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Antimicrobial Activity And Tetrahydrofuran From Medicine "Sidabiyai".

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Abstract: The antibacterial and antifungal activities of various extracts from the medicine "Sidabiyai" were screened by microdilution method on the standard and isolated strains of gram negative bacteria i.e. *Escherichia coli*, *Pseudomonas putida*, *Pseudomonas auriginosa* , *Klebsiella pneumoniae* and Gram positive *Staphylococcus aureus* against broad spectrum antibiotic Amphoterecine and the fungal species i.e. *Aspergilus flavus*, *Aspergilus niger*, *Aspergilus fumigatus*, *Candida albicans* and *Microsporum gypseum*,against well known antibiotic Chloramphenicol. Sidabiyai extracts manifested explicit antibacterial activity on specific strains i.e. ethanol extract on *Staphylococcus aureus* and *Pseudomonas auriginosa;* chloroform extract on *Pseudomonas putida* and *n- butanol* extract on *Staphylococcus aureus* that elucidate conspicuous impact. No significant upshot with any of the extracts for an exclusive antifungal activity to test fungi in comparison to Chloramphenicol. In detection of compounds, the traditional home made medicine the "Sidabiyai" patented tetrahydrofuran, an antimicrobiant that exhibited the sui generis associativeness in antimicrobial potency of the medicine.
Key Words: Antibacterial activity, antifungal activity, tetrahydrofuran, medicine "Sidabiyai".

INTRODUCTION

Leucorrhoea of white yellowish or bluish a common gynecological problem faced by the gynecologist and is often difficult to treat is an abnormal excessive vaginal discharge, often associated with irritation and pruritus. Leucorrhoea could be physiological when associated with various phases of menstrual cycle or due to cervical/vaginal inflammation or diseases. It can be due to infection with *Trichomonas vaginalis*, *Candida albicans* or mixed bacterial infections, chronic cervicities, cervical dysplasia, malignancy, or due to senile vaginitis [1]. Broad symptoms including pain in the lumber region and the calves and a dragging sensation in the abdomen symptoms of constipation, frequent headaches and intense itching leucorrhoea associated with pelvic [2]. In chronic leucorrhea patient feels disruptive disturbance irritable and develops black patches under the eyes inflammatory disease pelvic Inflammation disease (PID) an upper genital tract infections encompasses with endometritis-salpingitis pentonitis, caused more complexity.

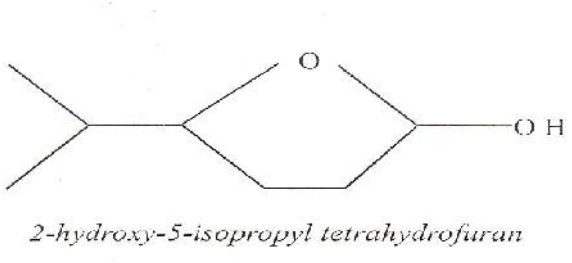

Fig.1. 2-hydroxy-5-isopropyl tetrahydrofuran

"Sidabiyai", a widely used common safe phytomedicine for women, which is prescribed by traditional medicinemen, to Leucorrhoea and Leucorrhoea associated PID. It is crude plant drug composed of premium phytonutrients non-hormonal rational combination of valuable indigenous formulation from the bark and wood of *Terminalia arjuna, Santalum album* and *Adenanthera paronina* at the ratio 4:2:1. The herbal extract drug is known to have direct action on the utchine musculature, improve its circulation and tone up the female genital tract. However, in depth, therapeutically active chemicals based on scientific research have not yet been undertaken in this indigenous herbal drug of Manipur. It is worthwhile to detect the therapeutic organic compounds presents in the medicines to enhance the good forth to traditional herbal drugs; to revitalize the indigenous technological knowledge of life saving herbals drugs and thereof the holistic health cares system, its tradition and traditional value.

