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ANTIBACTERIAL POTENTIAL AND PHYTOCHEMICAL ANALYSIS OF PIPER BETEL AND TINOSPORA CORDIFOLIA

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ABSTRACT

The unimpeded use of antibiotics even over a small infections, progresses the development of superbugs and this started with one of the broad spectrum beta lactam antibiotics ampicillin many years ago. Small skin infections need to be treated with traditional Indian herbal therapy which will lead a better way to stop the emergence of antibiotic resistant bacteria. The present study focuses on the antibacterial effect of two herbal extracts of leaves of *Piper betel* and *Tinospora cordifolia* and their phytochemical analysis. The water and ethanol extracts of *Piper betel* and *Tinospora cordifolia* were prepared to assess the activity against pathogenic bacteria isolated from otitis externa. The broad spectrum antibiotic imipenem was taken as control. Both water and ethanolic extracts of *Piper betel* exhibited good activity against *Klebsiella pneumoniae* strain CIFRI-OBBKP2, *Staphylococcus simiae* CCM 7213, *Bacillus subtilis* strain NCDO 1769 and *Acinetobacter pittii* DSM 21653 strain ATCC 19004 as compared to *Tinospora cordifolia.*

KEYWORDS: Piper betel, Tinospora cordifolia, Antimicrobial, Phytochemical.

INTRODUCTION

Medicinal plants have great importance in ancient Ayurveda. As the pathogens of various diseases exhibited resistivity against potent antimicrobial agents like antibiotics, the value of medicinal plants increases.[1] The *Piper betel* is an evergreen perennial plant belong to the family Piperaceae and also called as Golden Heart of the Nature.[2] The utilizable parts of *Piper betel* are leaves, roots, stems, stalks and fruits. The leaves of *Piper betel* exhibited anticancer, anti-diabetic, antioxidant, gastro-protective, antifungal, anti-filarial, anti-allergic and anti-hemolytic activities.[3–7] *Piper betel* leaves contains various phytochemicals like alkaloids, flavonoids, glycosides, tannins, saponins and some reducing sugars.[8] It contains copious bioactive molecules (Hydroxy Chavicol, Chavicol, Eugenol) which enhances the antibacterial activity.[9]

Tinospora cordifolia belong to family Menispermaceae, geographically distributed in Shrilanka, Bangladesh, China and all over India.[10] It is perennial, climbing shrub with fleshy stem and utilized widely among all medicinal plants. It is identified by various local names like (Gurcha (Hindi), Garo, Galac (Gujarat), Thippateega (Telugu), Amrutavalli (Kannada), Amrita, Gilo (Kashmiri), Chittamrutu (Malayalam), Gulvel (Marathi), Guluchi (Oriya), Gilo (Punjabi), Seendal, Seendil Kodi (Tamil), Siddhilata and Amarlata (Assamese) in India.[11] It purifies blood and also acts as anti-infectious agent. Several diseases like Jaundice, rheumatoid arthritis, diabetes, gout, viral hepatitis general weakness can be treated with *Tinospora.*[12] *Tinospora cordifolia* manifests anti-cancer activity, anti-toxin, anti-diabetic, immunomodulatory, anti-oxidant activity, antimicrobial and anti-osteoporotic activity.[12–16]

Present study evaluated the antibacterial potential of ethanolic and aqueous extracts of two medicinal plants, *Piper betel* and *Tinospora cordifolia* against pathogenic bacteria *Klebsiella pneumoniae* strain CIFRI-OBBKP2, *Staphylococcus simiae* CCM 7213, *Bacillus subtilis* strain NCDO 1769 and *Acinetobacter pittii* DSM 21653 strain ATCC 19004 of otitis externa (external ear infection).[17]

MATERIALS AND METHODS

The study was conducted in the Research Laboratory, Department of Microbiology, Shri Shivaji College, Akola (Maharashtra), India. The antibacterial effect of dried leaves extract of *Piper betel* and *Tinospora cordifolia* was evaluated against pathogenic bacteria isolated from external ear infection.

Collection and processing

The *Piper betel* leaves were purchased from local market and the *Tinospora leaves* were collected from College garden. Both the leaves were shed dried for eight days and the powdered form of leaves was used for the preparation of extract. Soxhlet apparatus was used for the extraction procedure. The extract was prepared in 75% ethanol and water as a solvent. After extraction the excess ethanol from the extract allowed to evaporate at the room temperature while for the evaporation of water, the extract was kept in hot air oven at 55^0C for 2 days and the remains was used for evaluation of antibacterial activity and phytochemical analysis.

