, constipation, bronchitis, congestion, cough etc (Mitali, *et al.,* 2020) It contain a large number of biologically active compounds depending on the range of the plant, season, and climate(Satish AB, *et al.,* 2013) It is a rich source of various minerals as well as vitamins such as calcium, carotene, niacin riboflavin and thiamine. A natural phenylpropene known as chavicol, which is useful for killing germs. There are several betel leaf research projects where the purified chemicals and leaf extract fractions are to play a function in oral hygiene and to have immense features such as anti-diabetic, cardiovascular, anti-inflammatory, anti-ulcer, hepato-protective, anti-infective, antiplatelet, etc (Maira *et al.,* 2021) patents were also awarded for some biological activities like anti-inflammatory, anti-cancer, and immunomodulatory associated with the leaf extract and purified compounds. Many studies reported that the fatty acid in piper betel act as anionic surfactants which is selective against the structure and function of Gram-positive bacterial cell membranes (Arif and Diah, 2020).

With this background the study was conducted with the objectives of evaluation of the antimicrobial activity using agar well diffusion method, agar disc diffusion method for antifungal activity, antidiabetic activity by alpha-amylase inhibition assay and MTT assay for anticancer.

MATERIALS AND METHODS

Collection of the plant

Healthy *Piper betel* Leaves were collected from local Nursery located in Chennai and authenticated by the taxonomist Prof. P. Jayaraman [Certificate No. PARC/2019/4147]

Collection of bacterial and fungal isolates for antimicrobial activity

Clinical isolates bacteria, like *Escherichia coli* and *Klebseilla sp* and fungi like *Candida albicans and Trichoderma* where collected from a hospital. Samples were transported to the laboratory for further processing in an ice box.

Cell Line and Cell Culture for Anticancer Activity of Piper betel Leaves Extract

VERO cell lines (PC3) Prostate cancer cell line was obtained from the laboratory of NCCS Pune. The cells were maintained in Minimal Essential Medium supplemented with 10% Fetal Bovine Serum (FBS), Penicillin (100 µg /ml), and Streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml carbon-di oxide at 37°C.

Sample Preparation:

Fresh leaves of *Piper betel* plant were softly eroded in deionized water by which the dust particles were removed, dried under sunlight for seven days. By using mortar and pestle dried leaves were ground and sieved to get very fine powder. Take 100gms of powdered leaves and soaked in 500 ml of methanol, allowed to stand for overnight and filtered to obtain methanolic extract of betel leaves (Figure 1).

Figure 1: Preparation of Methanol Extract from Piper betel Filtered Methanol Extract

Phytochemical Screening of Piper betel Leaves Extract

First, a qualitative phytochemical investigation was done to identify a number of active components in the methanolic extract of piper betel leaves. The components of methanol extract of betel leaves were screened using standard methods. The components analyzed were carbohydrates, tannins, saponins, flavonoids, alkaloids, quinones, glycosides, cardiac-glycosides, terpenoids, phenols, coumarins, steroids and phytosteroids, phlobatannins and antraquinones

Antibacterial Activity

Stock cultures [*Escherichia coli and Klebseilla sp* were maintained at 4°C nutrient agar slant and incubated at 37˚C, for 24 hrs. The Agar Well diffusion method was utilized to conduct the antibacterial assay. 3.8gms of Muller Hinton Agar medium was dissolved in 100ml of distilled water and sterilized. After sterilization, the medium was poured in to sterile petriplates and were allowed to solidify for 1hr. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. Disc were prepared with 20 µl, 40 µl, 60 µl and 80 µl of *Piper betel* leaves extract, 20 µl of Tetracyclin was used as control for Antibacterial Activity respectively. These plates were incubated at 37ºC for 24 hrs. Then the bacterial growth was determined by measuring the diameter of zone of inhibition.

Antifungal activity

Antifungal activity of *Piper betel* leaves extract against fungal species, was carried out by Well diffusion method by 3.9 grams of potato dextrose agar medium was dissolved in 100ml of distilled water and add 1 gm of agar and sterilized. After sterilization, the medium was poured in to sterile petriplates and were allowed to solidify for 1hr. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension. Well were prepared with 20 µl, 40 µl, 60 µl 80 µl of *Piper betel* leaves extract, 20 µl Flucanazole was used as control for antifungal activity respectively, these plates were

incubated at 37ºC for 24 hrs. Then the fungal growth was determined by measuring the diameter of zone of inhibition.

Antidiabetic Activity

Alpha-Amylase Inhibition Assay: Yeast extraction is prepared by dissolving 10gms of yeast with 30ml of distilled water following with centrifugation at 4200rpm for 5mins until the supernatant appeared clear, supernatant is transferred to another test tube .The samples were added 1µl of glucose in all the concentrations following with concentrations of the samples (25, 50, 75, 100,200µl) along with the control incubated for 10mins at 37˚c to these concentrations 1µl of yeast extract is added and vortex for better dissolving and followed by incubation for incubation at 37˚c for 60mins, 3800rpm for 5mins and the supernatant is collected.

