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Isolation and characterisation of cryptolepine from *Sidaacuta*Burm and its antimycobacterial activity

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ABSTRACT

Background

*Sida acuta*is a shrub indigenous to pantropical areas, widely distributed in regions and found in pastures, waste lands, cultivated lands, roadsides, lawns and planted forests. *Sidaacuta is* ethanomedically used as treatment of diuretic, asthma, fever, headache, cough, cold, ulcer, anthelmintic, snake bite, urinary disease, female disorders, sedative, eczema, kidney stone, elephantiasis, testicular swelling, poultice for dandruff, rheumatic affections, facial paralysis, pulmonary tuberculosis and gonorrheae.

Methods

Hydro-alcoholic extract of *Sidaacuta* was chromatographed (Column) to isolate cryptolepine. The isolatetd SA-I was subjected to various spectral studies such as ultraviolet spectroscopy, infrared spectroscopy, nuclear magnetic resonance spectroscopy and mass spectroscopy. The hydro-alcoholic extract of *Sidaacuta* and isolated SA-I were evaluated for its anti-mycobacterial studies by Luciferase reporter phage assay method using mycobacterial tuberculosis standard strain of H_37R_V.

Results

The isolated compound (SA-I) showed the spectral data of cryptolepine. The percentage inhibition of hydro alcoholic extract of *Sidaacuta* (500 & 1000µg/ml) against mycobacterium tuberculosis showed antimycobacterial effect was found to be 99% in comparison with isoniazid (1ug/ml) 92% and rifampicin (1µg/ml) 96%. Whereas isolated cryptolepine did not any sensitiveness towards mycobacterium tuberculosis.This research indicatecryptolepine did not show any antimycobacterial effect. It specifyantimycobacterial effect of hydro-alcoholic extract of *Sidaacuta* may be due to the combined nature of cryptolepine with other phytoconstituents.

Conclusion

The present research draws the conclusion that this *Sida acuta* plant showed extreme antimycobacterial effect, which may be due to the phytoconstituents.

Keywords: Antimycobaterial, Cryptolepine, *Sida acuta*, Malvaceae

INTRODUCTION

Sida acuta is shrub indigenous to pantropical areas, weed is frequently found in pastures, waste lands, cultivated lands, roadsides, lawns and planted forests.

Sidaacuta used in ayurvedic preparation as diuretic, sedative, abortifacient for the treatment of, asthma, fever, headache, cough, cold, ulcer, anthelmintic, snake bite, urinary disease, female disorders (Mudaliar, 1998) [19] eczema, kidney stone, elephantiasis, testicular swelling, poultice for dandruff, rheumatic affections, azoospermia, oligospermia, spermatorrhea, leucorrhoea, wounds, sciatica, nervous and heart disease, facial paralysis, pulmonary tuberculosis, gonorrheae (Ananil, K, *et al* 2000, [4]Silja, VP *et al.*,2008, Oliver Tene*et al*.,2017, Saraswathy*et al*.,1998)

The literature survey of the plant revealed the presence of tannin, saponin, flavonoid, terpenoids, cardio glycoside, vitamin composition was thiamine, niacin, ascorbic acid, tocopherol, riboflavin and mineral composition was calcium, magnesium , zinc, steroids (ecdysterone, β-sistosterol, ampesterol), phenolic compounds (evofolin-A and B, scopoletin, loliolid and 4-ketopinoresinol, polyphenol, sesquiterpene, alkaloid cryptolepine, quindoline and quindolinone and fixed oil (Nwankpa, P et al.,2015 [20], Konate, K *et al*., 2010 [15], Jang, DS *et al*.,2003, [15]Palaksha, MN *et al* 2012) [27, 28]