Further, traditional and folklore medicines play an important role in health service around the globe, about three quarter of the world's population relies on plants and plant extracts for health care. The state Manipur is rich in medicinal plants and is one of the richest regions in the northeast hot spot concerning to genetic resources of medicinal plants. Several plants have been used in folklore medicine[3]. The traditional design of novel drugs from traditional medicine offers new prospects in modern health care. However, the research work on the scientific principle is extremely meager. The present investigation on the antimicrobial activities in different extracts of the medicine "Sidabiyai" have undertaken with the objectives to examine the antimicrobial activities of plant extracts; to determine the effectiveness of medicine "Sidabiyai" extracts on different five species of fungi and bacteria to isolate purity and identify the therapeutic compounds to renovate the value of aged old traditional medicine.

MATERIALS AND METHODS

Extraction

Medicine and plant materials: The medicine "Sidabiyai" was freshly prepared with the constituents of bark and woods of *Terminalia arjuna, Santalum album* and *Adenanthera paronina*. The plant parts were collected from the Wabagai, Thoubal District; Moreh, Chandel District, and Langthabal, Imphal District, Manipur; India during February to March 2009. The identity of the selected plant species was confirmed and voucher specimens were deposited at the Post Graduate Study Centre HRDRI Canchipur for future reference. The collected masses were individually washed thoroughly with tap water and with distilled water then they were chopping and dried under shade and grounded into mixture to obtained powder of the bark or wood. The powder was mixed in the ratio 4:2:1 of *Terminalia arjuna, Santalum album* and *Adenanthera paronina*.

The homogeneously mixed powder were extracted three times with 99% MeOH (each time 48 hrs at 45^0C) and methanol was removed under reduce pressure to give MeOH extract (70g). These extract (40g) were dissolved in water - MeOH then extracted with diethyl ether, Chloroform, ethyl acetate and n-butanol followed by the removal of the solvents under reduce pressure to give diethyl ether extract (3.8g) $CHCl_3$ extract (3.1g), ethyl acetate extract (3.4g) and n-BuOH extract (18.9g), and water extract (12.2g).

Isolation and purification:

The $CHCl_3$ extract (2.1g) was subjected to column chromatography on a 2 X 30cm silica gel open column using a step wise gradient n-hexane and increasing amount of chloroform 10 % each step; chloroform with increasing amount of MeOH 1% each step and finally 80% MeOH. The collected fractions were evaporated under vacuum and examined by TLC for further investigation on identification of compounds .

Test Micro-organisms

Antimicrobial activities of the different extracts of medicine "Sidabiyai" include 5 bacteria: *Escherichia coli* (Gram negative), *Pseudomonas putida* (Gram negative), *Pseudomonas auriginosa* (Gram negative), *Staphylococcus aureus* (Gram positive), *Klebsiella pneumoniae* (Gram negative) 5 fungi; *Aspergilus flavus,* ITCC number [5175], *Aspergilus niger* ITCC number [2146], *Aspergilus fumigatus,* ITCC number [6050], *Candida albicans* ITCC number [3179] and *Microsporum gypseum,* ITCC number [5277]. The microorganism were chosen

based on their chemical and pharmaceutical significance.

Media: Luria bertani agars, Luria bertani broth, potato dextrose agar, trusty products of Hi-media Laboratories, Mumbai (India) were appropriately used for the concerned microbes.

Antimicrobial agents : Amphoterecin-B (75µg in 2.5ml of water) and Chloramphenicol (90 µg in 3 ml of MeOH) were used as control for bacteria and fungi respectively.

Antimicrobial activity: Antimicrobial activity was accounted using modified paper disc method. [4, 5, 6,7] .

The paper disc was prepared by taking aliquots of 1 ml each of the different extracts in the separate eppendorf tubes. The sterilized paper disc prepared from the Whattman paper (diameter of 5.42 mm) was dipped in the different extracts at different concentration for 1hr. After thoroughly wetted the paper discs were incubated in the oven at 45^0C overnight to evaporate the solvent from the paper disc. Approximately 13 ml of nutrient agar poured in each sterilized petri-plate (90mm) for base agar. Cell suspensions with strength of 10^8CFU/ml cells for bacteria and 10^7CFU/ml cells for fungi were prepared. The 24 hrs old broth culture of each bacterium and 3 days old fungus culture were inoculated in previously melted and cooled soft agar (5ml) at about 45- 50^0C. After well mixing the soft agar was poured over the base agar plate and after solidification of soft agar, the paper disc previously prepared with its control is placed over the solidified agar plate. Then bacterial plates thus made were incubated at 35^0C for 24hrs and fungal plates at 25^0C for 3 days.