Anthrone inhibition method: Sample of required concentrations (25,50,75,100,200 µl) are taken in different test tubes and distilled water of 1ml as control followed by adding 2ml of anthrone reagent to all the test tubes and mixed thoroughly to observe the colour change to bluish-green and calculated the wavelength at 630nm

The Increase in Glucose uptake is calculated by the Formula:

$$\frac{\textit{Absorbance of control} - \textit{Absorbance of sample}}{\textit{Absorbance of control}}$$

Anticancer Activity of Piper betel Leaves Extract

Cells [20,000/well] were plated in 96-well plates and incubated in 37^0C with 5% CO_2 condition. After the cell reaches the confluence, the sample was added and incubated 37^0C for 24hrs. After incubation, the Sample (Piper betel) was taken out of the well and rinsed in MEM without serum or phosphate-buffered saline (pH 7.4). Three hours were spent incubating 100 l of 0.5% 3-[4,5-dimethyl-2-thiazolyl]-2,5—-tetrazolium bromide (MTT) in each well at a concentration of 5 mg/ml. After incubation, remove the MTT reagent and add 100 l of solubilization solution DMSO to each well. The plates were then wrapped in aluminum foil to protect them from light. Gently stirred with gyratory shaker for enhancing dissolution. The absorbance at 570nm and 630nm was measured with UV- Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition [IC50] The IC50 value was determined by using linear regression equation

Statistical analysis

The data obtained were subjected to SPSS package. Mean, Standard Deviation and ANOVA was carried out.

RESULTS

Phytochemical screening of Piper betel.

The results of the preliminary study of the phytochemical screening of *Piper betel* with Methanolic extract are given (Figure 2 and Table.1).

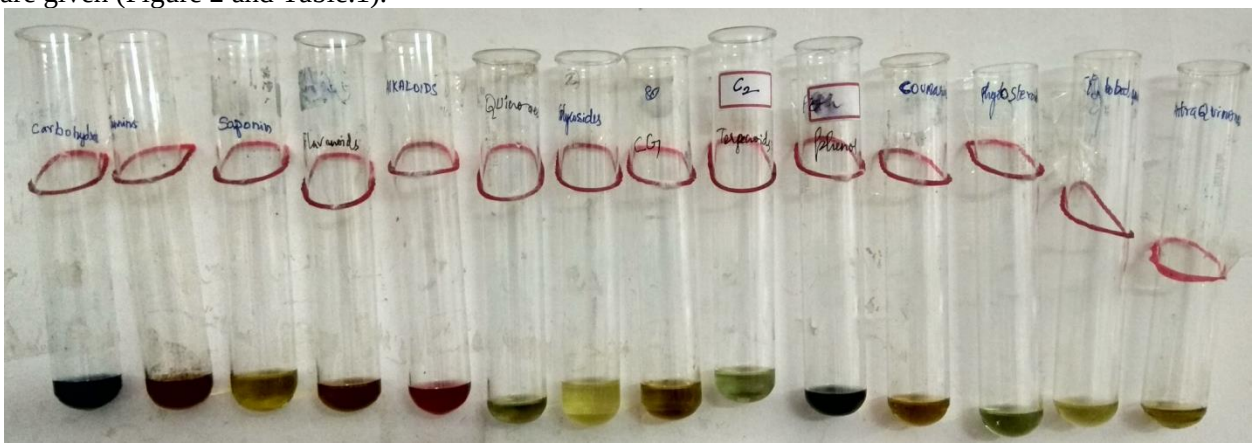

Figure 2: Quantitative Phytochemical Screening of Methanol Extract of *Piper betel*

Qualitative phytochemical screening of Piper betel.

Phytochemical analysis of piper betel with Methanolic extract showed the presence of carbohydrates, Tannins, flavonoids, phenol and Coumarins Since there was no significant colour changes in saponins, alkaloids, quinones, glycosidase, cardiac glycosidase, terpenoids, steroids and Phlobatannis and Anthroquinones indicates to be negative.

Table 1: Phytochemical screening of *Piper betel* leaves extract

S.No	Tests	Methanol Extract
1.	Carbohydrates	+
2.	Tannins	+
3.	Saponins	–
4.	Flavonoids	+
5.	Alkaloids	–
6.	Quinones	–
7.	Glycosides	–
8.	Cardiac glycosides	–
9.	Terpenoids	–
10.	Phenol	+
11.	Coumarins	+
12.	Steroids and phytosteroid	–
13.	Phlobatannis	–

Antibacterial activity of Piper betel

Antibacterial activity of piper betel with Methanolic extract against two bacterial isolates namely Gram-Negative *E. coli* and Gram Negative *Klebseilla sp* as revealed in (Plate1a, 1b, Table 2a, 2b, 2c and fig. 3a, 3b). The antibacterial activity of *Piper betel* showed lower concentration of inhibition towards *E. coli* (Plate. 1a, Table. 2a and 2b and Figure 3a) to be 6.7 in 20µl and higher concentration of inhibition of 17.4 in 80 µl. In *Klebseilla sp* (Plate1b, Table 2a, 2c and Figure 3b). The maximum zone of inhibition was found to be 17.4 in 80 µl and minimum zone of inhibition was found to be 9.3 in 20 µl. Statistically, In *Klebseilla sp* and *E. coli* the zone of inhibition at different concentration was significant. Statistically ANOVA shows significant with difference in *E. coli* and *Klebseilla sp* at higher concentration (80µl) the zone of inhibition is more when compared to the lower concentration values (20µl). Though the Methanolic extract of *Piper betel* shows significant inhibition effect against both the Bacterial species but slightly higher rate of inhibition was recorded in *Klebseilla sp* by Methanolic effect of *Piper betel* rather than *Escherichia coli.*

