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Itmad Awad Elhassan
Pharmaceutical Industries Department, Industrial Research and Consultancy Centre, P.O. Box 268, Khartoum, Sudan.

Nuha Yousif Ibrahim
Sudan National Public Health Laboratory-Tuberculosis Reference Laboratory, Khartoum, Sudan.

Omer Mohammed Mahmoud
Pharmaceutical Industries Department, Industrial Research and Consultancy Centre, P.O. Box 268, Khartoum, Sudan.

Asrar M Salam
Sudan National Public Health Laboratory-Tuberculosis Reference Laboratory, Khartoum, Sudan.

Rahmaali Hasab Elrasoul
Sudan National Public Health Laboratory-Tuberculosis Reference Laboratory, Khartoum, Sudan

Correspondence
Itmad Awad Elhassan
Pharmaceutical Industries Department, Industrial Research and Consultancy Centre, P.O. Box 268, Khartoum, Sudan.

Anti-tubercular activity of essential oils from *Cymbopogon citratus*, *C. nervatus* and *C. proximus*

Itmad Awad Elhassan, Nuha Yousif Ibrahim, Omer Mohammed Mahmoud, Asrar M Salam, Rahmaali Hasab Elrasoul

Abstract
Tuberculosis (TB) remains a major global health problem, especially with the emergence of drug resistance. It ranks as the second leading cause of death from an infectious disease worldwide, after the human immunodeficiency virus (HIV). This research was conducted to evaluate *in vitro* anti-tubercular activity of the essential oils from three aromatic herbs belong to the Genus *Cymbopogon* (Family Poaceae), *Cymbopogon citratus* (leaves), *C. nervatus* (inflorescences) and *C. proximus* (leaves and inflorescences). The essential oils were tested *in vitro* for their activity against nine clinical isolates and a reference susceptible strain ($H_{37}R_v$) *M. tuberculosis*, using Lowenstein-Jensen (LJ) medium containing glycerol, at concentrations of 75-15 µl/mL. Evaluation of antitubercular activity was determined using the absolute concentration method which is expressed in terms of the minimal inhibitory concentration (MIC). The investigated essential oils from the genus *Cymbopogo*n exhibited anti-tubercular activity, in Lowenstein-Jensen medium, towards all the tested strains of *M. tuberculosis* isolates, up to concentration of 15 µl/ml for each oil. Some strains showed resistance towards Rifampicin, a marker drug for MDR-TB. This research findings represents additional challenges for essential oils as promising natural products leads for tuberculosis (specially MDR) drug discovery and development.

Keywords: *M. tuberculosis*, Cymbopogon, essential oils, absolute concentration, drug resistant.

1. Introduction
Tuberculosis (TB) remains a major global health problem, responsible for ill health among millions of people each year. TB ranks as the second leading cause of death from an infectious disease worldwide, after the human immunodeficiency virus (HIV). Moreover, the emergence of multi-drug resistant (MDR) and extensively drug resistant (XDR) strains of *M. tuberculosis* has complicated the problem.
According to WHO latest Global Tuberculosis Report in 2013, an estimated 9.0 million people developed TB and 1.5 million died from the disease, (13%) of whom were HIV-positive. About 64% of the estimated 9 million people who developed TB were notified as newly diagnosed cases. Globally, 3.5% of new and 20.5% of previously treated TB cases were estimated to have had MDR-TB in 2013. On average, an estimated 9.0% of patients with MDR-TB had XDR-TB [1].
The recent increase in the widespread existence of XDR-TB especially in the developing nations emphasized the need for the development of new drugs to treat this infection. Therefore, development of new treatments is the major focus of current tuberculosis research worldwide [2].
On the other hand, natural products play a most significant role in the drug discovery and development process; Phytochemicals may also offer an effective and cheaper alternative treatment for tuberculosis [3]
Many studies were conducted to evaluate anti- tubercular activities of many medicinal plants extracts [4-9].
Essential oils of *Cymbopogon* species are easily available, have a pleasant aroma, are non-toxic and safe; the active principles are therefore becoming increasingly popular in pharmaceuticals and medicines. Their chemical composition was well investigated [10-22].
Essential oils of *Cymbopogon* species have outstanding antifungal activities and significant antibacterial activities. Their antimicrobial properties have been well documented [11, 15, 17-19, 21-23].

