

Plants as sources of anti-gonococcal agents

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Background and motivation

- Neisseria gonorrhoeae*, the causative agent of a sexually transmitted infection (STI), gonorrhea, has developed resistance to available antibiotics [1,2].
- The resistance is partly due to the bacteria's adaptability to antigenic variability which allows it to escape host defenses and often causing asymptomatic infections.
- To date, no vaccine is available for gonorrhea, necessitating ongoing research to find effective, affordable, and safe anti-gonococcal agents.
- Plants are a good source of lead compounds for drug developed. Extracts from various plants have shown *in-vitro* growth inhibition of *N. gonorrhoeae* [3,4].
- Various communities in Namibia commonly use plants for STI treatment including gonorrhea (Figure 1). However, these plants have not yet been evaluated for their anti-gonococcal activity and human safety.

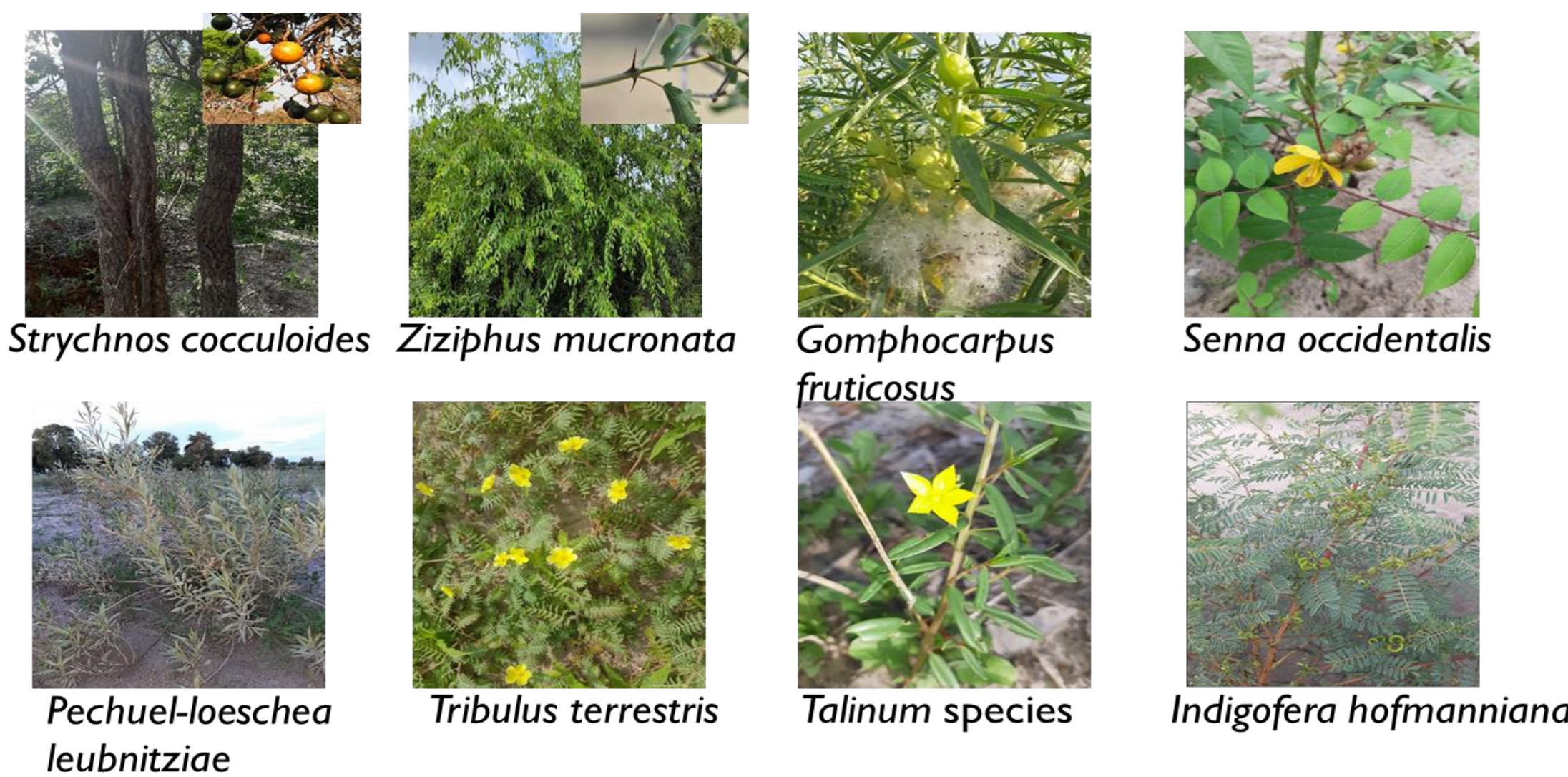


Figure 1: Some of the plants and plant parts that are used to treat STIs in Zambezi region

Objectives

- To screen plant species that are traditionally used to treat STIs in Namibia for anti-gonorrheal activity. To ascertain their anti-gonococcal activity.
- To isolate and characterize bioactive compounds from extracts that show activity against *N. gonorrhoeae*.
- To employ computational approaches including density functional theory and molecular docking to investigate the interaction of isolated compounds with *Neisseria gonorrhoeae* carbonic anhydrase (NgCA).

Methodology

- Plant species (13) were selected based on ethnomedicinal uses reported in literature and some herbalist's reports on their use in treating gonorrhea/STIs.
- Plants were taxonomically identified at the National Botanical Research Institute (NBRI) of Namibia. A total of 16 plant parts (leaves, bark, roots) from these species were analyzed.
- Chromatographic techniques were used to isolate compounds
- NMR and x-ray diffraction was used for structure determination (Figure 2)
- Anti-gonococcal activity of the extracts and isolated compounds was done on 4 strains of *N. gonorrhoeae*, tetracycline was used as a standard antibiotic.