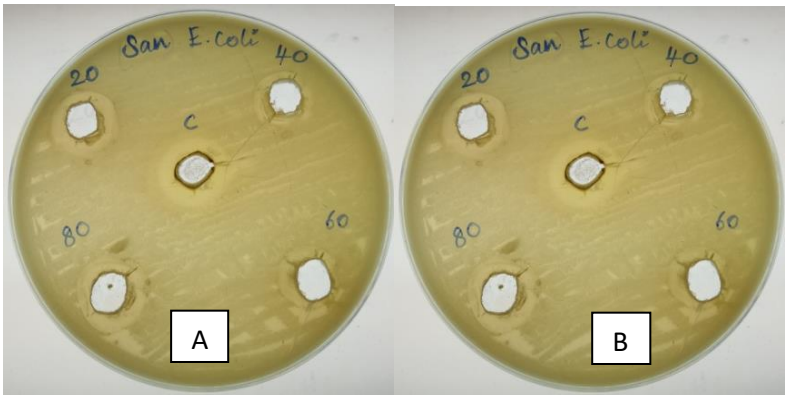

Plate 1: ***Antibacterial Activity of Piper betel Leaves against*** *A) E. coli B) Klebseilla sp*

Table 2a: Antibacterial activity of methanol extract of *Piper betel* against *E. coli and Klebseilla sp*

		Zone of inhibition (mm)		
Name of the Organisms	**Sample Concentration (µg/ml)**	**Sample Mean**	**Control (Tetracyclin)**	**S.D.**
	20	6.7		0.251
	40	7.8		0.152
	60	10.2		0.482
E. coli	80	11.2	6	0680
	20	9.3		0.351
	40	10.2		0.251
Klebsiella sp.	60	13.3		0.353
	80	17.4	7	0.513

Table 2b: ANOVA Antibacterial activity of *Piper betel* using *E. coli*

Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	38.09333	3	12.69778	46.45528	2.09E-05	4.066181
Within Groups	2.186667	8	0.273333			
Total	40.28	11				

Table 2c: ANOVA Antibacterial activity of *Piper betel* using *Klebseilla sp*

Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	120.0425	3	40.01417	279.1686	1.96E-08	4.066181
Within Groups	1.146667	8	0.143333			
Total	121.1892	11				

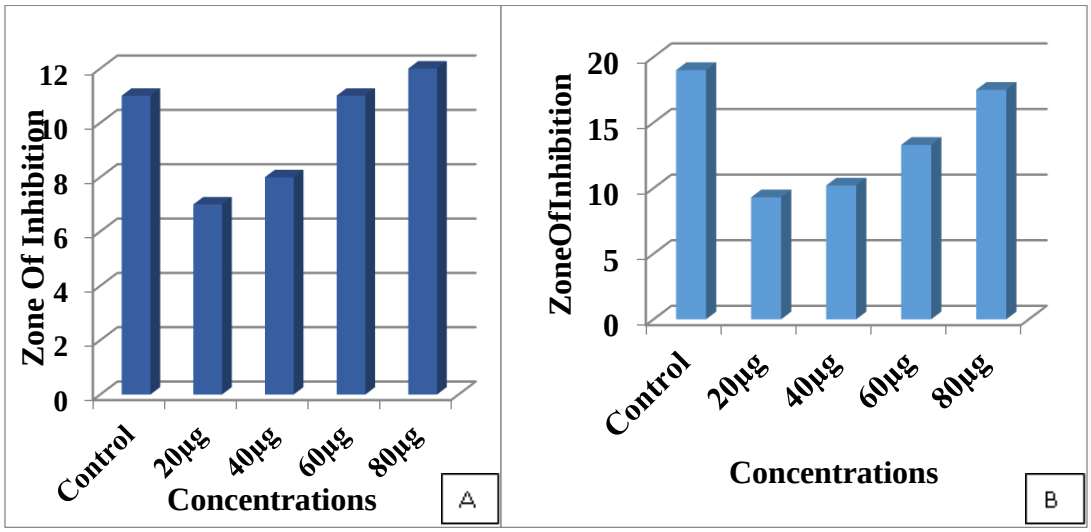

Figure 3: Antibacterial Activity of *Piper betel Leaves against* *A) E. coli B) Klebseilla sp*