This research was conducted to evaluate the *in vitro* anti-tubercular activity of the essential oils from three plants belong to the Genus *Cymbopogon*, *Cymbopogon citratus*, *C. proximus* and *C. nervatus*.

2. Materials and Methods

Materials

Plant materials

The three herbs parts used in the study (*C. citratus* leaves, C. *nervatus* inflorescences, *C. proximus* leaves were collected from Industrial Research and Consultancy Centre (IRCC) farm, Khartoum State, Sudan.

Mycobacterial strains

Nine clinical isolates and reference susceptible strain ($H_{37}R_v$) *M. tuberculosis*, provided by -Tuberculosis Reference Laboratory – Sudan National Public Health Laboratory.

Methods

Essential oil preparation and analysis

Essential oils were hydro-distilled from the different *Cymbopogon* herbs and dried over anhydrous sodium sulphate. The essential oils were analyzed by gas chromatography coupled with mass spectrometry (GC-MS) using HP 6890 (GC) and HP 5973 (MSD).

Identification of components

The essential oils constituents were identified by their retention time and computer matching of their mass spectra with those found in NIST and Wiley libraries database.

The percentage of composition of the identified compounds was computed from the GC peak area.

Antimycobacterial activity assessment

The essential oils were tested *in vitro* for their activity against nine clinical isolates and reference susceptible strain ($H_{37}R_v$) *M. tuberculosis*, using the conventional growth-based method that utilized egg-based media, Lowenstein-Jensen (LJ) medium containing glycerol.

The media containing different concentration of essential oils (75, 50, 30, and 15 µl/ml) were inoculated with the different strains of *Mycobacterium* suspension the concentration of which was equivalent to McFarland No. 0.5, then were incubated at 37 ºC for up to six weeks, with weekly observation. All isolates were tested for susceptibility to the antitubercular first-line drug rifampicin (RIF).

Evaluation of antitubercular activity was determined using the absolute concentration method which is expressed in terms of the minimal inhibitory concentration, MIC [24].

3. Results and Discussion

Gas chromatogram of the hydrodistilled essential oils of *C. proximus* (Fig 1) revealed detection of piperitone (43.2, 45.8%), elemol (13.45, 14.43 %), 4-carene (7.55, 9.75%), β-eudesmol (5.41, 4.32%) as the main components in the essential oil of *C. proximus* leaves and inflorescence, respectively.

