



Evaluating the anti-microbial activity, bioactivity and chemical constituents of essential oil distilled from the seeds of *Trachyspermum roxburghianum*, used to impair gastrointestinal disorders.

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Introduction

Trachyspermum roxburghianum, generally known as 'Asamodagam' in Sinhala, and Ajwain or Bishop's weed in English. *T. roxburghianum* are generally employed in the product of 'Siddhalepa Asamodagam spirit' (Sid.AS), which is the distilled product deduced from these seeds.



Figure 1. Exterior view of *Trachyspermum roxburghianum* a) View of the plant; b) View of the seeds; c) View of the authenticated specimen sample of *T. roxburghianum*

The current investigation aimed to determine the anti microbial activity, *in-vitro* anti-urease activity (Urease inhibition assay), anti-inflammatory activity (HRBCM stabilization assay) and analysis of Chemical constituents of Sid.AS obtained from the seeds of *Trachyspermum roxburghianum*. The Chemical constituents of Sid.AS were discovered and quantified using the GC-MS technique.

The anti-inflammatory assay was conducted using the human red blood cell stabilization method, which uses the heat induced hemolysis principle. The assay was conducted for Sid.AS, and the positive control (Aspirin) separately. The absorbance was measured at 560 nm using the Microplate Reader (Biotek, USA).

In Urease inhibitory assay, test solutions of Sid.As, and positive control (Thiourea) were prepared with concentrations of 0.625, 1.25, 2.50, 5.00 and 10.00 mgmL⁻¹. The absorbance of each well was measured at 625 nm by the Microplate reader (Biotek, USA).

In vitro antimicrobial studies were carried out against human pathogenic microorganisms including two strains of Gram-positive(+) bacteria (*B. subtilis* (ATCC-6633) and *S. aureus* (ATCC-25923)) and one strain of Gram-negative bacteria(-) (*E. coli* (ATCC-25922)) and one fungal strain (*Candida albicans* (ATCC-10231)) using well diffusion method. The prepared concentrations from Sid.AS samples were ranged between 3 mg/mL – 15 mg/mL and Amoxycillin and Fluconazole used as positive controls for ABST and AFST, respectively.

Future works

This study only reflects the *in vitro* investigations of the distillate of *T. roxburghianum* (Sid.AS). Therefore, further experiments should be designed to conduct a clinical trial to provide *in vivo* evidence of the anti-microbial activity & bioactivity of the distillate of *T. roxburghianum* (Sid.AS) in the future.

Results of (Sid.AS) the anti-microbial activity against pathogenic microorganisms

Concentration (mg/mL)	Antibacterial Sensitivity Test (ABST) / Antifungal Sensitivity Test (AFST)			
	Zone of inhibition (mm) / Mean $\pm$ standard deviation			
	<i>E. coli</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>C. albicans</i>
3	11.00 $\pm$ 0.50	NI	NI	NI
5	12.77 $\pm$ 0.63	11.83 $\pm$ 0.29	NI	NI
7	15.07 $\pm$ 0.29	13.67 $\pm$ 0.76	10.33 $\pm$ 0.58	NI
9	15.67 $\pm$ 0.67	15.67 $\pm$ 0.76	14.50 $\pm$ 0.87	11.33 $\pm$ 0.29
11	17.67 $\pm$ 0.29	17.00 $\pm$ 1.00	19.17 $\pm$ 1.16	11.83 $\pm$ 0.76
13	18.17 $\pm$ 0.29	21.00 $\pm$ 1.00	22.83 $\pm$ 1.26	18.67 $\pm$ 0.28
15	21.17 $\pm$ 1.26	23.39 $\pm$ 1.34	23.33 $\pm$ 1.04	21.50 $\pm$ 0.50
Positive(+)	25.17 $\pm$ 0.29	39.17 $\pm$ 1.26	34.64 $\pm$ 0.58	22.00 $\pm$ 0.50
Negative	NI	NI	NI	NI

NI - NO Inhibitory zone

Three replicates are used for each analysis.