Sample preparation and extraction

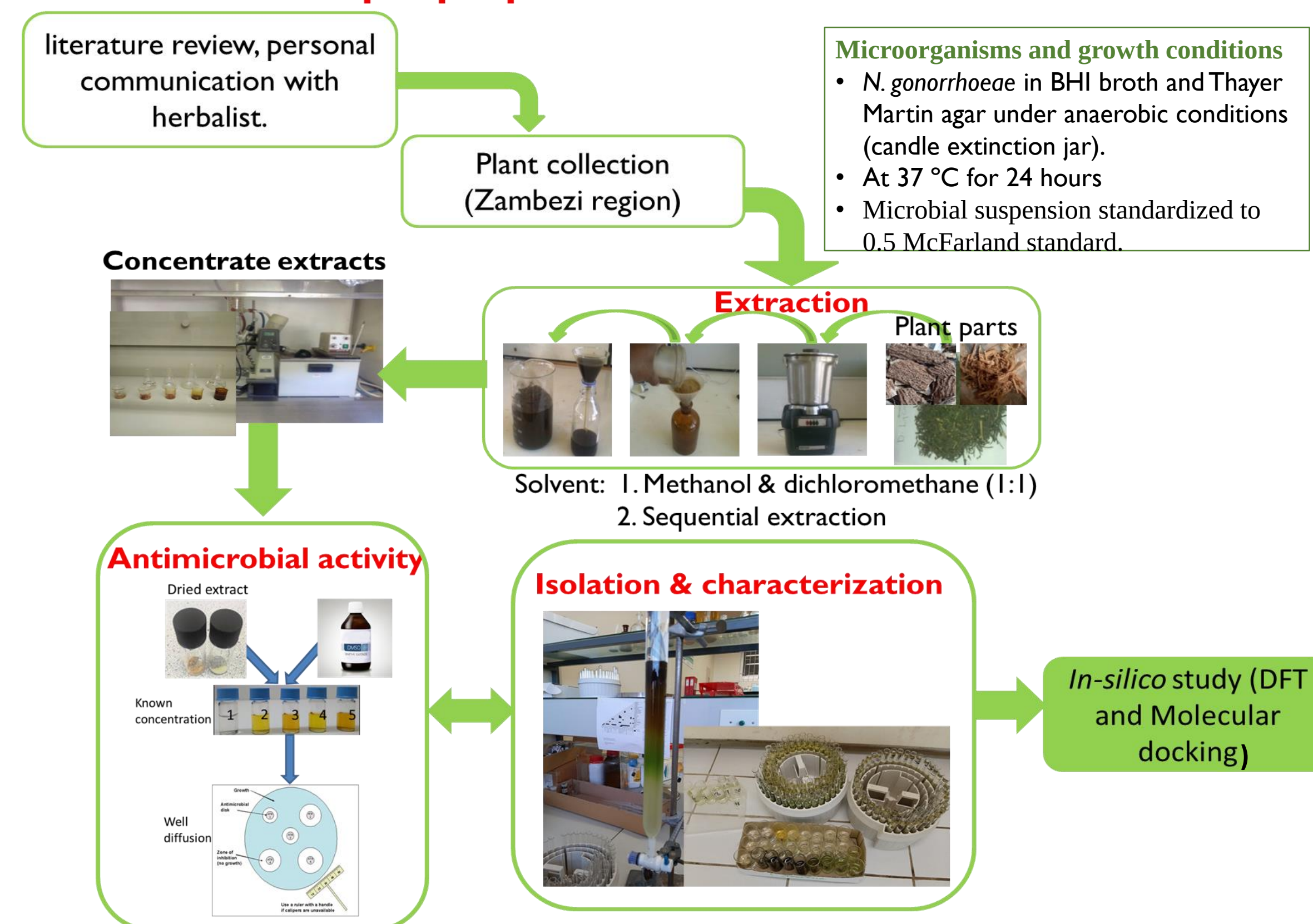


Figure 2: Sample preparation, extraction and bioassay guided fractionation from selected plants