Minimum inhibitory concentration (MIC) : The determination of minimum inhibition concentration (MIC) was carried out by placing the paper discs in increasing or decreasing concentration of the extract over the Petri plate containing soft agar layered over base agar plate [8].

Statistical analysis : The Experimental results were recorded in tabulated form for further in-depth analysis of the research to make coordinating relationship among different extracts, microbial types and concentrations of bioactive etc. All the accorded data of antimicrobial activity have statistically tested and graphically represented to verify the trueness of the findings.

Antimicrobial activity index: Antibacterial index (A_bI) and antifungal index (A_fI) for individual chemical extract of medicine Sidabiyai were calculated as the mean value of zone of inhibition obtained against all individual bacterial and fungal test strains respectively [9].

RESULTS AND DISCUSSION

Medicine “Sidabiyai” in ethanol extract accord low suppression of 10, 12, 13 mm on *Escherichia coli* at concentrations of 10, 20 and 30 mg/ml respectively while the control suppressed 14 mm. Next, in the bacteria *P. putida*, the impact of ethanol extract accords 5, 6, 13mm at 10, 20, 30 mg/ml concentration while control accord 15mm. In *P. auriginosa* the minimum inhibition zone (MIZ) accords 10, 15, 20mm at 10, 20, 30mg/ml concentration while control attained 15 mm. Similarly in *S. aureus* the MIZ accords 19, 20, 25mm at 10, 20, 30 mg/ml concentration while the control record 17mm; next the *K. pneumoniae* accord low impact of 12, 13, 13 mm by the extract at 10, 20, 30 mg/ml while the control accord 14mm. The accorded data has presented in table B.I.1 and graphically verified in fig. B.I.1.

In the antibacterial test of the chloroform extract of medicine “Sidabiyai” four types of bacteria viz., *E. coli*, *P. auriginosa*, *S. aureus*, *K. pneumoniae*, demonstrate the MIZ of low suppression of 10, 12, 13 mm, at concentrations of 10, 20, 30mg/ml respectively except *P. putida* which accord 20, 20, 20 mm at 10, 20, 30mg/ml concentration while the control accord 14, 15, and 17mm. The observed data has presented in table B.1.1. and graphically represented in fig. B.1.1. Medicine 'Sidabiyai' extract in n-butanol accord 5,6, 10 mm MIZ in *E. coli* at concentrations of 10, 20 and 30mg/ml respectively and control accord 14 mm. In *P. putida* the MIZ accord 10, 13, 15mm suppression at 10, 20, 30 mg/ml concentrations while control accord 15mm. Similarly *S. aureus* accord 13, 13, 20mm MIZ at 10, 20, 30mg/ml concentration while the control accord 17mm. In *K. pneumoniae* the antimicrobial low action shows 5, 6, 10 mm at 10, 20, 30 mg/ml concentrations while control accord 14mm.The accorded data were represented in table B.1.1 and graphically demonstrated in fig. B.1.1.

Table B.I.1. Antibacterial activity (zone of inhibition) of solvent extracts (ethanol, chloroform, n – butanol) of medicine "Sidabiyai".

Microorganisms	Medicine Sidabiyai (10, 20, 30mg/ml)				
	Concentration mg/ml	Sample 1 (mm)	Sample 2 (mm)	Sample 3 (mm)	Amphoterecine 0.3mg/ml (mm)
Escherichia coli	10	10±0.63	10±0.63	5± 0.44	14±0.75
	20	12±0.69	12±0.69	6± 0.44	14±0.75
	30	13±0.72	13±0.72	10±0.63	14±0.75
Pseudomonas putida	10	5± 0.44	20±0.89	10±0.63	15±0.7
	20	6± 0.44	20±0.89	13±0.72	15±0.77
	30	13±0.72	20±0.89	15±0.77	15±0.77
Pseudomonas auriginosa	10	10±0.63	12±0.63	5± 0.44	15±0.77
	20	16±0.77	13±0.72	6± 0.44	15±0.77
	30	20±0.89	13±0.72	10±0.63	15±0.77
Staphylococcus aureus	10	19±0.85	10±0.63	13±0.72	17±0.82
	20	20±0.89	12±0.69	13±0.72	17±0.82
	30	25±1.0	13±0.72	20±0.89	17±0.82
Klebsiella pneumoniae	10	12±0.63	10±0.63	5± 0.44	14±0.75
	20	13±0.72	12±0.69	6± 0.44	14±0.75
	30	13±0.72	13±0.72	10±0.63	14±0.75