Antifungal activity of *Piper betel*

Antifungal activity of *piper betel* with Methanolic extract against two fungal isolates namely *Candida tropicalis* and *Trichoderma* as revealed in (Plate1c,1d, Table 3a, 3b, 3c and Figure 3c, 3d). The antifungal activity of *Piper betel* showed lower concentration of inhibition towards *Candida tropicalis* (plate 1c, table 3a, 3b and figure 3c) to be 0.6 in 20µl and higher concentration of inhibition of 13.2 in 80 µl. In *Trichoderma* (Plate1d, Table 3a, 3c and Figure 3d). The maximum zone of inhibition was found to be 13.6 in 80 µl and minimum zone of inhibition was found to be 0.7 in 20 µl. Statistically, In *Candida tropicalis* and *Trichoderma* the zone of inhibition at different concentration was significant. Statistically ANOVA shows significant with difference in *Candida tropicalis* and *Trichoderma* at higher concentration (80µl) the zone of inhibition is more when compared to the lower concentration values (20µl). Though the Methanolic extract of *Piper betel* shows significant inhibition effect against both the Fungal species but slightly higher rate of inhibition was recorded in *Trichoderma* by Methanolic effect of Piper *betel* rather than *Candida tropicalis*.

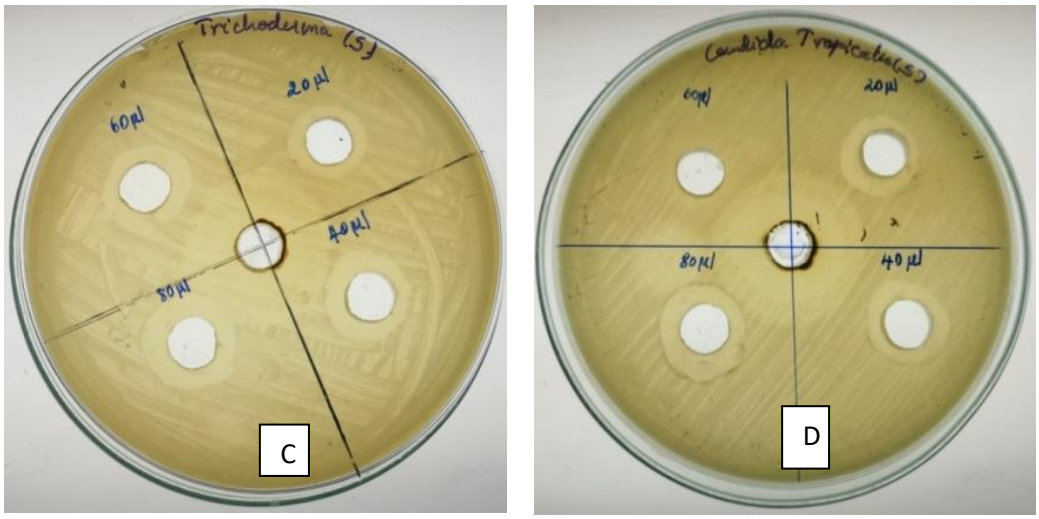

Plate 1: ***Antifungal activity of Piper betel leaves extract against*** C) *Candida tropicalis* D) *Trichoderma* sp.

Table 3a: Antifungal activity of *Piper betel* leaves extract against *Candida tropicalis* and *Trichoderma* sp.

		Zone of inhibition (mm)		
Name of the Organisms	**Sample Concentration (µg/ml)**	**Sample Mean**	**Control (Tetracyclin)**	**S.D**
Candida tropicalis	20	0.6	6	0.360
	40	1.7		0.404
	60	2.1		0.208
	80	3.2		0.264
Trichoderma	20	0.7	7	0.152
	40	2.0		0.264
	60	2.8		0.208
	80	13.66		0.305

Table 3b: ANOVA Antifungal activity of *Piper betel* extract using *Candida tropicalis*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	8.929167	3	2.976389	29.27596	0.005115	4.066181
Within Groups	0.813333	8	0.101667			
Total	9.7425	11				

Table 3c: ANOVA Antifungal activity of *Piper betel* extract using *Trichoderma* sp.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	7.749167	3	2.583056	44.92271	2.37E-05	4.066181
Within Groups	0.46	8	0.0575			
Total	8.209167	11				