The plant exhibited various pharmacological activities such as antibacterial (DamintotiKarou*et al* 2005), antimicrobial(Sanganuwan*et al*.,2006) larvicidal and reppellent (MarimuthuGovindarajan*et al*., 2010), gastric anti-ulcer (Akilandeswari*et al*., 2010), insecticidal (Adeniyi, SA *et* a.,2010) [1], hypoglycemic (Okwuosa, CN. *et al*.,2011) [22], anti-pyretic (Sharma, R.*et al*.,2012) [35], anthelmintic (Palaksha, MN. *et al*., 2012) [29], antioxidant and thrombolytic (EntazBahar*et al*.,2013) [8] electrolytes and organ function parameters (Enemor, VHA. *et al*., 2013) [9], diuretic and anti-urolithiatic (Palaksha, MN *et al*., 2015) [29], invitro stability and aggregatory (ObiomaBenedethEze, *et al*.,2016) [23, 24] anti – inflammatory (ObiomaBenedethEze. *et al*., 2016) [24, 25] alpha amylase Inhibitory (KemiFeyisayo*et al*.,2017), hepataprotective(Sridevi.*et al*.2009) calcium oxalate crystal growth inhibitory (Vimala,T*et al*., 2012) [38] corrosion inhibitory (Umoren., *et al*.,2016) [37], antiplasmodial (Benzouzi. *et al*., 2004) [5], analgesis (Oboh, IE.*et al*., 2005) [26], anti-venom (Otero.*et al*.,2000) [26], anti-malaria (DamintotiKarou*et al*., 2003) [6] anti-ulcer (Malairajan, P *et al*., 2006), wound healing (Akilandeswari, S *et al*.,2010) [2, 3], cytototoxicity (Pieme, *et al*., 2010) cardiovascular (Kannan, RR et al., 2012), antifungal (Jindal Alka., *et al* 2012) [12], anticancer (Mahesh Thondawada. *et al*., 2016) [18]

The modern medicine causes hepatitis, lethargy, rashes, fever, acne, arthralgia, headache, redness and bone pain.

An attempt was taken to investigate isolated SA-I and invitroantimycobacterial effect for this plant.

MATERIALS AND METHODS

Plant collection & authentication

Fresh leaf of *Sidaacuta*Burm were collected from the komanampatty village Dindigul (Dist), (Tamil Nadu) during the month of August-2017 was authenticated by DR. D.Stephen, M.Sc., Ph.D., Assistant Professor, Department of Botany, American College, Madurai-20.The herbarium of this specimen was kept in the department for further references

Preparation of hydro-alcoholic extract of *Sidaacuta*Burm. (HAESA)

Procedure

The shade dried and coarsely powdered leaf of *SidaacutaBurm.* (Leaf) was defatted with petroleum ether (60-80˚c). The residue was dried and extracted with hydro-alcohol (70%) by maceration until the complete extract of the material and filtered. The extract was concentrated under reduced pressure to obtain a solid residue (dark brown).

Isolation of compound SA-I

Powdered, oven-dried (60^0 C) leaves of *Sidaacuta* 50gm were defatted with 100 ml of petroleum ether for 8 hours. The marc was extracted by soxhlet with methanol 100 ml Methanol for 8 hours the extract was evaporated under reduced pressure to give a residue 3.4gm which was thoroughly mixed with AcOH (5%

500ml). The mixture diluted with water (250ml) and allowed to stand overnight. The mixture was filtered affording a clear solution and some insoluble material.The aqueous filtrate was made basic with aqueous ammonia to PH 9.5 and extracted with $CHCl_3$ (500ml). Extracts were dried over anhydrous $MgSo_4$, filtered and evaporated to afford a dark alkaloidal residue (450mg). The alkaloidal residue was dissolved in $CHCl_3$ (25ml) Adsorbed on to Al_2O_3 (20gm) and chromotographed over Al_2O_3 (200gm). Elution was conducted successively with petroleum ether 40-60 (500ml) followed by petroleum ether-$CHCl_3$ (500ml) and $CHCl_3$ (500ml) $CHCl_3$- MeOH (9:1) 500ml and finally MeOH (500ml). QUINDOLINE was obtained as yellow solid after column chromatography and preparative tlc on AlO_3 in chloroform. Which gave an orange colouration with Dragendorff's reagent, which is evidence for quindoline alkaloids. Elution of the column with MeOH (9:1) afforded a violet residue.it crystallized from aqueous ethanol as long violet needles shaped named as SA-I