Phytochemicals

For phytochemical analysis ethanolic extracts of *Piper betel* and *Tinospora cordifolia* were used.

Alkaloids: 2ml extract is taken and 2ml of Wagner's reagent was added in it, development of brownish precipitate indicates the presence of alkaloids.

Glycosides: The extract is hydrolysed with HCL solution and neutralised with NaOH solution. A few drops of Fehlings solution A&B are added red precipitate indicates the presence of glycosides.[1]

Flavonoids: 2 ml of 10%lead acetate added in 2ml of extract. Yellowish green colour indicates the presence of flavonoids.

Saponins: 2ml of extract is dissolved with 2ml of Benedict's reagent. Blue black precipitate indicates the presence of saponins.

Tanins: 2ml of extract is treated with 0.1% of ferric chloride. Brownish green indicates the presence of tannins.

Reducing sugars: The extract is shaken with distilled water and filtered. The filtrate is boiled with drops of Fehling's solution A&B for few minutes. An orange red precipitate indicates the presence of reducing sugars.

Antibacterial activity

The antibacterial activity of both aqueous and ethanol extracts of *Piper betel* and *Tinospora cordifolia* were tested using agar well diffusion method. An Imipenem antibiotic was taken as control. The pathogenic bacteria *Klebsiella pneumoniae* strain CIFRI-OBBKP2, *Staphylococcus simiae* CCM 7213, *Bacillus subtilis* strain NCDO 1769 and *Acinetobacter pittii* DSM 21653 strain ATCC 19004 isolated from external infected ears (otitis externa)[17] were inoculated in to sterilized nutrient broth and incubated at 37^0**C** for several hours unless 0.5 MacFarland turbidity (CLSI guidelines) is not achieved. The bacterial lawn was prepared on plates containing Muller Hinton agar and further the 0.8mm wells were prepared using cork borer. 40µl of (10mg/ml) each extracts were added into the wells with the help of micropipette and plates were incubated at 37^0**C** for 24hours to see the results. The zone of inhibition was measured

RESULTS AND DISCUSSION

Phytochemical analysis

Phytochemicals were assessed using ethanol and aqueous extracts of both *Piper betel* and *Tinospora cordifolia* leaves. Alkaloids, tannins and reducing sugars showed their presence in both aqueous and ethanol extracts of *Piper betel* leaves (Table 1). Alkaloids, flavonoids, saponins, tannins and reducing sugars showed their presence in both aqueous and ethanol extracts of *Tinospora cordifolia* leaves (Table 2). Alkaloids, tannins, reducing sugars and phenolics were found to be present in ethanol extract of *Piper betel* leaves while aqueous extract was found to be positive for alkaloids, tannins, glycosides, reducing sugars and saponins.[7] An ethanol, methanol, chloroform and aqueous extracts of *Tinospora cordifolia* were found to be positive for alkaloids, saponins, terpenoids whereas flavonoids were found to be present in ethanol, methanol and aqueous extract only.[19] An ethanol extract of *Piper betel* leaves were showed the presence of carbohydrates, proteins, phenolics, flavonoids and antioxidants.[4]

Table 1: Phytochemical analysis of *Piper betel* leaf extracts.

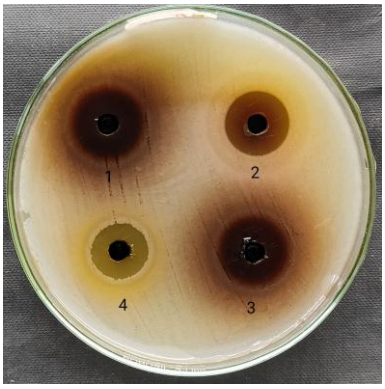
Sr. No.	Phytochemical compounds	Aqueous extract	Ethanol extract
1.	Alkaloids	+	+
2.	Glycosides	+	_
3.	Flavonoids	_	_
4.	Saponins	+	_
5.	Tannins	+	+
6.	Reducing sugars	+	+
Key: + :Present, -: Absent			