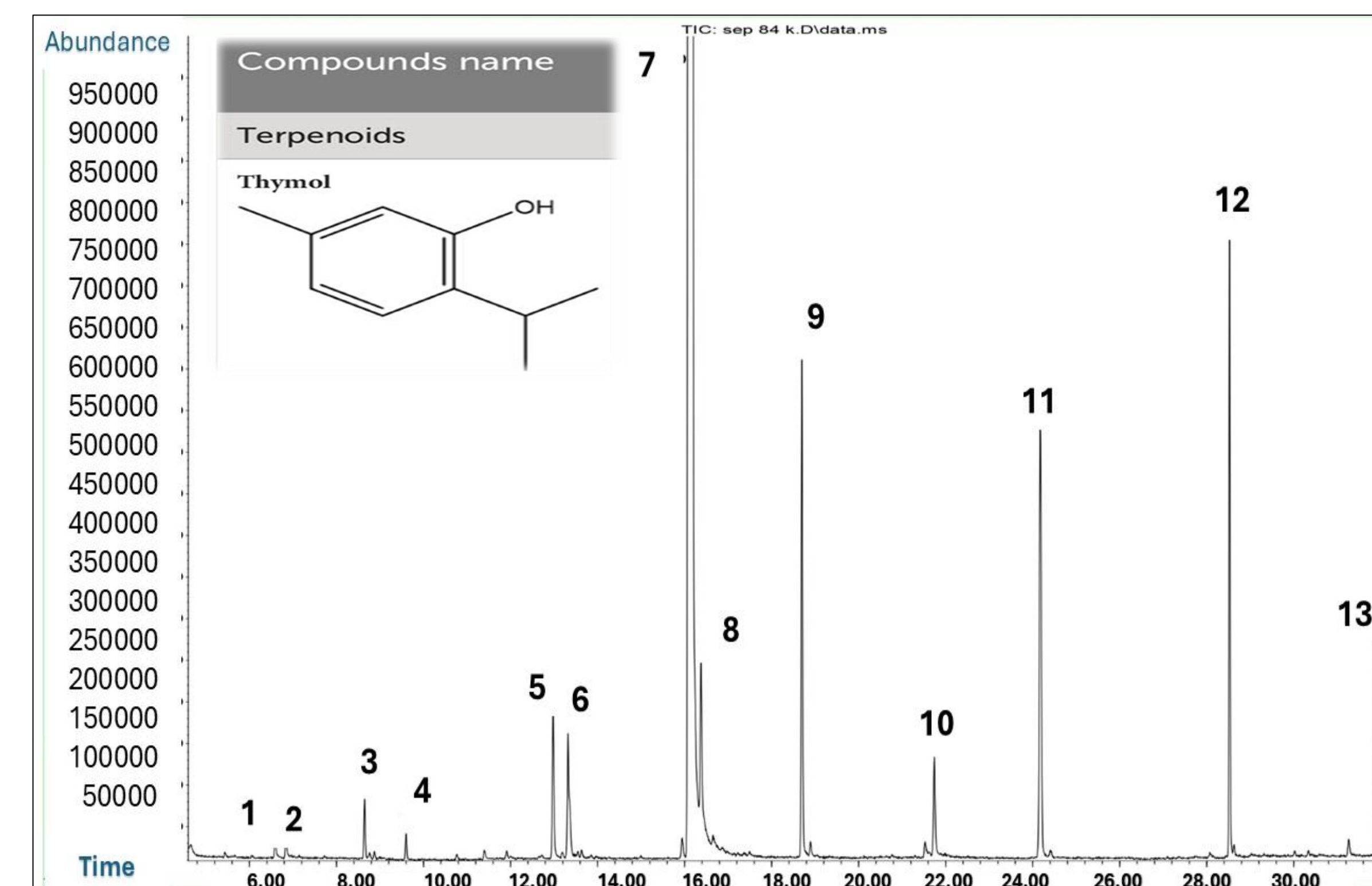
- The results revealed that Sid.AS was potentially effective in suppressing microbial growth of tested bacteria and the fungus with variable potency.
- It was dose dependent, and the highest inhibition zone (23.39 $\pm$ 1.34 mm) was observed for *S. aureus* and *B. subtilis* at 15 mg/mL concentration of the Sid.AS. Samples.

Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC) values of Sid. AS against test pathogenic microorganisms

MIC/MLC (mg/mL)	Test Microorganisms			
	<i>E.coli</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>C. albicans</i>
MIC	5.00	5.00	11.00	9.00
MLC	7.00	7.00	13.00	11.00