Spectral analysis of the Isolated SA-I subjected to ultraviolet spectroscopy infrared spectroscopy, nuclear magnatic spectroscopy, mass spectroscopy and the results are displayed in **Figures**:1,2,3,4 and 5.Tabulated in: **Tables:** 1,2,3,4 and 5

DETERMINATION OF ANTIMYCOBACTERIAL ACTIVITY OF HAESA

Luciferase reporter phage assay (LRP)

Antimycobacterial effect was determined forhydro-alcoholic extract of *Sidaacuta*Burm as per (Papitha.n*et al* 2013)

Procedure

Standard strain H37RV and a clinical isolate of *M.tuberculosis*resistant to S, H, R& E were grown in Middlebrook 7H9 complete medium 12 with and without extracts of *Sidaacuta*Burm. for 3 days at 37°C. Luciferase Reporter Phage Assay10 was done using concentrations of 500 and 1000 µg/ml of *Sidaacuta*Burm extracts and isolated SA-I. Fifty-microliter bacterial suspension equivalent to MacFarlands No.2 standard was added to 400 µl of G7H9 with and without the test compound. For each sample, two drug-free controls and two drug concentrations were prepared and this set up was incubated for 72 h at 37°C. After incubation, 50 µl of the high titer Luciferase reporter phage (phAE129) and 40 µl of 0.1 M CaCl2 were added to all the vials and this setup was incubated at 37°C for 4 h. After incubation, 100 µl of the mixture was taken from each tube into a luminometer cuvette and an equal amount of working D-luciferin (0.3 mM in 0.05 M sodium citrate buffer, pH 4.5) solution was added. The RLU was measured after 10s of integration in the Luminometer. Duplicate readings were recorded for each sample and the mean was calculated. The percentage reduction in the RLU was calculated for each test sample and compared with control. The experiment was repeated when the mean RLU of the control was less than 1000. The results are tabulated in **Table: 6** and displayed **Figure: 6**

RESULTS AND DISCUSSION

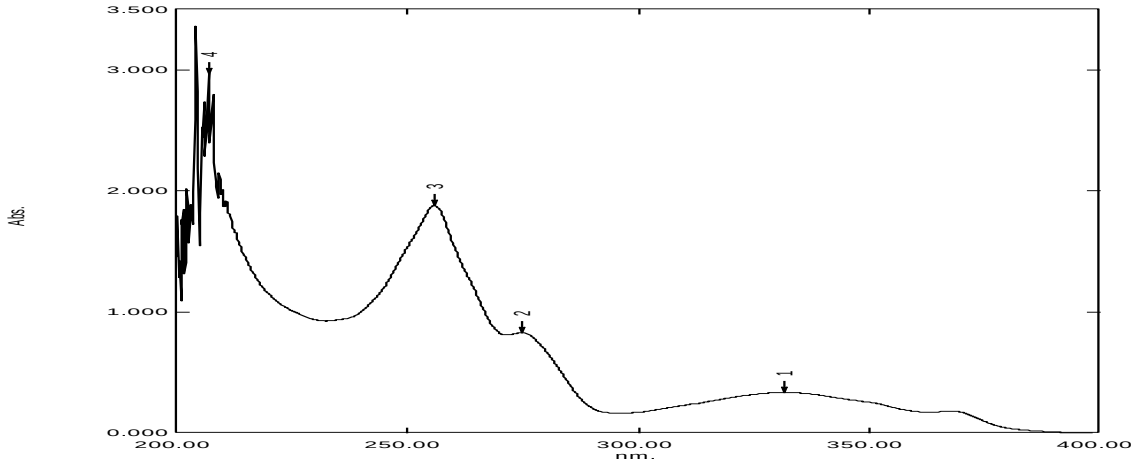


Figure :1 UV spectrum of SA-I

The methnolic(100%) fraction of HAESA was pooled and the named as SA-I.The isolated compound SA-I was dissolved in methanol and was scanned under UV range from 200-600 nm in the UV-Visibble spectrophotometer. The isolated compound SA-I showed absorbance maxima at 255nm, 274nm and 331,which indicates the presence of an aromatic ring.