Table 2: Phytochemical analysis of *Tinospora cordifolia leaf extracts.*

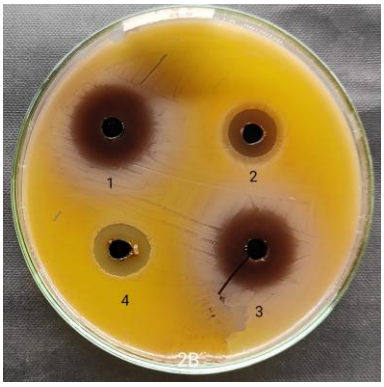
Sr. No.	Phytochemical compounds	Aqueous extract	Ethanol extract
1.	Alkaloids	+	+
2.	Glycosides	+	_
3.	Flavonoids	+	+
4.	Saponins	+	+
5.	Tannins	+	+
6.	Reducing sugars	+	+
Key: + :Present, -: Absent			

Antibacterial activity

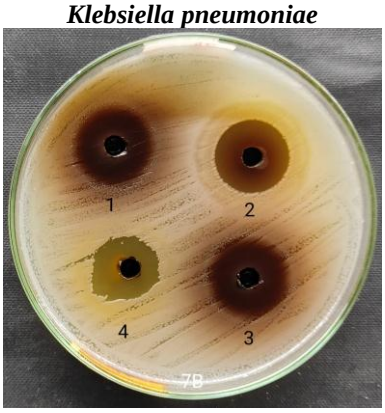
The antibacterial activity of herbal extracts were assessed and the results revealed that both ethanol and aqueous extracts of *Piper betel* and *Tinospora cordifolia* showed good antibacterial activity against test pathogenic bacteria (Table 3 and Figure 1). The maximum zone of inhibition observed against *Acinetobacter pittii* while minimum zone of inhibition observed against *Klebsiella pneumonia*. As compared to *Tinospora cordifolia*, *Piper betel* exhibited good antibacterial activity. The maximum zone of inhibition (29mm) observed against *Acinetobacter pittii* while ethanol and aqueous extracts of *Tinospora cordifolia* evinced minimum zone of inhibition (16mm) against *Staphylococcus simiae.*



Klebsiella pneumoniae



Staphylococcus simiae



Bacillus subtilis

Acinetobacter pittii

Figure 1: Muller Hinton agar plates showing antibacterial activity of *Piper betel* and *Tinospora cordifolia* leaves extracts. (Well number 1 and 3 represents antibacterial activity of aqueous and ethanol extract of *Piper betel* while 2 and 4 represents antibacterial activity of aqueous and ethanol extract of *Tinospora cordifolia* respectively.)

Table 3: Antibacterial activity of *Piper betel* and *Tinospora cordifolia* leaves extracts.

Herbal extracts	Solvent	Pathogenic bacteria and zone of inhibition (mm)			
		Klebsiella pneumoniae	*Staphylococcus simiae*	*Bacillus subtilis*	*Acinetobacter pittii*
Piper betel	Ethanol	21	22	22	28
	Aqueous	20	22	22	29
Tinospora cordifolia	Ethanol	18	16	21	29
	Aqueous	17	16	20	29
Imipenem		23	23	20	28

The antibacterial activity of two different concentrations (50µg/ml and 100 µg/ml) of aqueous and ethanol extracts of *Piper betel* leaves evaluated against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa.* Both the extracts showed significant zone of inhibition against tested pathogenic organisms.[7]

In one study, an ethanol, methanol and aqueous extracts of *Tinospora cordifolia* were tested against Gram negative pathogenic bacteria *Escherichia coli.* Four different concentrations (0mg/ml, 25mg/ml, 50mg/ml and 100mg/ml) of all three extracts were prepared for evaluation of antibacterial activity by using slip disc method. Both ethanol and methanol extracts showed good zone of inhibition at concentrations 50mg/ml and 25mg/ml respectively.[20]

Against seven pathogenic bacterial strains, antimicrobial activity of *Tinospora cordifolia* and *Cassia fistula* were evaluated. Antimicrobial activity of aqueous, methanol, chloroform, acetone and ether was assessed against *Klebsiella pneumoniae* (ATCC 15380), *Escherichia coli* (ATCC 25922), *Micrococcus luteus* (ATCC 9341), *Streptococcus pneumoniae* (ATCC 12755), *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778) and *Lactobacilus acidophilus* (ATCC 53103). Aqueous extracts of both *Tinospora cordifolia* and *Cassia fistula* showed prominent zone of inhibition as compared to all other solvents.[12]

CONCLUSION

From present work it can be concluded that herbal extract contains potent phytochemicals having dynamic antimicrobial activity. Further formulations prepared from purified herbal antibacterial compounds for skin infections, may minimizes the overuse of oral and local antibiotics. Pharmodynamics and pharmacokinetic study of both two herbal formulations may lead to development of cost effective new herbal therapy which will treat skin infections.

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