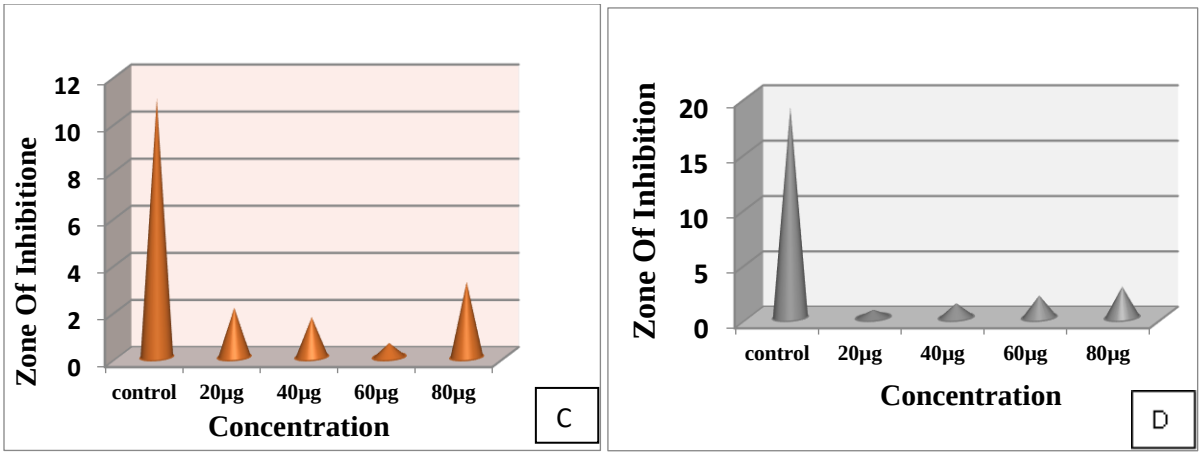

Figure 3: ***Antifungal activity of Piper betel Leaves Extract against*** C) *Candida tropicali* D) *Trichoderma*

Antidiabetic activity of Piper betel

The result of Antidiabetic activity with Methanolic extract with Alpha –Amylase Inhibition Assay revealed through showing higher concentration of 90.45 in 1000µl and lowest concentration of 70.39 in 200 µl with positive control and with the Methanolic extract of *Piper betel* shows the highest concentration of 20.81 in 1000 µl and lowest concentration of 5.19 in 200 µl. The concentration of the positive sample is higher when compared to Methanolic plant extract is due the purity of the samples and the change of colour to blue significantly proves the presence of Antidiabetic activity in Piper *betel* through Anthrone inhibition [Figure 4a, 4b and Table. 4]

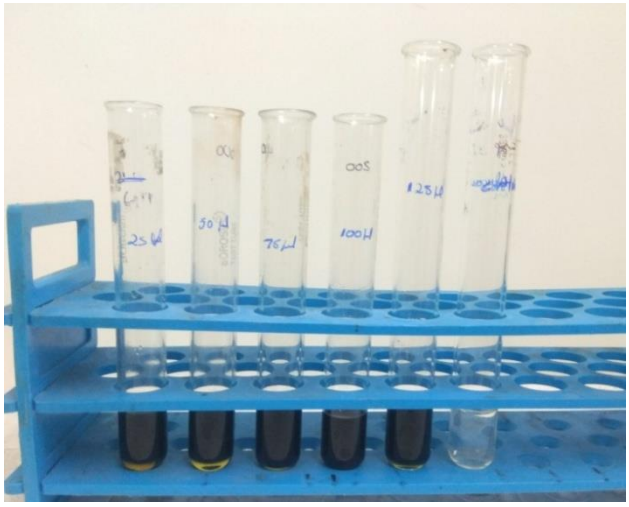

Figure 4a: Antidiabetic Activity of *Piper betel*

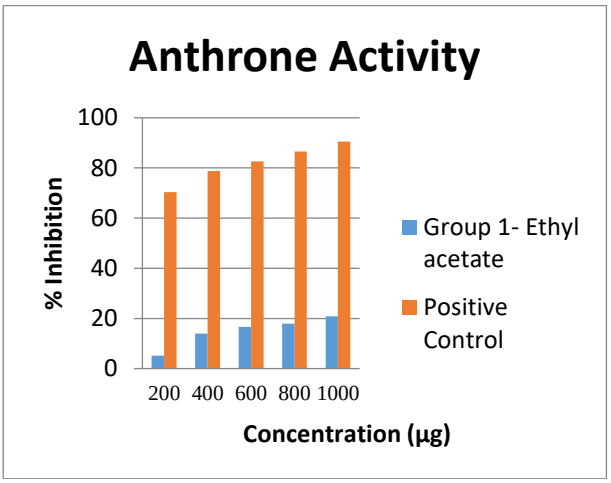

Figure 4b: Antidiabetic Activity of *Piper betel* extract

Table 4: Antidiabetic activity of *Piper betel* extract

Concentration in µg	Sample	Positive Control
200 µg	5.19	70.39
400 µg	13.95	78.78
600 µg	16.68	82.62
800 µg	17.93	86.56
1000 µg	20.81	90.45

Anticancer activity of Piper betel

The anticancer activity reveals the result that *Piper betel* with Methanolic extract showed good anticancer activity which has IC_{50} at 692 µg. The maximum cell viability was observed in 3.4 µg with 98.4. Concentration of the sample was followed by a decrease in 1000 µg with 32.4. Through this result it has been

proved that the extract shows high percentage of cell viability in lower concentration and has good toxic potential against PC3-Prostate cancer cell line based on its dosage of the drug [Figure 5a, 5b, 5c and Table 5]