Values are expressed as mean ± SEM; n = 3 in replicate for each treatment.
Sample 1:DMSO dissolved in Ethanol extract of medicine "Sidabiyai"
Sample 2: DMSO dissolved in Chloroform extract of medicine "Sidabiyai"
Sample 3: DMSO dissolved n - butanol extract medicine "Sidabiyai"

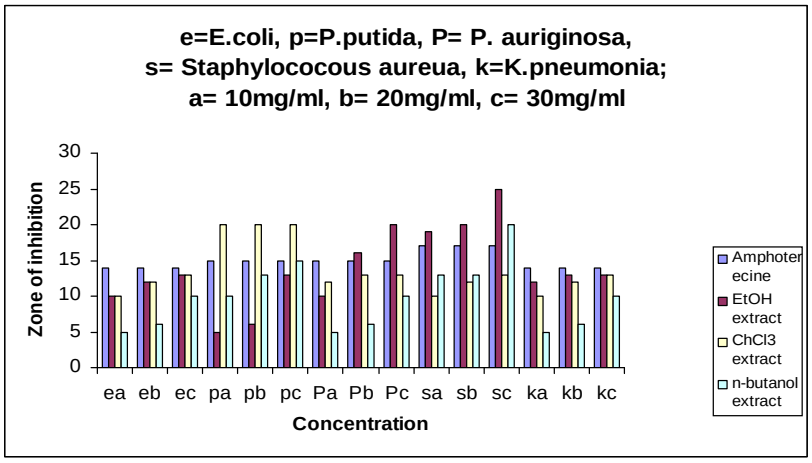

Fig. B.I. 1.Antibacterial activity (zone of inhibition) of various extracts viz ethanol, chloroform and n-butanol of medicine "Sidabiyai" with control against *E.coli, P.putida, P.auriginosa, S.aureus* and *K. pneumoniae.*

Table B.1.1 revealed the antimicrobial activity of ethanol extract of medicine Sidabiyai with low MIZ 10, 12, 13mm on *E. coli*, 5, 6, 13 mm on *P. putida*, 12, 13, 13 mm MIZ on *K. pneumoniae* at 10, 20, 30 mg/ml concentration respectively whereas on *P. auriginosa* a suppression of 10, 15, 20 mm MIZ and 19, 20, 25mm MIZ on *S. aureus* at three different level of concentrations i.e. 10, 20, 30 mg/ml against the 15mm, 17 mm MIZ of control. The finding indicates the ethanol extract of medicine "Sidabiyai" on *S. aureus* and *P. auriginosa* accord better then control at higher concentration of MIC viz at 20 and 30 mg/ml concentration. Whereas the antibacterial activity has low and insignificant antibacterial activity against *E.coli, P. putida* without barrier of concentration upto 30mg/ml from 10mg/ml. The finding evident that the ethanol extract of medicine 'Sidabiyai' can replace the Amphoterecin in controlling *S. aureus* and *P.auriginosa*, at 20 mg/ml and above MIC and cannot control. *P. putida*. and has no significant impact on *E.coli*, *P. putida* and *K. pneumoniae.* The finding further proves the selectiveness activity of ethanolic extract of medicine 'Sidabiyai' and specific in nature.

The same table B.I.1. demonstrate the antibacterial activity of Chloroform extract of the medicine "Sidabiyai" with 10, 12, 13 mm; 12, 13, 13 mm; 10, 12, 13 mm; 10, 12, 13 mm MIZ at 10, 20, 30 mg/ml on *E.coli, P.auriginosa, S. aureus, K. pneumoniae* respectively except 20mm MIZ each to 10, 20 30 mg/ml concentration on *P. putida*. against the 15mm MIZ of control conveying the activities of bioactive in the extract. It is evident from the present finding that chloroform extract of medicine "Sidabiyai" have best antibacterial activity on *P. putida* however negligible or very low activity on *E.coli, P. auriginosa, S.aureus, and K. pneumoniae* with comparison to control.