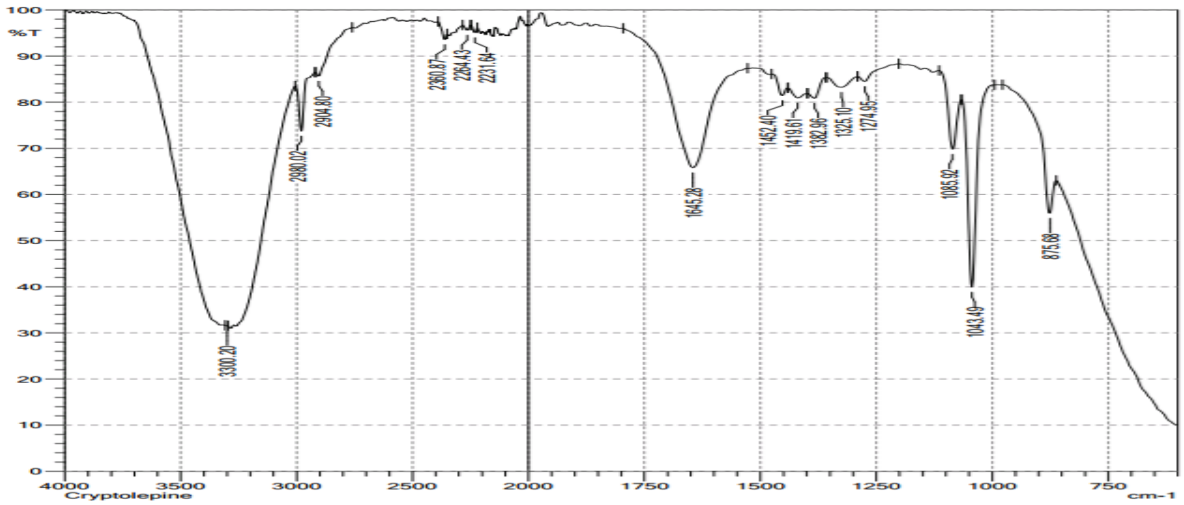


Figure: 2 Infrared spectroscopy of isolated SA-I

Table: 2 Interpretation of Infrared spectrum of SA-I

S.no	Standard IR Range (cm^{-1})	Observed IR range(cm^{-1})	Intensity	Types of band	Functional group
1	3200-3600	3300	Strong, Broad	Stretching	OH, Alcohol
2	2850-300	2904, 2980	C-H-, Alkane Strong	Stretching	C-H-, Alkane
3	2100-2270	2264, 2231	Variable not present in symmetrical alkynes	Stretching	-C≡C-, Alkyne

4	1620-1680	1645		Variable	Stretching	-C=C, Alkane
5	1350-1480	1452, 1419	Variable	bending	-C-H, Alkane	
6	1345-1385	1382	Strong two bonds	Stretching	N-O, Nitro	
7	1080-1360	1325, 1274,1085	Medium	Stretching	C-N, Amine	
8	1000-1300	1043	Strong	Stretching	C-O, Ether	
9	675-1000	875	Strong	Stretching	=C-H, Alkane	

The isolated compound SA-I was scanned under IR range from 600cm^{-1}- 4500cm^{-1} in the IR spectrophotometer. The IR spectrum of isolated SA-I shows a broad absorption band at 3300 cm^{-1} indicates (alcoholic –OH), 2904cm^{-1} and 2980cm^{-1} (alkane-C-H), 2264 cm^{-1} and 2231cm^{-1} (Alkyne-C≡C) 1645cm^{-1} (Alkane C-C,) 1452cm^{-1} and1419cm^{-1} (-C-H alkane), 1382cm^{-1} (nitro N-O), 1325cm^{-1},1274cm^{-1} and1085cm^{-1} (Amine-C-N),1043cm^{-1} (Ether-C-O), 875cm^{-1} (Alkane-C-H),which indicates unsaturated compound with tertiary amine linkage.