Results

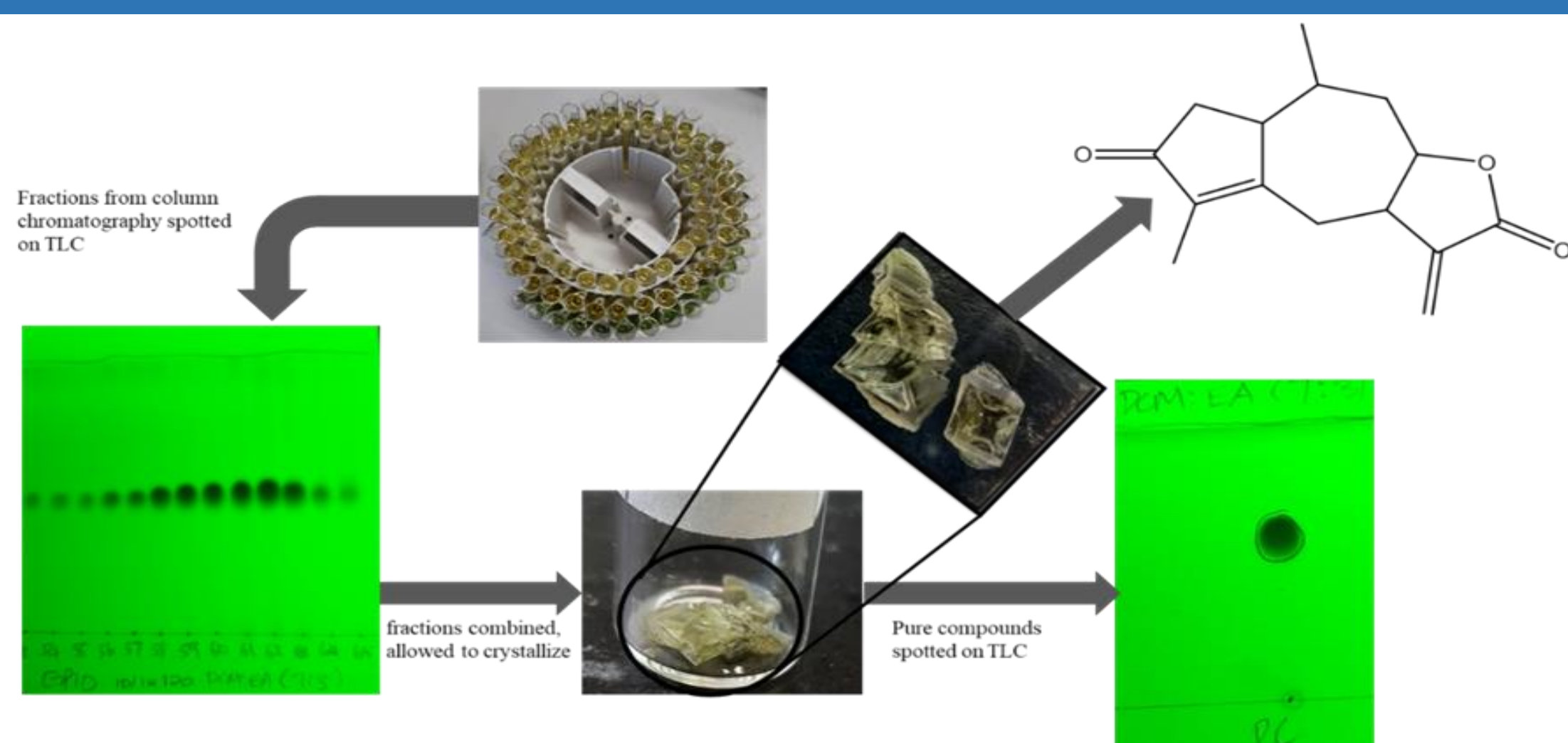


Figure 3: TLC profile of *Pechuel-loeschea leubnitziae* leaf fractions collected from column chromatography and the resulting crystals that formed. The structure of xerantholide was determined by x-ray diffraction

Anti-gonococcal activity of isolated compound

Table 1. The percentage inhibition of *Neisseria gonorrhoeae* strains and concentration leading to 50% inhibition (MIC₅₀) of extracts and pure compound from *Pechuel-loeschea leubnitziae* leaves

	ATCC19424		ATCC31426		ATCC43069		ATCC49226	
Extracts	MIC ₅₀ (mg/mL)	% inhibit	MIC ₅₀ (mg/mL)	% inhibit	MIC ₅₀ (mg/mL)	% inhibit	MIC ₅₀ (mg/mL)	% inhibit
GP10 ^a	0.400	61.4±3.6	0.095	55.4±6.6	0.096	55.1±1.0	0.808	58.2±1.8
DCM	1.16	51.0±12	0.829	50.0±9.1	0.730	52.7±2.2	>5.00	<30
DCM:MeOH	1.41	50.0±5.6	1.68	49.1±4.3	0.998	50.0±4.3	>5.00	<30
MeOH	3.07	42.0±11	2.00	42.4±2.1	1.56	40.7±2.3	>5.00	<30
Tetracycline	0.21	88.3±0.2	0.019	55.4±2.9	0.013	55.5±3.1	0.341	53.1±0.1

Results expressed as % mean ± standard deviation (n=4)

GP10 = xerantholide; DCM = dichloromethane; MeOH = methanol

Table 2. Zone of inhibition and minimum inhibitory concentrations of 16 plant crude extracts against four strains of *Neisseria gonorrhoeae*