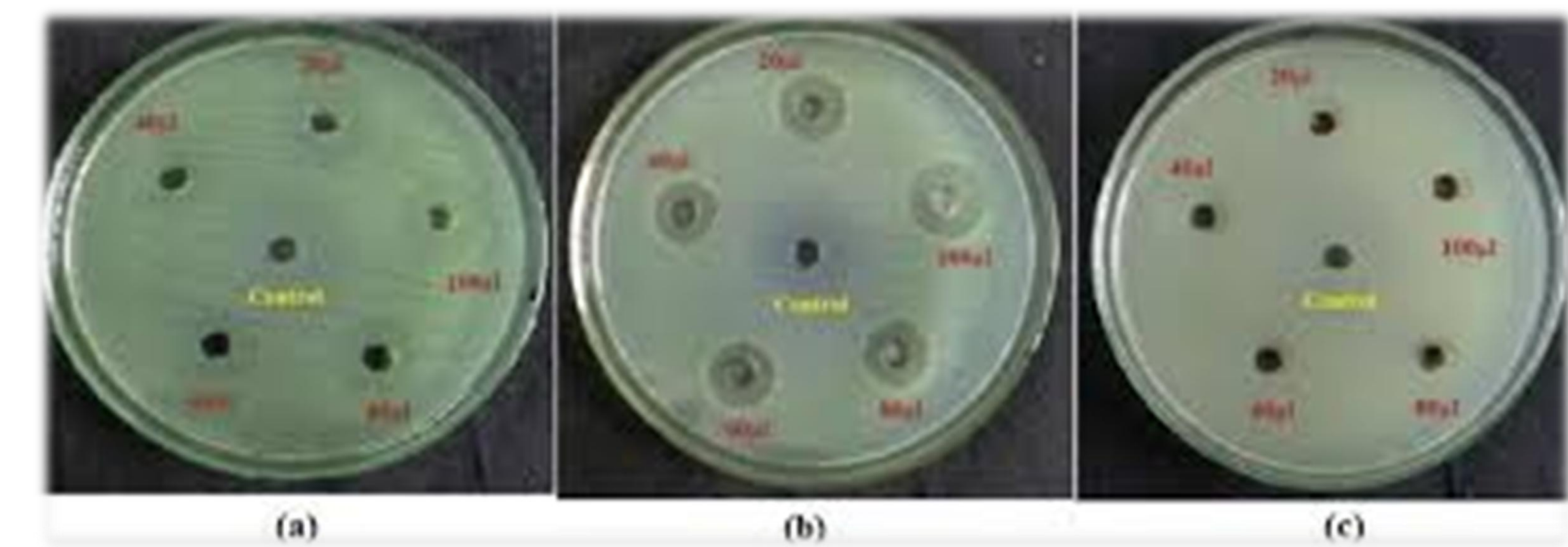
- The concentration of the Sid.AS is inversely proportional to MIC and MLC values and the lowest MIC and MLC observed were 5 mg/mL and 7 mg/mL respectively for the both pathogenic bacteria as *E.coli* and *S. aureus* indicating the potent antibacterial effect of the Sid.AS.