Further table B.I.1 exemplify the n-butanol extract of medicine "Sidabiyai" with less than 11 mm MIZ on *E. coli, K. pneumoniae* and *P. auriginosa* while 14 to 17 mm MIZ in control however on *P. putida* and *S.aureus* they manifested right activity upto 15 and 20 mm MIZ while control suppressed 15 and 17 mm at higher concentration of 30 mg/ml. The finding blaze a new room of bioactivities to the concentration of bioactives of the extracts. The present finding also put frame necessity of higher dose application for increasing the efficiency to LD_{50} toxicity.

Fig. B.1.1. displayed the bars representing antibacterial activity of test extracts of ethanol, chloroform and n-butanol of medicine 'Sidabiyai' on five test bacterial types viz. *E.coli, P. auriginosa, P. putida, S.aureus,* and *K. pneumoniae* with control elucidating a comparative distinction of activities of bioactives in the extracts. The highest zone of inhibition strike to *S. aureus* with value range to 25mm by ethanol extract, followed by ethanol extract on *P. auriginosa*; chloroform extract on *P. putida*; n-butanol extract on *S. aureus* with 20mm each of MIZ. The finding shows that the bioactives of the extract of medicine 'Sidabiyai' have efficacies specifically to selective bacterial types. The finding was in corroborative with the results of other workers in different plant at different places. The ethanolic extract of *Mentha longifolia* have the most active against the tested bacteria *E. coli, S. aureus, B. cerus, P. auriginosa* and *B. subtilis* [10]. The multidrug resistant (MDR) strains of *Streptococcus mutans, S. aureus, E. facculis, S. bovis, P. auruginosa, S. typhinurium, E. coli, K. pneumonia* and *C. albicans* were sensitive to the antimicrobial activity of extracts of *A. nilotica, S. aromaticum* and *C. zeylanicum,* whereas they exhibited strong resistance to the extracts at *Terminalia arjuna* and *Eucalyptus globules*[11].

The present finding vividly showed that antibiotic effect of different extracts of medicine Sidabiyai have divergence activities against certain types of bacterial growth in comparison to control amphoterecin. The ethanol extract ranking top in inhibiting three test microbes viz. *P. putida, P. auriginosa, S. aureus* , which was followed by n-butanol extract inhibiting two viz. *P. putida, S. aureus* and chloroform extract to one viz. *P. putida* . Thus, the efficacy of medicine extract evaluation as antibacterial agent absolutely dependent on the bioactives dissolve in solvent of extractions and thereof their impact to bacterial types. This finding is in agreement with the result of antimicrobial activities of extracts from different plants of different places [12]. WHO found that ethanolic extract of some Nigerian spices were more potent than the aqueous extract against common food borne microorganisms including *S.aureus* and *K. pneumoniae, Proteus vulgaries* and *Streptococcus faecalis*.

The bioactivities of ethanol extract of medicine "Sidabiyai" accord low inhibition lesser than 11mm MIZ on *A. flavus* and *A. fumigatus,* at different concentration of 10, 20 and 30mg/ml against 20mm MIZ on control while the fungus *A. niger* accord 10, 10, 12mm at 10, 20, 30mg/ml concentration against 25mm on control. Next in fungus *C. albicans* the MIZ accord 13, 15, 16mm at 10, 20, 30mg/ml concentration while the controlled attained 25 mm; similarly *M. gypseum* accord 15, 20, 20mm at 10, 20, 30mg/ml concentration against 25mm on control. The observed accorded records were presented in table B.I.2. and graphically hand out in bar in fig. B.I.2.

