



Original Article

Phytochemical Screening and Antibacterial Activity of *Chloroxylon swietenia* DC.

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Objective: *Chloroxylon swietenia* traditionally used as antimicrobial, antifertility, analgesic, insecticidal, antifeedant in traditional medicine system hence the present work is conducted to evaluate antibacterial activity. **Experimental Approach:** Qualitative and quantitative phytochemical screening and antibacterial properties of *Chloroxylon swietenia* leaves, stem bark and seeds extract isolated using the solvents viz: pet ether, chloroform and ethanol by agar well plate method and MIC by resazurin dye method. **Findings:** The tree *Chloroxylon swietenia* possess alkaloids, terpenoids, tannins and phenolic compounds, and cardiac glycosides. The quantitative total phenolic content found at 22µg/mg in seed ethanolic extract, total alkaloids 17.32µg/mg in stem bark ethanol extract and total terpenoids 47.332µg/mg in leaf pet ether extract. The plant extracts showed appreciable antibacterial activity at a concentration of 80mg/mL against selected bacterial strains. Seed chloroform extract presented 17.67±0.33mm inhibition zone and MIC at 20.83µg against *S aureus*. Seed pet ether extract showed 16.67±0.33mm inhibition zone and MIC at 33.33 µg against *K. pneumonia*, while as, stem bark chloroform extract has shown 12.33±0.33mm inhibition zone and MIC at 133.33µg against *V. cholerae*, seed pet ether extract revealed 19.67±0.33 mm inhibition zone and MIC at 20.83µg towards *S. pneumonia*, *P. aeruginosa*, seed ethanol extract effect was valued at 11.67±0.33mm inhibition zone and MIC at 133.33 µg on its action to *S. typhi*. **Discussion:** The plant *Chloroxylon swietenia* containing beneficial phytochemical profile, among all extracts seed pet ether and chloroform extracts showed good antibacterial activity comparatively with other extracts and displayed prominent results on comparison with standard streptomycin. **Conclusion:** The results concluded that *Chloroxylon swietenia* is possessing important secondary metabolites that have a significant antibacterial property.

Keywords: *Chloroxylon Swietenia*, Phytochemical, Agar Well Plate, Antibacterial.

1. INTRODUCTION

All over the world people preparing herbal medicine using several hundred genera of medicinal plants producing potential drugs for the benefit of primary health care since thousands of years. The plants produce bioactive secondary metabolites like alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, glycosides that hold various pharmacological properties¹. WHO reports that 80% of world population using traditional medicine which are being prepared from plant extracts. Microorganisms create huge

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clinical problems due to indiscriminate use of antibiotics and have become resistant to antibiotics. Antibiotics also create harmful effect on host such as allergic reaction, hypersensitivity, weakening of gut etc. This leads to search alternative medicine for the treatment of microbial diseases. Analysis of medicinal plants for antimicrobial drugs is one of such approaches².

Plant medicines have often not been tested systematically, but have come into use informally over the centuries. By 2007, clinical trials had demonstrated potentially useful activity in nearly 16% of herbal medicines; there was limited *in vitro* or *in vivo* evidence for roughly half the medicines; there was only phytochemical evidence for around 20%; 0.5% were allergenic or toxic; and some 12% not yet been studied scientifically³.

Chloroxylon swietenia DC (East Indian satinwood) belongs to family Rutaceae commonly known as Cylonsatin wood or East Indian satinwood native to South India and Srilanka. In India this plant is commonly found in dry deciduous forests, tree is having pinnate leaves, straight cylindrical stem⁴. It is commonly used as antimicrobial, antifertility, analgesic, insecticidal, antifeedant activities. Leaves are used to treat worm infected wounds, fungal infected skin and also to treat inflammation, rheumatism. The plant root and bark possess astringent property⁵. On the basis of available literature, the leaves and bark essential oils having the potential inhibiting microbial activities⁶. The leaves, stem bark and seeds using the solvent petroleum ether, chloroform and ethanol have not been exposed yet and need to be evaluated which may yield a novel phytoconstituent for the prevention of infections caused by clinical pathogens. The present study was conducted on preliminary phytochemical analysis and the antibacterial potential of *Chloroxylon swietenia* DC.