Results of (Sid.AS) Chemical constituents using GC-MS method



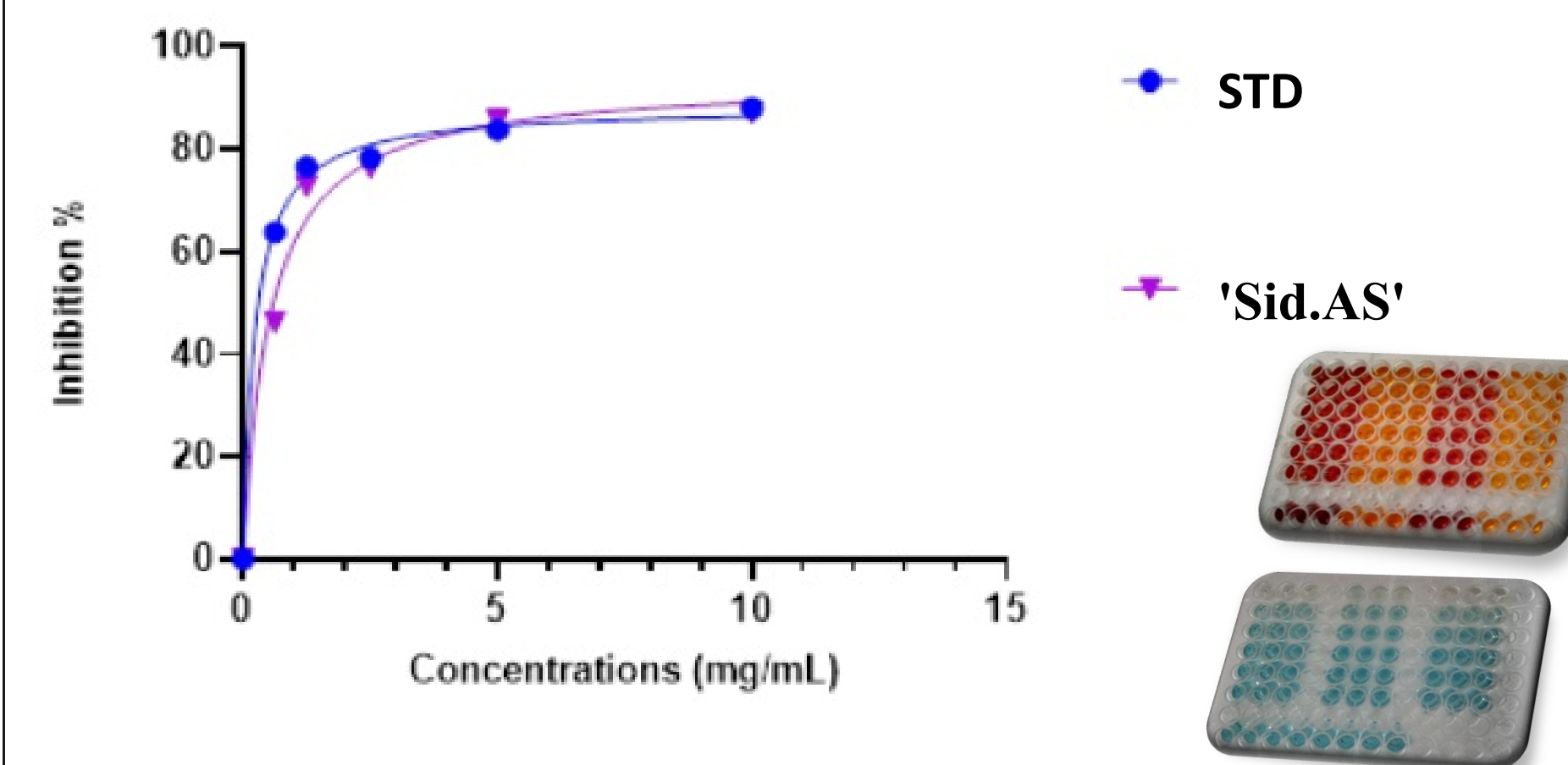
The chemical constituents are listed in order of their elution on the DB-5ms column.

Peak #	Compound	Retention time (min.)	EtOAc Extract of Sid.As Area %
1	α - Thujene	6.46	0.10
2	α - Pinene	6.65	0.10
3	α phellandrene	8.70	0.18
4	1,8- Cineole	9.66	-
5	terpinolene	12.76	0.47
6	trans-sabinenhydrate	12.95	0.53
7	Thymol	16.00	92.73
8	carvacrol	16.60	0.89
9	2-Tetradecene	18.70	1.32
10	Phenol, 2,4-bis-(1,1-dimethylethyl)	21.70	0.36
11	Cyclohexadecane	24.20	1.58
12	E-15-Heptadecenal	28.50	1.34
13	Stearic acid	31.90	0.18



Results of (Sid.AS) the Urease & anti-inflammatory inhibitory activity

The graph of inhibition % vs concentration (mg/mL)