Plant name	Inhibition zone, (mm)				MIC (mg/mL)			
	ATCC 19424	ATCC 31426	ATCC 43069	ATCC 49226	ATCC 19424	ATCC 31426	ATCC 43069	ATCC 49226
<i>Strychnos cocculoides</i> (r)	13±2	13±1	9±1	9±2	5	5	5	2.5
<i>Ziziphus mucronata</i> (r)	18±5	13±4	10±7	12±3	5	5	5	10
<i>Tribulus terrestris</i> (f)	13±5	13±3	8±1	10±1	5	5	5	2.5
<i>T. terrestris</i> (l)	11±1	13±2	10±2	11±3	10	10	10	10
<i>Talinum sp.</i> (t)	-	8±0	8±1	10±2	-	5	5	10
<i>Gomphocarpus fruticosus</i> (r)	-	14±6	9±2	12±2	-	5	10	10
<i>Indigofera hofmanniana</i> (r)	12±3	16±6	9±2	9±1	5	5	5	5
<i>Senna occidentalis</i> (r)	12±2	15±4	9±6	11±1	> 10	> 10	> 10	> 10
<i>Pechuel-loeschea leubnitziae</i> (r)	10±2	13±2	11±1	10±2	5	5	> 10	2.5
<i>P. leubnitziae</i> (l)	10±2	10±1	11±3	8±0	5	5	> 10	2.5
Plant 11 (unidentified) (r)	-	-	-	-	-	-	-	-
<i>Solanum delagoense</i> (r)	-	11±1	9±1	-	-	5	> 10	-
<i>Kigelia africana</i> (r)	-	-	8±0	8±5	-	-	> 10	> 10
<i>K. africana</i> (b)	-	10±0	9±1	9±2	-	10	10	5
<i>Aloe maculata</i> (r)	-	13±2	8±0	10±2	-	10	10	5
<i>Harpagophytum procumbens</i> (t)	-	-	-	-	-	-	-	-
Tetracycline*	30±2	29±2	35±2	10±1	NT	NT	NT	NT
Dimethylsulfoxide	-	-	-	-	NT	NT	NT	NT

Results are expressed as the mean of two trials done in duplicate (n=4); mean ± standard deviation; * = standard antibiotic; (-) no activity; (NT) = not tested. f, l, r, b = fruits, leaves, roots, and bark respectively.