2. MATERIALS AND METHODS

Plant Material Collection and Extraction

The leaves and stem bark of *Chloroxylon swietenia* were collected in the month of November and December. Seeds were collected in the month of April from Shimoga district, Karnataka, India. The plant was taxonomically identified and authenticated by a Botanist Dr. Y. L. Krishnamurthy, Professor, Post Graduate Studies and Research in Botany, Kuvempu University, Shimoga, Karnataka. The collected plant material was primarily washed with tap water and subsequently with distilled water. The plant material was air dried under shade and then powdered with a mechanical blender to obtain a coarse powder. The powder was subjected for extraction with different solvents viz: petroleum ether, chloroform and ethanol using soxhlet apparatus. Extraction process was carried out till the completion of 24 cycles for individual solvent. The solvent was completely removed by rotary vacuum evaporator. The extract was stored in a vacuum desiccator.

Preliminary phytochemical screening

Qualitative Analysis

Phytochemical screening of *Chloroxylon swietenia* extracts, was performed to detect the presence of alkaloids, flavonoids, terpenoids, saponins, tannins, phenolic compounds and glycosides using standard procedures^{7,8}.

Quantitative Analysis

Quantitate analysis of all extracts was carried out for determination of total phenolics, alkaloid and terpenoids.

Determination of Total Phenolic Content

Total phenol content of extracts was measured by the Folin–Ciocalteu method⁹. 1 ml of each extract (100µg, 200µg, 300µg) was mixed with Folin–Ciocalteu reagent (2 ml) (diluted 1:10, v/v) and 2 ml of sodium carbonate (7.5%, w/v) was added then mixed, the reaction mixtures was maintained for 90 min at room temperature and absorbance was measured against the blank at 750 nm using spectrophotometer. Total phenolic content of the extracts was determined using Gallic acid standard.

Total Alkaloid Content Determination

1gm of plant extract was dissolved in 40ml 10% acetic acid in ethanol allowed to stand for 4hrs by covering tightly, then filtered and filtrate was concentrated on water bath until total volume became 1/4th of the original and ammonium hydroxide was added drop wise until precipitation of extract completes. Precipitate was collected and washed with dilute ammonium hydroxide then filtered, the residue was dried and weighed¹⁰.

Total Terpenoids Content Determination

Total terpenoid content was determined by soaking 100mg of plant extract in ethanol for about 24hrs, then filtered the extract and filtrate was fractioned with petroleum ether using separating funnel. The extract separated in petroleum ether was considered as total terpenoids¹¹.

Antibacterial Activity

Bacterial Strains

Gram negative bacterial strains (PA) *Pseudomonas aeruginosa*, (KP) *Klebsiella pneumonia*, (ST) *Salmonella typhi* and Gram positive bacterial strains (SP) *Streptococcus pneumonia*, (SA) *Staphylococcus aureus*, (VC) *vibrio cholerae* were obtained from Shimoga Institute of Medical Sciences, Shimoga, Karnataka, India for evaluating antibacterial activity.

Agar Well Diffusion Method

The plant extracts dissolved in 10% DMSO were individually tested against a panel of clinically pathogenic microbial strains selected. A loop full of Bacterial strains were sub-cultured in a conical flask and incubated at 37°C for 24 h in nutrient broth media day before conducting the experiment. 100µl of bacterial culture (10⁵ cells/ml) was inoculated on the nutrient agar culture plates in order to determine their viability by making wells of 6 mm diameter for loading the plant extracts of 20µl with different concentrations and standard streptomycin 10µl (20µg) into respected wells and were kept for incubation at 37°C. After 24h zone of inhibition was recorded^{12,13}.

MIC by Micro Titer Plate Method

This method was used to determine the minimum inhibitory concentration (MIC) of the plant extracts using modified resazurin microtitre plate assay in nutrient broth (NB) as specified by National Committee for Clinical Laboratory Standard (NCCLS,1998). In this assay, 50µl of nutrient broth was added to all the wells under sterile conditions. Subsequently,50µl of each extract at a concentration of 400 µg was added into six wells of first row in the plate then continued with the serial dilution down the column to get concentrations 200,100,50,25,12.5,6.25,3.125µg/50µl. Then, wells of first row were inoculated by 10µl (5×10^6 cfu/ml) of different microbial strains followed by the addition of 10µl (0.015 %) resazurin dye to all the wells as an indicator. Seventh well of first row was used as reference positive control (load streptomycin instead of extract), eighth well as negative control (without extract) and another one column as normal control (only media and dye without strains)¹⁴. Thereafter, 30µl of iso-sensitized broth to all the wells and covered with clean wrap to avoid the dehydration of bacteria, individual concentration was maintained in triplicates. plates were incubated at 37°C for 24 h, later change of the color was observed, the conversion of purple to pink color indicated the microbial growth, whereas, no change of color indicates inhibition of microbial growth. The results were expressed by taking mean from the lowest concentration at which no color change appeared was taken as the MIC value^{15,16}. the activity index is calculated as per the following formula¹⁷.