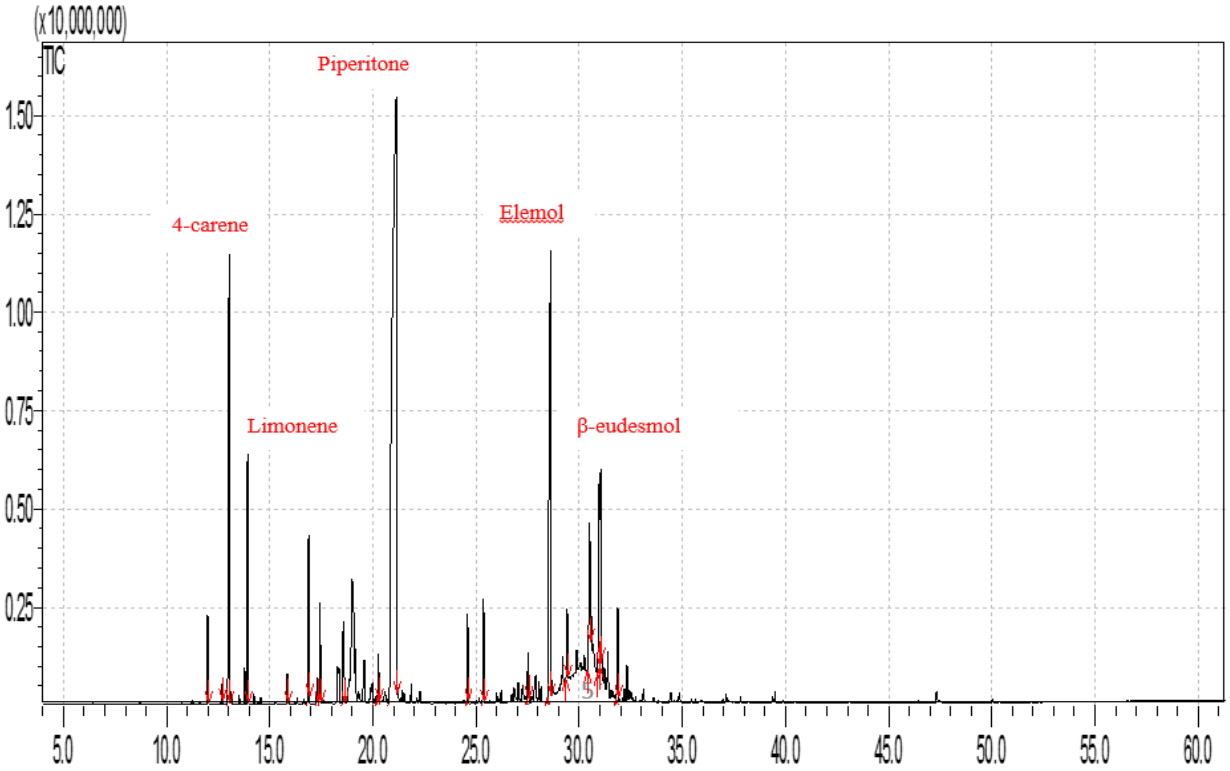

Fig 1: Gas chromatogram of *C. proximus* leaves essential oil

Gas chromatographic profile of lemongrass essential oil (Fig. 2) showed that the oil composed mainly of citral b (32.74%), citral a (26.23%) and β-pinene (9.36%).