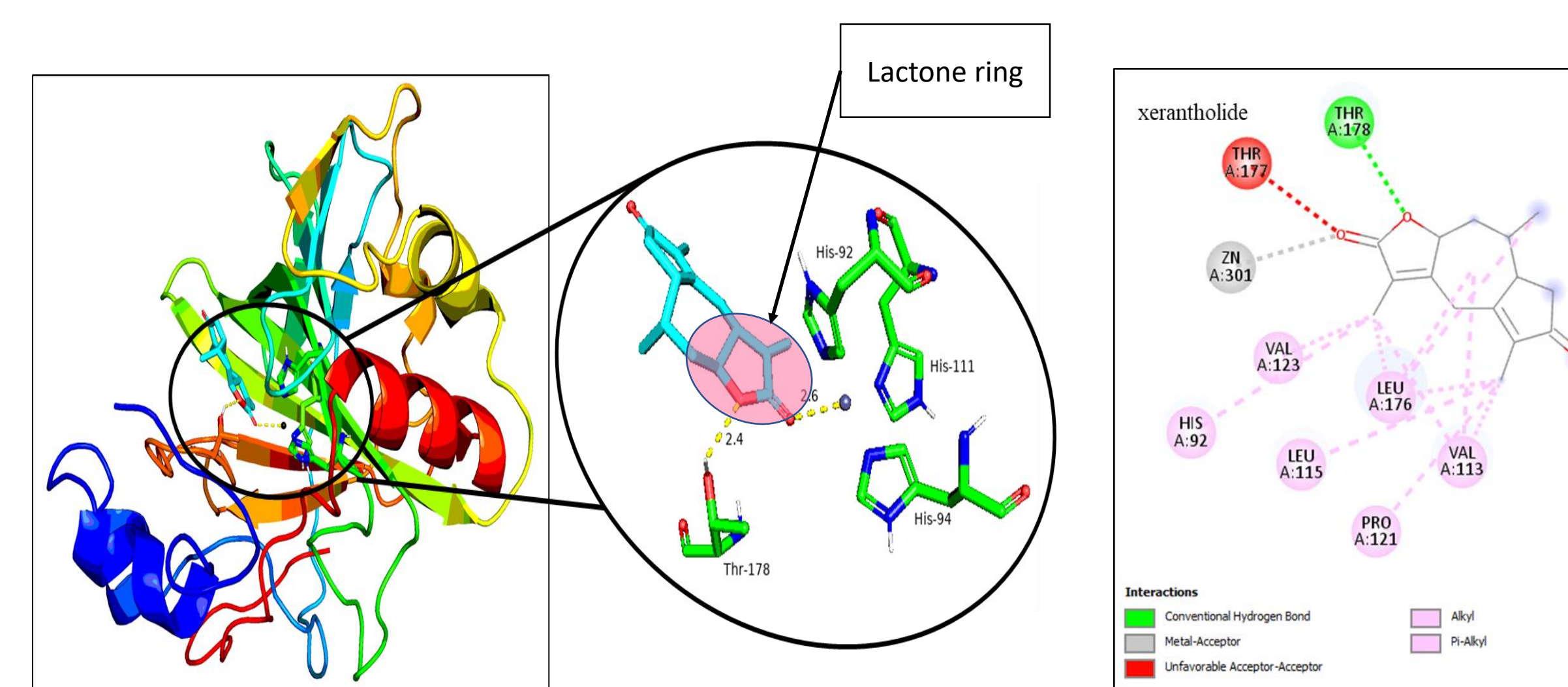


Figure 4: Interaction of xerantholide with NgCA at the active site and 2D view of binding mode and amino acids involved

Discussion

- Antimicrobial activity:** 14 (88%) extracts showed some degree of activity against at least two of *N. gonorrhoeae* strains (Table 2). Eight extracts inhibited the growth of all tested strains, suggesting that the tested plant extracts have anti-gonococcal activity. The varying degree in activity can possibly be attributed to the nature of the active ingredients as well as the level of concentration in the extracts.
- Six of the 16 plant extracts have *in-vitro* anti-gonococcal activity with MIC of 5 mg/mL (Table 2). *Strychnos cocculoides* is the most active extract with MIC of 2.5 - 5 mg/mL, followed by *Pechuel-loeschea leubnitziae* with MIC range of 2.5 – 10 mg/mL. This activity support their traditional use for treating gonorrhea.
- Isolation and structure elucidation:** A compound (xerantholide) was isolated from *P. leubnitziae* and showed good *in-vitro* anti-gonococcal activity (MIC₅₀ = 0.095 mg/mL) validating the ethnomedicinal use of this plant for gonorrhea (Table 1)^[4]. Xerantholide is a sesquiterpene lactone (Figure 3).
- Molecular docking** analysis shows that xerantholide interacts with NgCA via hydrogen bonding, metal-acceptor interaction, and non-polar alkyl and pi-alkyl interactions (Figure 4) with binding affinity of -6.8 kcal/mol which compares well with known NgCA inhibitor acetazolamide ⁽⁴⁾. The interaction mode involve the lactone ring and several amino acids.

Conclusion and recommendation

- Antimicrobial analysis results support the traditional use of 10 of the 13 plants for gonorrhoea treatment, six (*S. cocculoides*, *Z. mucronata*, *T. terrestris*, *I. hofmanniana*, *P. leubnitziae* and *Talinum* sp.) extracts exhibited anti-gonococcal activity against the four strains of *N. gonorrhoeae* with MIC ranging from 2.5-10 mg/mL and the other four extracts showed antimicrobial activity with MIC of ≥10 mg/mL.
- An active compound, xerantholide isolated from a plant proved to have both anti-gonococcal activity *in-vitro* and the potential as an enzyme inhibitor.
- Further studies to isolate and identify the active compounds from other plants is recommended
- To confirm binding of xerantholide to NgCA, co-crystallization should be done
- Work is on-going to identify other plants used ethnomedicinally and isolate active compounds

References

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