AI (Activity Index) = ZI of Test/ZI of Standard

Statistical Analysis

The analysis of variance (ANOVA) was performed using ezANOVA (version 0.98) software and Microsoft excel to determine the mean and standard deviation of zone of inhibition values between the extracts against bacterial culture¹⁸.

3. RESULTS

Preliminary Phytochemical Screening

The Plant crude extracts were subjected for Preliminary qualitative and quantitative analysis for the detection of various phytochemicals. The results showed that alkaloids, phenolic compounds and terpenoids are present in all extracts. whereas, cardiac glycosides are absent in LPE and BPE. It is also found that flavonoids and saponins are completely absent in all the extracts screened results are shown in Table 1. The quantified total phenolics, alkaloids and terpenoids contents of *Chloroxylon swietenia* extracts have shown that terpenoid content is found to be more in the selected plant. The seed ethanolic extract has more phenolic content i.e up to 22µg/mg whereas, alkaloid content 17.32µg/mg in stem bark ethanol extract similarly leaf pet ether extract showed more terpenoid content of 47.32µg/mg. The results are depicted in Fig. 1. The above results indicate that ethanol extracts contain more amount of phenolics and alkaloids compared to pet ether and chloroform extracts

whereas, the total terpenoids content is more in pet ether and chloroform extracts compared to ethanol extracts.

Antibacterial Activity and MIC by Micro Titer Plate Method

The antibacterial activity of all the extracts has been observed against all the bacterial strains selected. The zone of inhibition is presented in table 2 while as, activity index values are tabulated in table 3. Comparatively the chloroform and pet ether extracts showed significant inhibition for selected pathogenic bacterial strains. The test was conducted in triplicate and results presented by calculating the mean of the zone of inhibition. Zone of Inhibition of standard Streptomycin against *S. aureus*, *K. pneumonia*, *V. cholerae*, *S. pneumonia*, *P. aeruginosa* and *S. typhi* calculated at 29.67±0.33, 27.67±0.33, 28.67±0.33, 29.00±0.58, 28.33±0.33 and 27.67±0.33 respectively. Seed chloroform extract showed **17.67±0.33mm** inhibition zone and MIC value has been calculated at **20.83µg** against *S. aureus*. Seed pet ether extract showed **16.67±0.33mm** inhibition zone and MIC value at **33.33 µg** against *K. pneumonia*, while as, stem bark chloroform extract has shown **12.33±0.33mm** inhibition zone and MIC value tabulated at **133.33µg** against *V. cholerae*, seed pet ether extract revealed **19.67±0.33** mm inhibition zone and MIC value observed is at **20.83µg** towards *S. pneumonia*, *P. aeruginosa*, seed ethanol extract effect was valued at **11.67±0.33mm** inhibition zone and its MIC value has been reported at **133.33 µg** on its action to *S. typhi*. The MIC values are graphically represented in Fig. 2.

4. DISCUSSION

The medicinal value and biological activity of plants are usually being attributed to the presence of phytochemicals, whose composition is totally dependent on geographical and environmental factors¹⁹. People prefer medicinal plants for the treatment of various diseases due to the negligible harmful side effects. Synthetic drugs are known to cause side effects such as rashes, stomach pain etc. The plants belonging to genus *Swietenia* that possess good antibacterial activity are *Swietenia macrophylla* and *Swietenia mahagoni*^{20,21}. The plant *Chloroxylon swietenia* selected in current investigation is a traditional medicinal plant is traditionally being used for the cure of worm infected wounds, fungal infected skin and also to treat inflammation and rheumatism⁵. As per the literature survey no evidence is found on antibacterial activity from leaves, stem bark and seed extracts of pet ether, chloroform and ethanol of this plant.

In the present study, preliminary qualitative and quantitative analysis of the plant extracts has been done. Subsequently, the antibacterial efficacy has been tested by employing standard protocols. The qualitative phytochemical screening of petroleum ether, chloroform and ethanol extracts of *Chloroxylon swietenia* from leaves, stem bark and seeds indicated the presence alkaloids, terpenoids, phenolic

compounds and tannins in all extracts, whereas, cardiac glycosides are absent in LPE and SBPE found to be present in all other extracts. It is also found that flavonoids and saponins are completely absent in all the extracts screened. Among these groups alkaloids and terpenoids are the major secondary metabolites which have a substantial role in treating various diseases²². Therefore, the presence of these groups in *Chloroxylon swietenia* is considerable prospect of its significant role in pharmacology specifically can act against pathogenic microorganisms. Additionally, presence of important groups in this plant viz: phenolic compounds, cardiac glycosides, and tannins already been proved to be prominent in pharmacological studies encouraged to explore this plant for challenging public health issues.