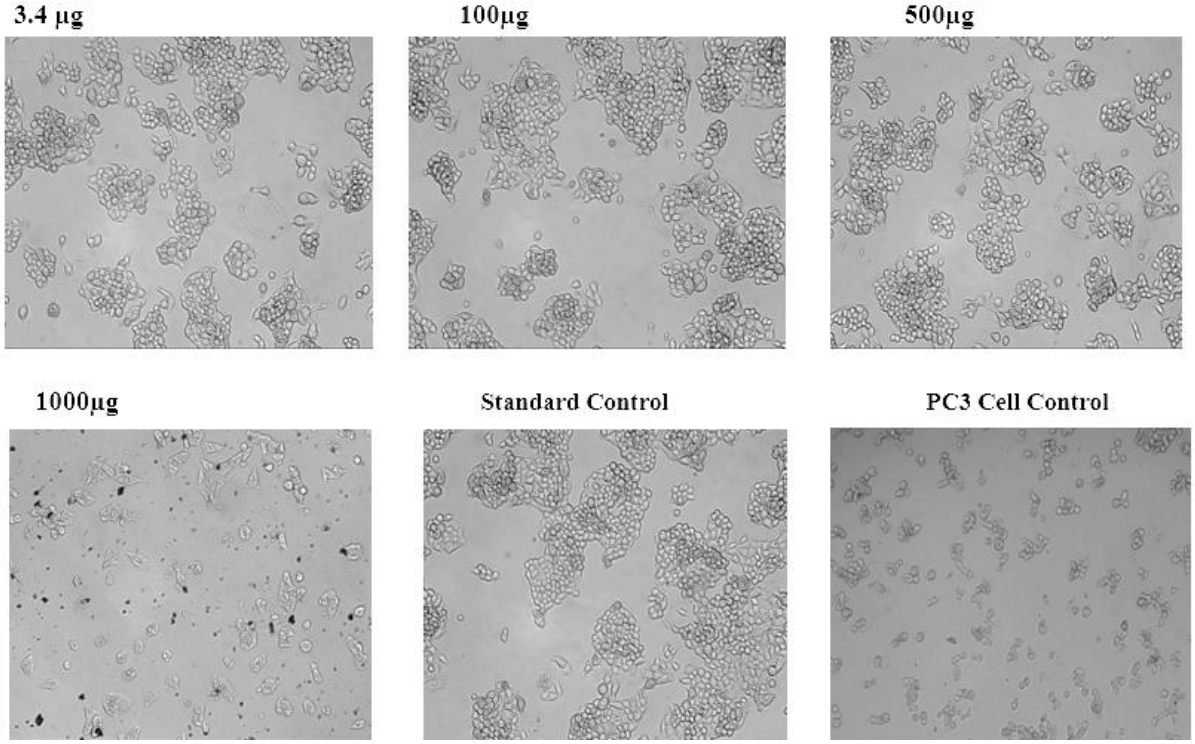

Figure 5a: Anticancer activity of *Piper betel* leaves extract on normal *VERO* cell lines

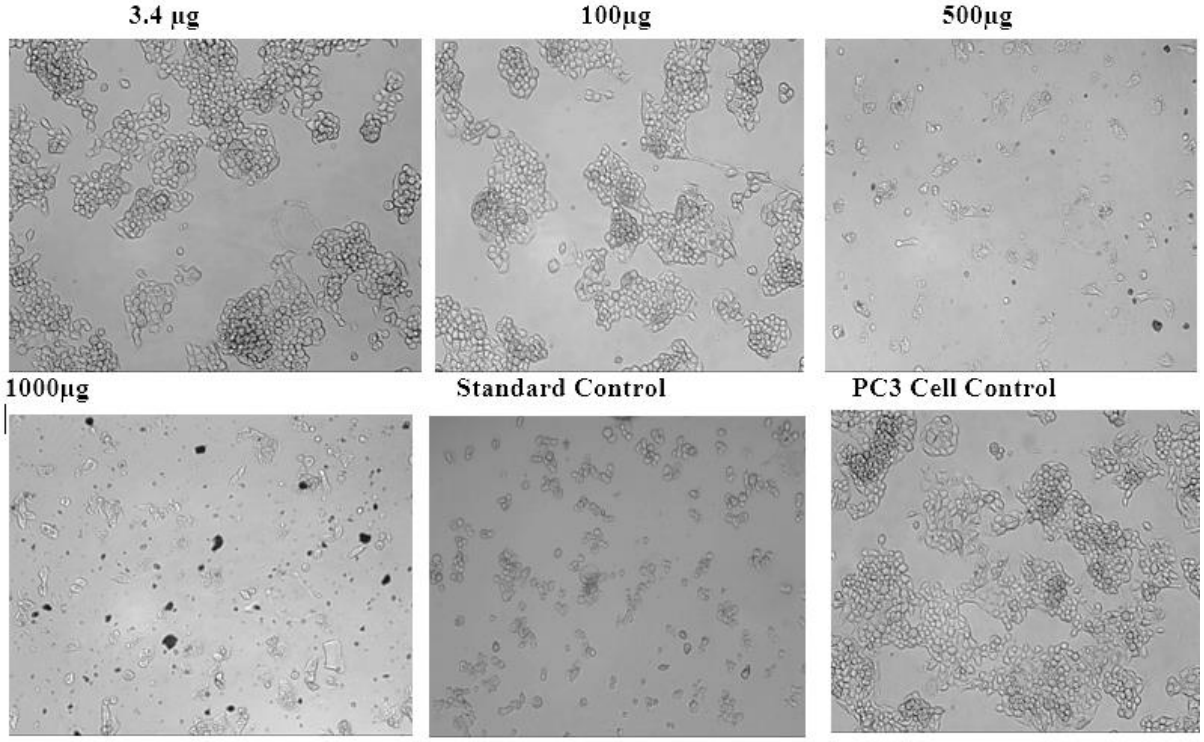

Figure 5b: Anticancer activity of *Piper betel* leaves extract on *PC3-Prostate* cell lines