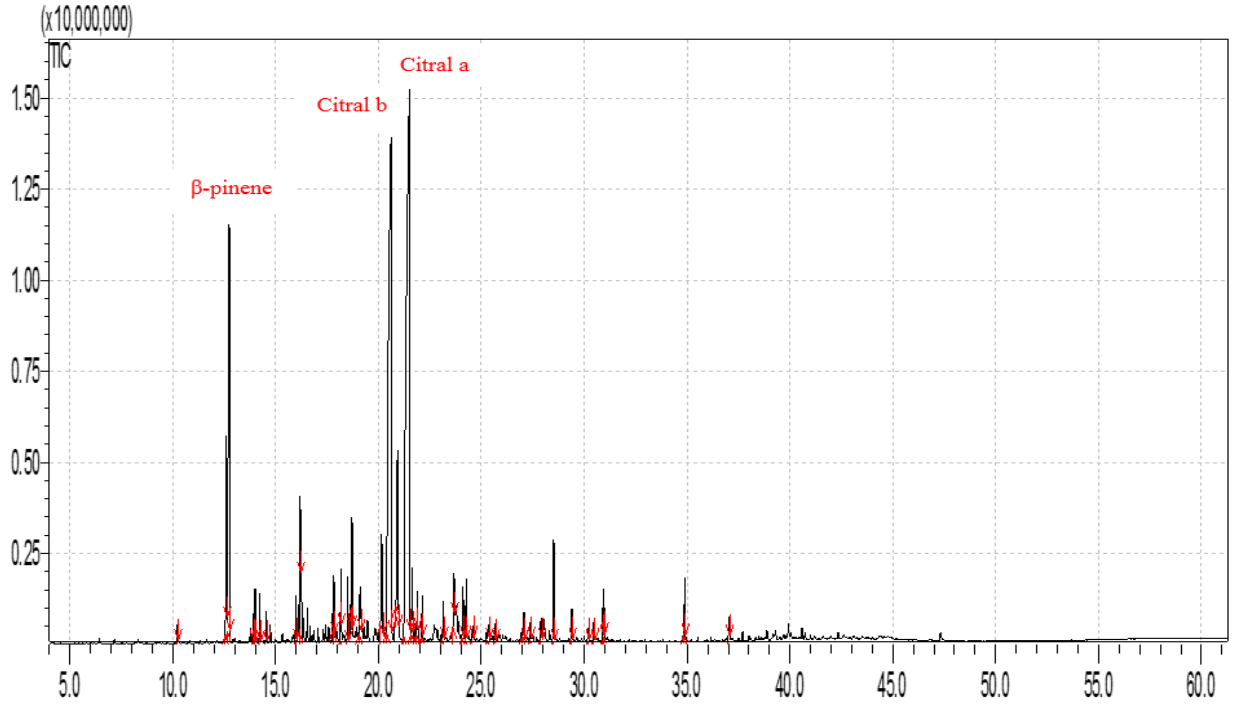

Fig 2: Gas chromatogram of *C. citratus* leaves essential oil

The main identified constituents in the investigated C. *nervatus* inflorescences essential oil were α- verbenol (20%), trans-pincarveol (19.41%), trans- p-menthe-2,8-dien-ol (14.14), d-Limonene (8.49%) among others (Fig. 3).

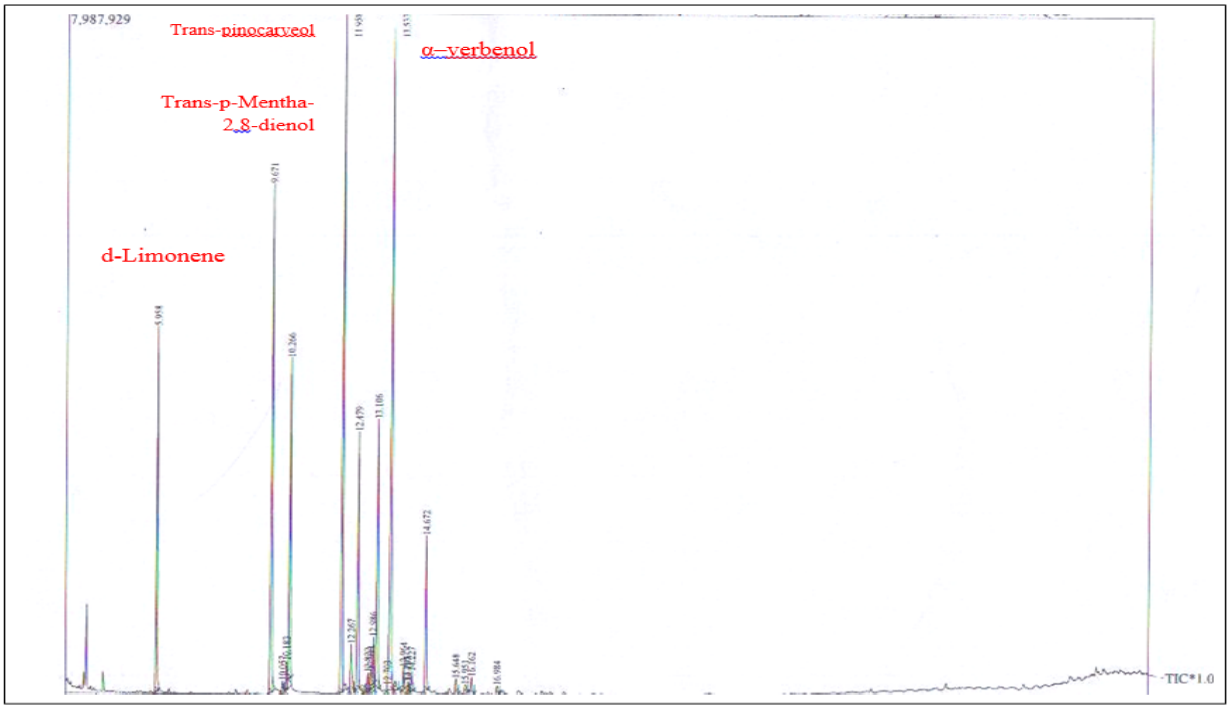

Fig 3: Gas chromatogram of *C. nervatus* inflorescence essential oil

Evaluation of antitubercular activity

The essential oils were tested *in vitro* for their activity against nine clinical isolates and a reference susceptible strain ($H_{37}R_v$) *M. tuberculosis*, using L-J medium containing glycerol, at concentrations of 75-15 µl/mL.