The plant extracts of *Chloroxylon swietenia* have been undertaken to determine the amount of prominent bioactive groups like total phenolics, alkaloids and terpenoids present in this plant. The seed ethanolic extract has more phenolic content i.e. up to 22µg/mg whereas, alkaloid content was observed at concentration of 17.32µg/mg in stem bark ethanol extract, whereas, leaf pet ether extract showed more terpenoid content of 47.32µg/mg. The above results indicate that ethanol extracts of all plant parts contain more amount of phenolics and alkaloids compared to pet ether and chloroform extracts, whereas, the total terpenoids content is more in pet ether and chloroform extracts compared to ethanol extracts. The antibacterial activity of all the extracts has been observed against all the bacterial strains selected. The test was conducted in triplicate and results are presented by calculating the mean of the zone of inhibition. Seed chloroform extract showed **17.67±0.33mm** inhibition zone and MIC value has been calculated at **20.83µg** against *S. aureus*. Seed pet ether extract showed **16.67±0.33mm** inhibition zone and MIC value at **33.33 µg** against *K. pneumonia*, while as, stem bark chloroform extract has shown **12.33±0.33mm** inhibition zone and MIC value tabulated at **133.33µg** against *V. cholerae*, seed pet ether extract revealed **19.67±0.33** mm inhibition zone and MIC value observed is at **20.83µg** towards *S. pneumonia* and *P. aeruginosa*. The effect of seed ethanol extract is found to be **11.67±0.33mm** of inhibition zone and its MIC value has been reported to be **133.33 µg** on its action against *S. typhi*. The calculated activity index of SPE showed good AI of **0.602, 0.678 and 0.694** against *K. pneumonia*, *S. pneumonia* and *P. aeruginosa* respectively. Similarly, SCE showed good AI **0.595** against *S. aureus*. Among the tested extracts, all the extracts showed significant AI when compared to standard streptomycin. In this study seed chloroform extract showed good inhibition of growth against *S. aureus*, seed pet ether is also effective against *K. pneumonia*, *S. pneumonia* and *P. aeruginosa*. Stem bark chloroform extract has induced considerable inhibition against *V. cholerae* and seed ethanol extract showed average inhibition of growth against *S. typhi* when compared with the standard antibiotic streptomycin. Comparatively, the chloroform and pet ether extracts showed

significant inhibition for selected pathogenic bacterial strains. The antibacterial property of plant may be due to the presence of secondary metabolites mainly terpenoids, alkaloids, glycosides, phenolic compounds and tannins¹¹. This potential antibacterial nature of plant may be helpful in the development of new class of natural antibiotics to cure infectious diseases caused by pathogenic bacteria.

Table 1: Qualitative Phytochemical Analysis of Extracts of *Chloroxylon Swietenia*

Tests	LPE	LCE	LEE	SBPE	SBCE	SBE	SPE	SCE	SEE
Alkaloids	+	+	+	+	+	+	+	+	+
Flavonoids	-	-	-	-	-	-	-	-	-
Phenolic compounds	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+
Cardiac Glycosides	-	+	+	-	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+
Saponins	-	-	-	-	-	-	-	-	-

LPE-Leaves pet ether extract, LCE-Leaves chloroform extract, LEE-Leaves ethanol extract, SBPE- Stem Bark pet ether extract, SBCE- Stem Bark chloroform extract, SBE- Stem Bark ethanol extract, SPE-Seed Pet ether extract, SCE-Seed chloroform extract, SEE-Seed ethanol extract.

(+) indicate the presence and (-) the absence of the respective phytochemicals.