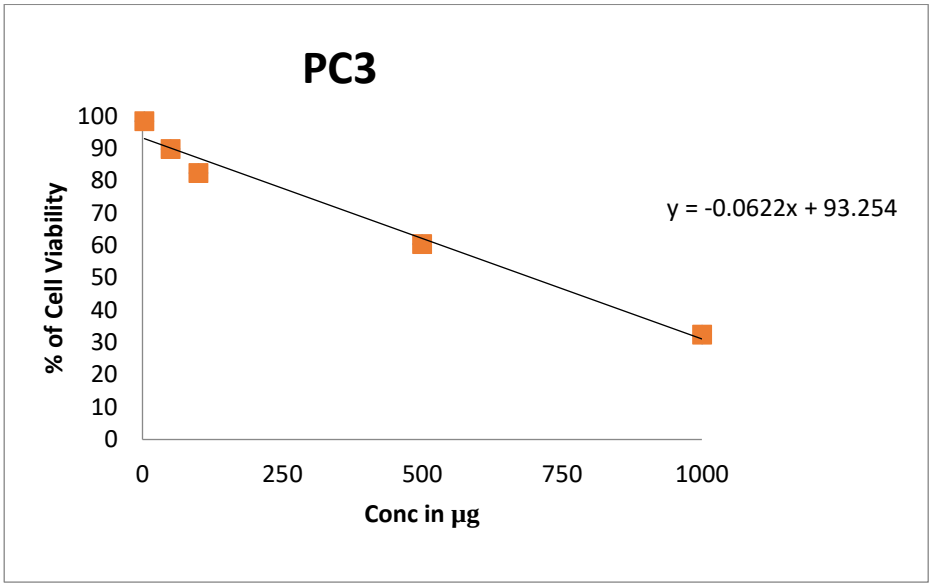

Figure 5c: Anticancer activity of *Piper betel* leaves extract on PC3-Prostrate cancer cell lines

Table 5: Anticancer activity of *Piper betel* leaves extract on PC3-Prostrate Cancer cell lines

Concentration Unit: µg		**Incubation: 24hrs**						
	BLANK	**UNTREATED**	**STD**	**3.4**	**50**	**100**	**500**	**1000**
Sample	0.05	0.868	0.4685	0.855	0.785	0.724	0.544	0.3155
Mean		0.818	0.4185	0.805	0.735	0.674	0.494	0.2655
Standard Deviation		0.004242641	0.000707107	0.002828427	0.005656854	0.001414214	0.0028	0.0021
Standard Error		0.003000453	0.000500076	0.002000302	0.004000604	0.001000151	0.002	0.0015
Viability %		100	51.16136919	98.41075795	89.85330073	82.39608802	60.3912	32.45721

DISCUSSION

Most plants have been employed as medicines for various diseases since the dawn of human history, and these plants form the foundation of contemporary medicine. Higher and aromatic plants have long been employed in folk medicine and for preserving food due to their ability to suppress the growth of bacteria, fungus, and yeast (Adilson *et al.*, 2004). Hence, usually bioactive substances derived from natural sources are always of significant interest to researchers studying infectious diseases.

Piper betel has been characterized throughout history as a fragrant stimulant, carminative, aphrodisiac, and astringent. Scientific studies have shown that this plant's leaves contain a variety of advantageous bioactive, and an extract made from betel leaves has a lot of promise for application in the creation of commercial products because of its various advantages.

The qualitative phytochemical screening of *piper betel* with Methanolic extract shows the result with the presence of Carbohydrates, Tannins, Flavonoids, Phenol and Coumarins and the Absence of Saponins,

Alkaloids, Quinones, Glycoside, Cardiac- glycosides, Terpenoids, Steroids and Phlobatannis and Anthraquinones. Flavonoids possess antidiabetic activity for the treatment of type-2 patients (Vessal *et al.*, 2003). Phenolic compounds possess natural antioxidants, antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, anti-inflammatory and immunomodulatory properties. Plant based phenolic compounds are one of the vital phytochemicals with a positive response to several disease preventions (Thangaiyan and Anupam, 2009). Quinones show purgative, antimicrobial and antiparasitic, anti-tumor and anti-cardiovascular disease. Triterpenoids show particular promise as anti-cancer agents (Sharif *et al.*, 2020)