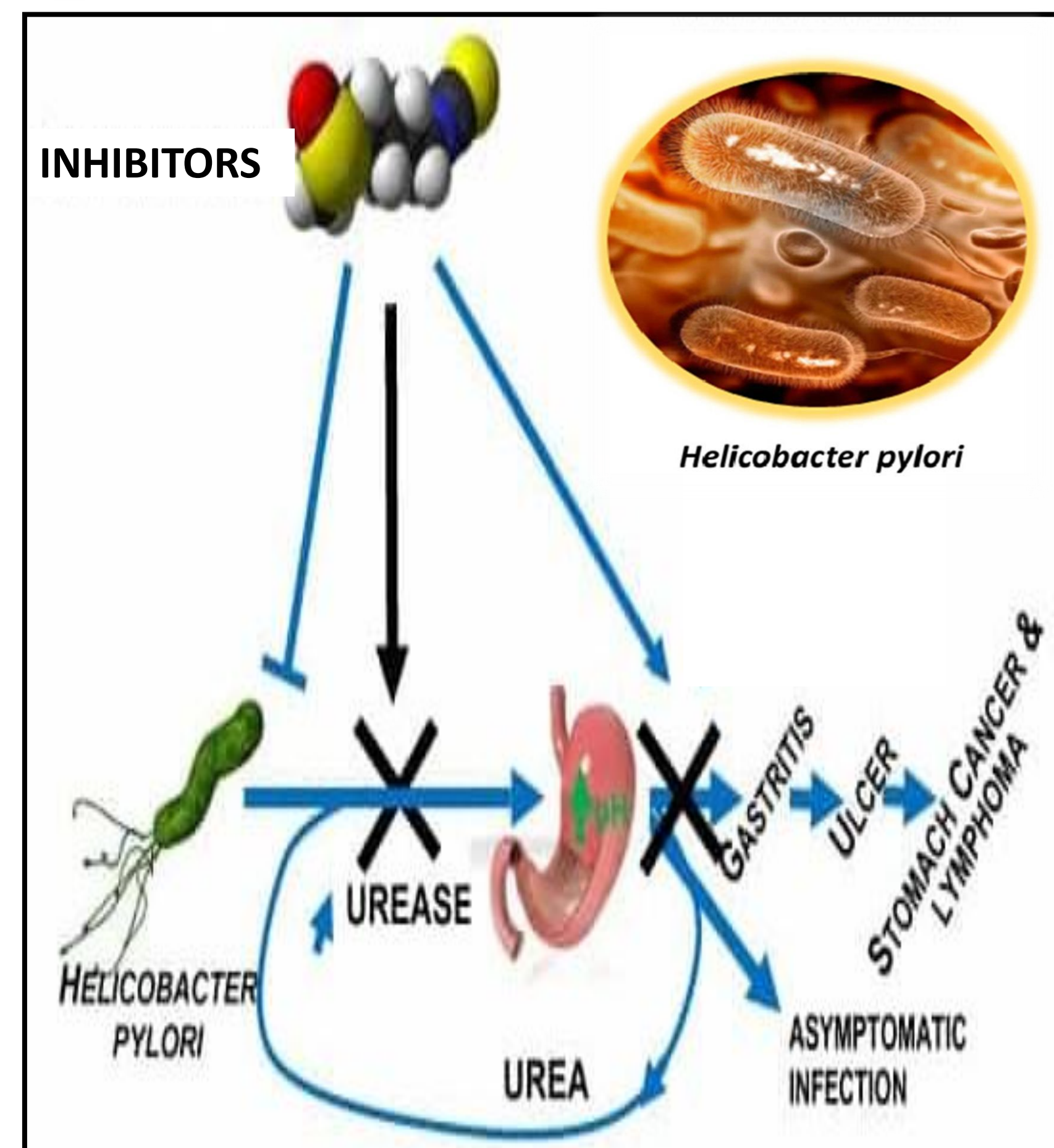
Samples	Urease assay IC ₅₀ values (mg/mL)	anti-inflammatory as. IC ₅₀ values (mg/mL)
(+) Control (STD)	0.08 $\pm$ 0.01 ^a (Thiourea)	0.24 $\pm$ 0.02 ^a (Aspirin)
'Sid.AS'	0.17 $\pm$ 0.01 ^b	0.57 $\pm$ 0.09 ^b

- The study revealed that the bioactive constituents present in the 'Sid.AS' contribute to increasing the urease inhibitory activity compared to the (Thiourea) positive control.
- These suggests that Sid.AS may exert its anti-inflammatory activity through mechanisms similar to those of Aspirin & potential of Sid.AS as a natural anti-inflammatory agent, offering a promising avenue for the management of inflammatory conditions and related diseases.

- Helicobacter pylori* is a type of gram(-) rod-shaped bacteria that lives in the human digestive tract & they can survive in acidic conditions by producing the urease enzyme and causes gastric tract syndromes.
- WHO has considered *H. pylori* as a class I carcinogen.
- These studies revealed that antibacterial effect of thymol causes the permeabilization & depolarization of cytoplasmic membrane of *E. coli*, *H. pylori* and other pathogenic bacteria.

References

- Jayawantha D, Hettigoda L, Mudalige TD, Paranagama PA. Exploring the Bioactivity of Siddhalepa Asamodagam Spirit from Seeds of *Trachyspermum roxburghianum* (DC.) H. Wolff. *Natural Product Communications*. 2024;19(8). doi:[10.1177/1934578X241271629](https://doi.org/10.1177/1934578X241271629)



Conclusions

- The major compound (92.73%) present in 'Sid.AS' was identified as thymol.
- Due to the presence of thymol as a major constituent in 'Sid.AS', it showed significant antibacterial & antifungal activity.
- These findings suggest that Sid.AS exhibits promising antimicrobial properties against various human pathogenic microorganisms, highlighting its potential as a natural antimicrobial agent.
- Finally, Its multifaceted remedial properties make it a precious element in various medicinal and culinary applications, pressing its potential for promoting good health and well-being.