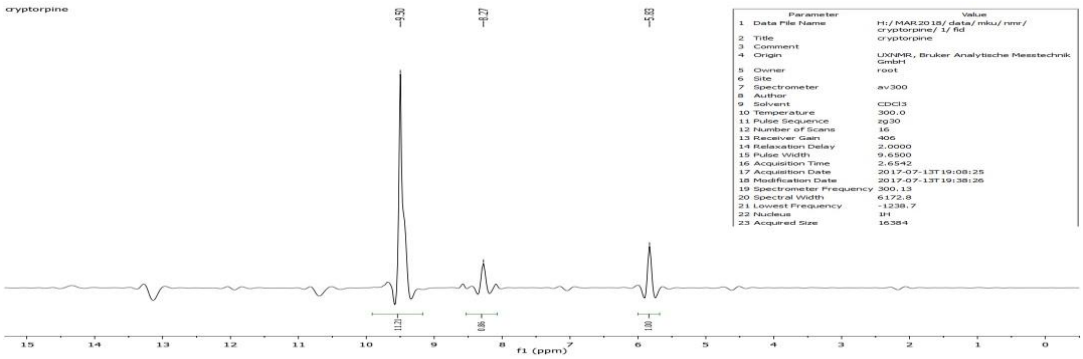


Figure: 3 ^{1} H NMR spectrum of SA-I

Interpretation of ^{1}H NMR spectrum of SA-I

The ^{1}H NMR of isolated SA-I showed an intense singlet at δ 5.83ppm with integral of 36mm corresponding to 3H of the methyl group of SA-I at position 5.Since the methyl group at position 5 of SA-I has no neighbouring protons to couple it. It gave a singlet peak at 5.83ppm typical of signal produced by methyl group attached to tertiary nitrogen.

The ^{1}H NMR of isolated SA-I showed aromatic multiples from δ 7.467 to 8.808 ppm aromatic singlet at δ 9.50 ppm corresponding to eight aromatic proton and isolated aromatic hydrogen of SA-I respectively. The aromatic singlet at δ 9.50 ppm from TMS may be due to the hydrogen at position 11 of cryptolepine (H-11) which is the only H without neighbouring hydrogen atoms to split its signal.

Table: 3 Interpretation of NMR spectrum of SA-I

S.no	Delta value ppm	Position of Hydrogen atom	Type of peak
1	5.83	H-5	Singlet
2	8.27	H-6	Aromatic multiplet
3	9.50	H-11	Singlet aromatic proton & isolated aromatic proton.