The developing countries uses plant derived medicine traditionally for curing antimicrobial infections and used as crude plant extracts for their treatment (Sebastian *et al.*, 2009).Antibiotic resistance is one of the huge problem of public health and the rise of multi-drug resistant organisms also causes a challenge in the treatment of infective diseases (Donadu *et al.*, 2019) .Hence the present investigation was attempted to study of antibacterial efficiency against bacterial pathogenic isolates, Gram negative *Escherichia coli* and Gram negative *Klebseilla sp* with well diffusion method revealed the least zone of inhibition towards *E.coli* to be 6.7 in 20µl and maximum zone of inhibition to be 17.4 in 80µl. In *Klebseilla sp* the maximum zone of inhibition was found to be 17.4 in 80µl and minimum zone of inhibition was found to be9.3 in 20µl. Though *piper betel* shows significant inhibition against both bacterial species but there was slightly high rate of inhibition was shown in *Klebseilla sp* than that of E. *coli*. It is often reported that Gram positive bacteria are more sensitive than Gram negative bacteria to plant extracts (Pattanathu *et al.*, 2009). But in our study, both gram negative bacteria were found to be sensitive to *Piper betel* extracts. In present study, *Klebsiella sp* was found to be most sensitive while *E. coli* was found to be least sensitive to plant organic extract than other organisms. The findings agree with that of other workers (Jose *et al.*, 2019).

Antifungal activity showed least zone of inhibition towards *Candida tropicalis* to be 0.6 in 20 µl and maximum zone of inhibition was found to be in 3.2 in80 µl. In *Trichoderma* the maximum zone of inhibition was found to be 2.8 in 80µl and maximum zone of inhibition was found to be 0.7 in 20µl. Though *piper betel* shows significant inhibition against both fungal species but there was slightly high rate of inhibition was shown in *Trichoderma* than that of *Candida tropicalis*

In the present investigation, we present the antibacterial activity and antifungal activity of *piper betel* leaves shows effective rate of inhibition bacteria and fungus growth. The study aimed to evaluate activity of *piper betel* leaves extract against the bacteria and fungi of clinical relevance. *Escherichia coli, Salmonella paratyphii, lactobacillus* and *Staphylococcus aureus, Proteus vulgaris, Kebsiella pneumoniae, Saccharomyces cerevisiae* known to be causes of antibiotic-resistant infections. We performed the agar well diffusion method and indicated the bacteriostatic or bactericidal action and fungistatic or fungicidal action. We suggest that extracts derived From *Piper betel* leaves might be the effective source of antibacterial compounds and the promising alternative to antibiotic therapy (Sunil *et al.*, 2016).

Further observation of *piper betel* extract with antidiabetic activity proved that diabetics is a clinical condition characterized by hyperglycemia in which an elevated amount of glucose circulates in the blood plasma. In recent papers proved that in streptozotocin-induced diabetic rat model study, a significant reduction in blood glucose level was observed when treated with orally administered betel leaf suspension for thirty days and was observed that reduction of glycosylated hemoglobin (Arnab and Proshanta 2021). Alpha amylase and alpha glucosidase inhibitors are used to achieve greater control of type 2-diabetics.the present study intends to screen alpha amylase inhibitors with natural source like plants in order to minimize the toxicity and side effects of the inhibitors. The alpha amylase inhibition assay showed that methanolic activity of piper betel was found to be 90.45 in 1000µl at higher concentration. At lower concentration was found to be 70.39 in 200µl.with positive control. The sample of *Piper betel* extract showed 20.81 in 1000 µl concentration and 5.19 in 200 µl was recorded.

The study as further extended to anticancer activity of *piper betel*. Demographic studies have reported variations in prostate cancer incidences all over the world. Prostate cancer is one of the deadliest diseases, its mortality rates are high in western countries when compared to Asian countries (Parkin DM 2005). Betel leaves are traditionally used as a mouth freshener in India and China by nearly 600 million people in Asia,

leaves of piper betel are a focus of research due to their wide usage of medicinal properties (Kumar N, *et al.,* 2010). Our current study in prostate cancer proved by MTT assay for the investigation of anticancer activity of piper betel leaves extract with PC3 (Prostate Cancer) cell lines. The anticancer activity reveals that Piper betel with Methanolic extract showed good anticancer activity which has IC_{50} at 692 µg. The maximum cell viability was observed in 98.4 at 3.4 µg of the sample and minimum viability of 32.4 was recorded at 1000 µg.

The results obtained from the present study showed the plant extract of Piper betel have the Ability to inhibit the growth of various microorganisms using Bacteria (*E.coli* and *Klebseilla sp*) and fungus (*Candida tropicalis* and *Trichoderma*) *Piper betel* extract also acts as a therapeutic agent with great interest in traditional methods thus the present investigation reveals that *Piper betel* extract has more medicinal applications especially for Antidiabetic and Anticancer activities. In the treatment of diabetics *Piper betel* has specific mode of action where it contains flavonoids, tannins, Coumarins etc. which are implicated for diabetes effects. The research for diabetes will continue all over the world as the disease poses many challenges not only to the physicians but also to the researchers. *Piper betel* shows promising results in the concentrations of the compounds to have the capacity to kill 50% of viable cells against the PC3 cells at 692 µg/ml after 24 hours of incubation at 37˚C, thus the observation strongly suggests that the *Piper betel* extract have good toxic potential against the PC3 cells based on the dosage of the drug . This encouraging result provide useful information for designing a better compound using Piper betel with minimum side effects. Piper betel can be implemented as a dose for Antidiabetic and as chemotherapeutic agent in anticancer activity.

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