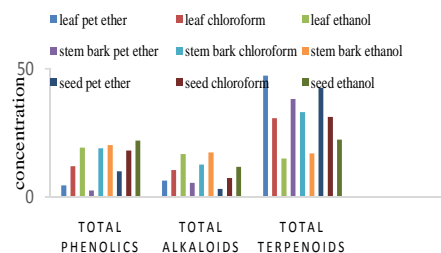


Fig 1: Quantitative Estimation of Total Phenolics, Alkaloids and Terpenoids

Antibacterial Activity

Table 2: Antibacterial Activity by Agar Well Plate Method

Sl. No.	Extracts Concentration 1600 µg					
	Zone of Inhibition(mm)					
	<i>S. aureus</i>	<i>K. pneumonia</i>	<i>V. cholerae</i>	<i>S. pneumonia</i>	<i>P. aeruginosa</i>	<i>S. typhi</i>
LPE	15.33±0.33	11.67±0.33	30.83±0.33	14.67±0.33	12.67±0.33	33.00±0.00
LCE	16.00±0.00	10.33±0.33	9.33±0.33	12.67±0.33	11.33±0.33	10.33±0.33
LEE	12.33±0.33	9.33±0.33	10.67±0.33	10.33±0.33	10.33±0.33	9.33±0.33
SBPE	13.00±0.00	9.33±0.33	30.83±0.33	12.67±0.33	9.33±0.33	30.83±0.33
SBCE	16.33±0.33	12.33±0.33	12.33±0.33	14.67±0.33	12.67±0.33	33.00±0.00
SBE	11.33±0.33	11.67±0.33	10.33±0.33	14.67±0.33	11.33±0.33	30.83±0.33
SPE	14.67±0.33	16.67±0.33	30.83±0.33	19.67±0.33	19.67±0.33	33.00±0.00
SCE	17.67±0.33	14.67±0.33	30.767±0.33	17.67±0.33	16.67±0.33	30.83±0.33
SEE	12.33±0.33	11.67±0.33	30.800±0.00	7.67±0.33	10.33±0.33	11.67±0.33

LPE-Leaves pet ether extract, LCE-Leaves chloroform extract, LEE-Leaves ethanol extract, SBPE- Stem Bark pet ether extract, SBCE- Stem Bark chloroform extract, SBE- Stem Bark ethanol extract, SPE-Seed Pet ether extract, SCE-Seed chloroform extract, SEE-Seed ethanol extract.

Table 3: Activity Index

Sl. No.	Extracts Concentration 1600 µg					
	Activity Index					
	<i>S. aureus</i>	<i>K. pneumonia</i>	<i>V. cholerae</i>	<i>S. pneumonia</i>	<i>P. aeruginosa</i>	<i>S. typhi</i>
LPE	0.516	0.421	0.290	0.505	0.447	0.289
LCE	0.539	0.373	0.325	0.436	0.399	0.373
LEE	0.415	0.337	0.372	0.356	0.364	0.337

SBPE	0.438	0.337	0.290	0.436	0.329	0.301
SBCE	0.550	0.445	0.430	0.505	0.447	0.289
SBEE	0.381	0.421	0.360	0.505	0.399	0.301
SPE	0.494	0.602	0.290	0.678	0.694	0.289
SCE	0.595	0.530	0.267	0.609	0.588	0.301
SEE	0.415	0.421	0.279	0.264	0.364	0.421

LPE-Leaves pet ether extract, LCE-Leaves chloroform extract, LEE-Leaves ethanol extract, SBPE- Stem Bark pet ether extract, SBCE- Stem Bark chloroform extract, SBEE- Stem Bark ethanol extract, SPE-Seed Pet ether extract, SCE-Seed chloroform extract, SEE-Seed ethanol extract.

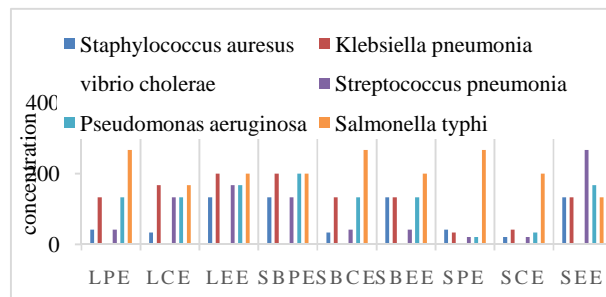


Fig 2: MIC by Micro Titer Plate Method

LPE-Leaves pet ether extract, LCE-Leaves chloroform extract, LEE-Leaves ethanol extract, SBPE- Stem Bark pet ether extract, SBCE- Stem Bark chloroform extract, SBEE- Stem Bark ethanol extract, SPE-Seed Pet ether extract, SCE-Seed chloroform extract, SEE-Seed ethanol extract.

5. CONCLUSION

Thus, from the present study it is clear that the plant extracts of *Chloroxylon swietenia* contain considerable amounts of bioactive secondary metabolites which are authenticable for the pharmacological properties. All the tested plant extracts possess antibacterial activity against selected clinical pathogenic bacterial strains. This confirms the traditional claim of the plant *Chloroxylon swietenia* against many ailments.

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