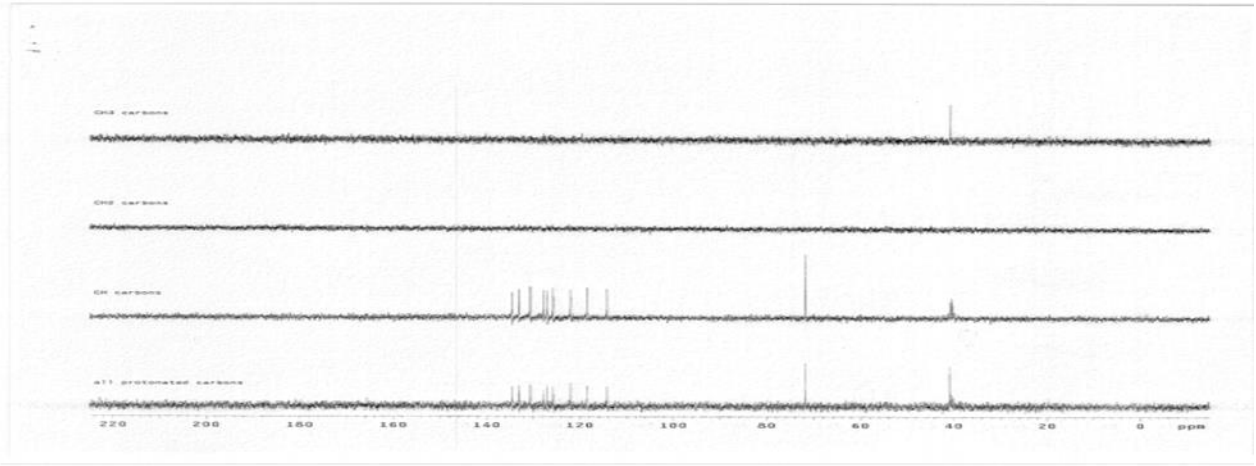


Figure: 4 ^{13}C NMR Spectrum of isolated SA-I

Interpretation of ^{13}C NMR spectrum of isolated SA-I

Hetero aromatic carbons give signals within the range δ 100-165ppm andnine peaks within this range of chemical shift under CH 13C DEPT analysis of isolated SA-I. This confirms the nine protonated hetero aromatic carbons in SA-I: C-1,2,3,4,6,7,8,9, and 11.sp3 hybridised carbons absorb at lowest field (δ 0-50ppm) as compared to sp2(δ100-160ppm aromatic) and sp (δ 65-90ppm) hybridised carbons. Isolated SA-1 showed a singlet at δ 40.3ppm which confirms the presence of the only methyl carbon on the quinoline nitrogen at position 5 of SA-I (>N-CH3).

Table: 4 Interpretation of ^{13}C NMR spectrum of Isolated SA-I

S. No	Delta value ppm	Types of Carbon
1	100-165	Hetero aromatic carbon
2	0-50	Hybridised carbon
3	100-160	Aromatic carbon
4	40.3	Methyl carbon

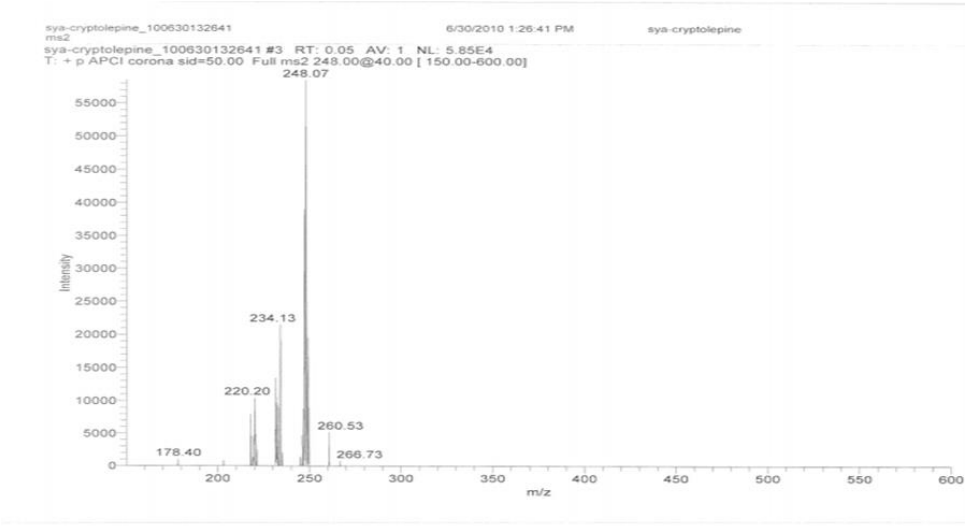


Figure: 5 Mass spectrum of isolated SA-I

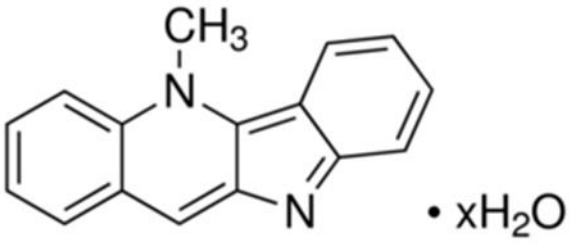
Interpretation of Mass spectrum of isolated SA-I

The base peak of mass spectrometric analysis of isolated SA-Iwas found to be at *m/z* Value of **248.07** corresponding to 100% relative intensity. Hence, the base peak of the mass spectrum of the isolated SA-I which also corresponds to the molecular ion actually occured at *m/z* **248.07**. This value is in agreement with the literature value of the molecular weight of SA-I which was 232g. The peak occuring at *m/z* **220.20**(29.36%).might correspond to N-demethylated fragment of SA-I.

