

# Pinus glabra: As a Potential Source of Anti-*Mycobacterium tuberculosis* Agent: Phytochemical and antimicrobial Studies of its Stem Extracts

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**Abstract**—With the increasing incidence of tuberculosis and rated second to HIV-AIDS by the World Health Organisation as a leading cause of death from infectious disease and increased resistance to drugs currently in use, there is therefore the need for alternative sources of drugs for the treatment of this disease. *Pinus glabra* presents as a potential candidate for such drugs discovery. Concoctions derived from the plant have been used to treat cases of rheumatism, cough, piles and catarrh. Sample extraction was performed by soaking the stem samples in ethanol for 172 h, which gave reddish-yellow oil after removal of the ethanol solvent. The oil was partitioned between 1:1 water/chloroform mixture. The aqueous layer was further partitioned separately with ethyl acetate and hexane. The phytochemical screening of the crude ethanol extract revealed the presence of alkaloids, saponins, tannins and flavonoids. Antimicrobial tests were performed on the crude ethanol extract, ethyl acetate and hexane fractions against clinical isolates *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella sp.* by measurement of zones of inhibition. All test samples exhibited significant antimicrobial activity against the organisms albeit to different extent.

**Keywords**—*Phytochemical studies; antimicrobial studies; anti-Mycobacterium tuberculosis; isolation and extraction; pinus glabra*

## I. INTRODUCTION

This template, You may ask why we need new TB drugs, when there are several drugs out there in the market.

There are three main reasons why we need new TB drugs: there is the complexity and toxicity of the current TB drug regimens; the issue of TB drug resistance; and the problem of the interaction of current TB drugs with antiretroviral drugs taken by HIV positive people [1].

So what are we looking for in the new drugs?

1. Shorter and simpler, but still affordable multi drug regimens for drug sensitive TB;
2. Shorter, more effective, less toxic and less expensive regimens for drug resistant TB;
3. Short, simple, easily tolerable and safe regimens for latent TB;
4. Drugs with few drug interactions so they can be safely provided to people with people with HIV.

*Mycobacterium tuberculosis* of the genus *mycobacterium* is the major cause of tuberculosis, which is the leading cause of death after HIV [2]. There was an estimated 8.8 million incidence of TB globally in 2010.

Nigeria has the fourth largest cases of tuberculosis in the world with more than 460,000 cases in 2007 [3]. The mortality cases of TB in Nigeria have been put at 320 in every 100,000.

Majority of the peoples of the developing countries of the world still rely on traditional medicine derived mainly from

medicinal plants. Medicinal plants remain important sources for finding new active drugs or new therapeutic agents [4]. Phytomedicines have shown great promise in the treatment of intractable infectious diseases including tuberculosis [5] [6].

*Pinus glabra* has been used to treat chronic rheumatism, catarrh and swollen testicles [7].

The failure of the Directly Observed Therapy, Shot Course Strategy (DOTS), the prevalence of multi drugs resistance tuberculosis (MDRTB) and the emergence of extensively drug resistant tuberculosis (XDR-TB) it has become necessary to look for alternative sources of drugs and the screening of herbal plants with anti-mycobacterial effect therefore needs to be paid serious attention.

With these observations in mind, it was decided to extract, isolate and characterise the secondary metabolites from the stem of *Pinus glabra* and to determine their anti-microbial potential.

Previous studies on the *Pinaceae* family have revealed that the chemical constituents were mostly terpenoids, flavonoids, phenols, steroids, fatty acids and fatty alcohols [8] [9] [10] and the extracts were found to possess bioactivities as anti tumour, anti-hypertensive and antitussive agents.

The family were also found to possess antimicrobial activity against *Bacillus megatherium*, *Bacillus subtilis*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Mycobacterium smegmatis*, *Proteus vulgaris*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Candida albicans*, *Candida tropicalis* and *Penicillium italicum* [11].

## II. MATERIAL AND METHOD

*Pinus glabra* used in this study was harvested from the university grounds and authenticated in the biological sciences department of the university.

The sample stem (1000 g) was soaked in ethanol (6.10 L) for 168 h. After which time the solution was decanted and filtered. The filtrate was distilled on a rotary evaporator to give yellow/red oil (189.5 g). The ethanolic extract (50 mL) was partitioned between water:chloroform mixture (1:1) in a separator funnel. After separation the chloroform fraction (F1) was kept and the aqueous layer was further partitioned between water and ethyl acetate.

After separation the ethyl acetate fraction (F2) was kept and the aqueous layer was again partitioned between water and n-hexane. After separation the aqueous layer (F3) and n-hexane fraction (F4) was kept for analysis.

Phytochemical screening was carried out on the crude ethanolic extract using standard methods as described by Trease and Evans [12]. A summary of the screening result is shown in Table 1.

Bacterial cultures of *E. coli*, *Staphylococcus aureus*, *Klebsiella sp.*, *Pseudomonas aeruginosa* were maintained on nutrient with broth at 37°C for 24 h prior to testing, used for antimicrobial analysis on an agar well diffusion technique.

Ethanol extract, n-hexane extract and ethyl acetate extract at 50 mg/mL was introduced into the wells. Chloramphenicol (100 mg/mL) was used as a positive control and 20% nutrient agar as negative control.

The outcomes of the measurements of the zones of inhibition for antimicrobial tests are recorded in Table 2.

## III. RESULTS AND DISCUSSION

The phytochemical screening of *Pinus glabra* stem revealed that crude ethanolic extracts of *Pinus glabra* stem contained flavonoids, alkaloids, tannins, saponins, phenol, cardiac glycosides and saponin glycosides at very high intensity while anthraquinones were moderately present.

The antimicrobial potential of crude extract and fractions during partitioning were assessed in terms of zone of inhibition of bacterial growth. The result antimicrobial activities is presented in Table 2. Activity were studied at 50 mg/ml concentration against four pathogenic bacterial strains, one Gram-positive (*Staphylococcus aureus*) and three Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). As compared with standard drugs, the results revealed that majority of the extracts were sensitive while Ethyl acetate extract showed no sensitivity towards *E. coli*.

Table 1. Summary of Phytochemical screening of ethanol extract of *Pinus glabra*

Test	Intensity
Flavonoids	+++
Alkaloids	+++
Tannins	+++
Saponins	+++
Anthraquinones	+
Cardiac glycosides	+++
Phenol	+++
Saponin glycosides	+++
Key: + = mild, ++ = moderate, +++ = intense	

Table 2. Zones of Inhibition

Test organism	Zone of inhibition (mm)		
	Ethanol	Ethyl acetate	n-hexane
<i>E. coli</i>	16	-	15
<i>Staphylococcus aureus</i>	15	10	13
<i>Pseudomonas aeruginosa</i>	25	22	19
<i>Klebsiella pneumoniae</i>	15	10	13

#### IV. CONCLUSION

The Phytochemical screening which revealed the presence of secondary metabolites and the antimicrobial activities of the various fractions against selected bacteria show that *Pinus glabra* is a potential candidate for the treatment of various ailments as has been reported for other members of the plant family [7].

We are therefore working on isolating and characterising the individual components of the extract and testing for their biological activity and tuberculosis in particular.

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