Evaluation of antitubercular activity was determined using the absolute concentration method which is expressed in terms of the minimal inhibitory concentration (MIC). All investigated essential oils from the genus *Cymbopogo*n exhibited pronounced anti-tubercular activity, in L-J medium, towards all the tested strains of *M. tuberculosis* isolates, at all concentration up to 15µl/ml for each oil (Table 1).

Table 1: Antitubercular activity of *Cymbopogo*n essential oils at 15 µl/ml against *M. tuberculosis* strains

No.	Strain code	Sensitivity				
		P	Pf	N	C	Rif
1	1303	S	S	S	S	S
2	150904	S	S	S	S	S
3	1098	S	S	S	S	S
4	1240	S	S	S	S	R
5	0115	S	S	S	S	S
6	1214	S	S	S	S	R
7	1081	S	S	S	S	S
8	150	S	S	S	S	S
9	153	S	S	S	S	R
10	H37 Rv	S	S	S	S	S

Rif = Rifampicin

It is surprising that three strains which were sensitive to *Cymbopogo*n essential oils showed resistance towards Rifampicin, a marker drug for MDR-TB, indicating that they were MDR-TB strain.

Based on this result, *Cymbopogo*n essential oils could offer an effective, safer and cheaper treatment for tuberculosis and may provide alternative drugs to current MDR TB drugs.

In many essential oils, the antimicrobial activity is due to the presence of terpenoids such as monoterpenes, sesquiterpenes or related alcohols and phenols. The lipophilic character of their hydrocarbon skeleton and the hydrophilic character of their functional groups are of main importance in the antimicrobial action of essential oil components. Possibly, the antibacterial activity of terpenes is due to a perturbation of the microorganism lipid fraction of the plasma membrane, and results in alterations of membrane permeability and in leakage of intracellular materials. This effect may be a consequence of the interaction between the major and minor components of essential oil [25-27].

Comparison of the minimum inhibitory concentration (MIC, µg ml^{-1}) of the *Cymbopogo*n essential oils against the reference susceptible strain (H37Rv) *M. tuberculosis*, with that of some previously studied plants essential oils, extracts or isolates [27, 28], revealed the pronounced antimycobacterial activity of *Cymbopogo*n essential oils among the others (Fig. 4).

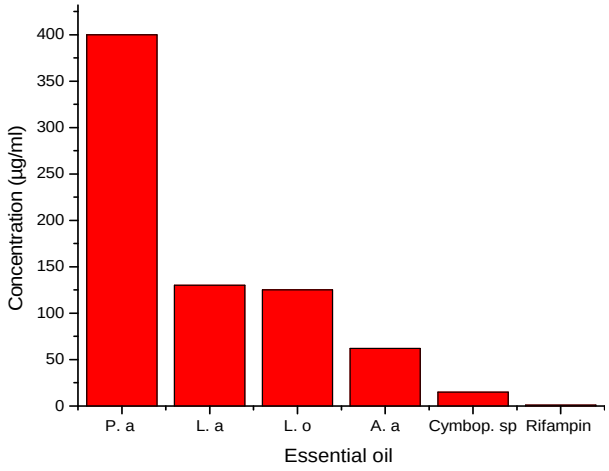

Fig 4: MIC of different essential oils against drug-susceptible reference strain *M. tuberculosis* H_37R_v
P. a = Piper auritum leave and stem s*; L. a = Lippia alba* leaves and stem
L. o -= Lippia origanoides leaves; *A. a = Achyrocline alata* leaves and stem

4. Conclusion

This research findings make *Cymbopogo*n essential oils promising natural products leads for tuberculosis drug discovery and development, specially for MDR TB, the treatment of which requires the use of second line drugs, which are more expensive and with even more serious adverse effects.

5. References

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