Table: 5 Interpretation of Mass spectrum of SA-I

S. No	Type of ion	m/z Value
1.	Molecular ion	248.07
2	Methylene fragment	234.13
3	Demethylated fragment ion	220.20

STRUCTURE OF SA-I



IUPAC NAME : 5-methyl 5-H indolo [3,2-b]quinoline.(hydrated cryptolepine)
Molecular Formula : $C_{16}H_{12}N_2$
Molecular Weight : 248.286g/mol

DETERMINATION OF ANTIMYCOBACTERIAL ACTIVITY OF HYDRO ALCOHOLIC EXTRACT OF *Sidaacuta*Burm (Leaves) (HAESA) by Luciferase reporter phage assay

The antimycobacterial effect determined for the hydro-alcoholic extract of *Sidaacuta*Burmand isolated SA-I as per **(Papitha.n*et al* 2013)** [38]

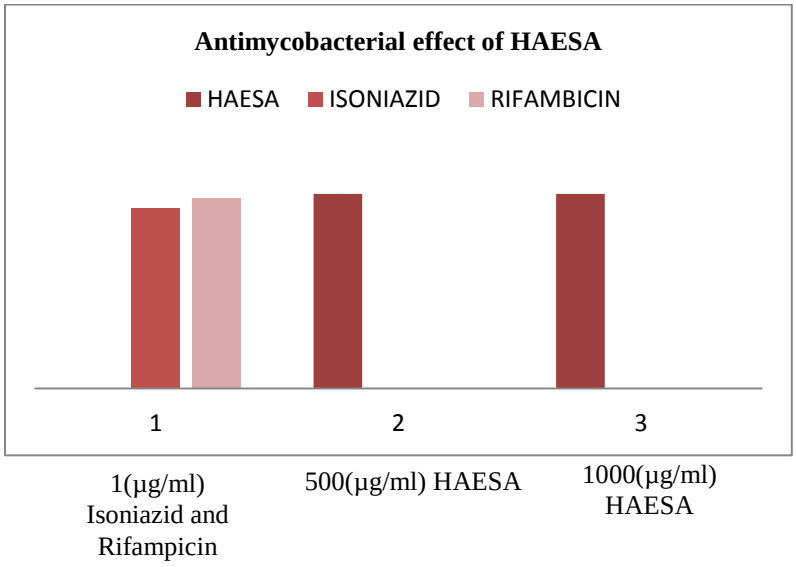


Figure:6 Antimycobacterial effect of Hydro-alcoholic extract of *Sidaacuta*burm (leaves) (HAESA)

Table: 6 Determination of Antimycobacterial activity of Hydro-alcoholic extract of *Sidaacuta*Burm leaves against standard strain of Mycobacterium tuberculosis H37Rv.

S.No	Compound	Concentration(µg/ml)	Relative light unit value	% of Inhibition
1	Isolated alkaloid SA-I	500	12715	0%
		1000	12596	0%
2	HAESA	500	110	99%
		1000	75	99%
3	Isoniazid	1	792	92%
4	Rifampicin	1	299	96%

The percentage inhibition of *Sidaacuta* Leaf (HAESA) against mycobacterium tuberculosis standard strain of H37Rv. HAESA (500µg/ml and 1000µg/ml) showed anti mycobacterial effect was found to be 99% in comparison with isoniazid (1ug/ml) 92% and rifampicin (1µg/ml) 96%.

The isolated compound (SA-I) showed the spectral data of cryptolepine. The percentage inhibition of hydro alcoholic extract of *Sidaacuta* (500 & 1000µg/ml) against mycobacterium tuberculosis showed antimycobacterial effect was found to be 99% in comparison with isoniazid (1ug/ml) 92% and rifampicin (1µg/ml) 96%, whereas isolated cryptolepine did not any sensitiveness towards mycobacterium tuberculosis.This research reports cryptolepine did not show any antimycobacterial effect. It indicated that antimycobacterial effect of hydro-alcoholic extract of *Sidaacuta* may be due to the combined nature of cryptolepine with other phytoconstituents.

CONCLUSION

The present research draws the conclusion that hydroalcoholic extract of *Sidaacuta*plant showed extreme antimycobacterial effect, which may be due to the combined nature phytoconstituents.

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