The antifungal activity of chloroform extract of medicine "Sidabiyai" on two fungi of *A. flavus, A. fumigatus* accord highest 12 mm inhibition while control attained 20 mm and on the test fungus *A. niger* accord 10, 10, 13mm at 10, 20, 30mg/ml concentration while the control accord 20mm. In fungus *C. albicans*, the MIZ extend to 10, 15, 17mm at 10, 20, 30mg/ml. concentration while control attained 25mm MIZ. Similarly the inhibition on *M. gypseum* accord 10, 15, 20mm at 10, 20, 30mg/ml concentrations while the control accord 25mm. The extract of medicine 'Sidabiyai' ranges from negligible to very weak antifungal activity to all test fungi. Very weak action of chloroform extract on *A. niger* and *C. albicans* correspond to low grade inhibition of MIZ lesser than their respective control. The accorded results were presented in table B.I.2.and graphically put forward by bar in fig. B.I.2. The antifungal action of n-butanol extracts of medicine "Sidabiyai", freely accord low inhibition in three fungi viz. *A. flavus, A. niger, A. fumigatus* whereas on *C. albicans* the antifungal activity was accorded with 10, 15, 15 mm *at* 10, 20, 30mg/ml and in control accord 25mm. Further the

antifungal activity on *M. gypseum* accord 10, 15, 15mm at 10, 20, 30mg/ml concentrations while the control accord 25mm. The data resulted from the experimentation were represented in table B.I.2.and graphically bestowed in bar in fig. B.I.2.

Table B.I.2. demonstrate the low inhibition of ethanol extract of the medicine "Sidabiyai" on *A. flavus* and *A. fumigatus* and lesser MIZ than control on *A. niger*, *M. gypseum* and *C. albicans* at all three tested concentration of 10,20, 30mg/ml. The finding indicates the bioactive activity of the ethanol extract of medicine "Sidabiyai" have no antifungal activity on the ubiquitous fungi *A. flavus* and *A. fumigatus* and too weak on *A. niger*, *M. gypseum* and *C. albicans.*

The result of the antifungal test of chloroform extract of medicine "Sidabiyai" reveals a remarkably low impact of antifungal activity even lower than the control to *A. niger*, *C. albicans*, *M. gypseum*, *A. flavus* and *A. fumigatus.* The finding vividly manifest the bioactive activity of the chloroform extract of medicine "Sidabiyai" acts as very weak antifungal activity to the test fungi. The finding was in concordance with that of the different workers in different places [13, 14].

TableB.I. 2. Antifungal activity (zone of inhibition) of solvent extracts (ethanol, chloroform, n – butanol) of medicine "Sidabiyai".

Microorganisms	Medicine Sidabiyai (10,20,30 mg/ml)				
	Concentration mg/ml	Sample 1 (mm)	Sample 2 (mm)	Sample 3 (mm)	Chloramphenicol 0.3mg/ml (mm)
Aspergilus flavus	10	5± 0.44	7± 0.51	5± 0.44	20±0.89
	20	7±0.51	10±0.63	7± 0.51	20±0.89
	30	10±0.63	12±0.69	10±0.63	20±0.89
Aspergilus niger	10	10±0.63	10±0.63	6± 0.47	25±1.0
	20	10±0.63	10±0.63	8±0.56	25±1.0
	30	12±0.69	13±0.72	10±0.63	25±1.0
Aspergilus fumigatus	10	5± 0.44	5± 0.44	10±0.63	20±0.89
	20	7± 0.51	7± 0.51	12±0.69	20±0.89
	30	10±0.63	10±0.63	13±0.72	20±0.89
Candida albicans	10	13±0.72	10±0.63	10±0.63	25±1.0
	20	15±0.77	15±0.77	15±0.77	25±1.0
	30	16±0.80	17±0.82	15±0.77	25±1.0
Microsporum gypseum	10	15±0.77	10±0.63	10±0.63	25±1.0
	20	20±0.89	15±0.77	15±0.77	25±1.0
	30	20±0.89	20±0.89	15±0.77	25±1.0

Values are expressed as mean ± SEM; n = 3 in replicate for each treatment.
Sample1:DMSO dissolved in Ethanol extract of medicine "Sidabiyai"
Sample 2: DMSO dissolved in Chloroform extract of medicine "Sidabiyai"
Sample 3: DMSO dissolved in n - butanol extract of medicine "Sidabiyai"

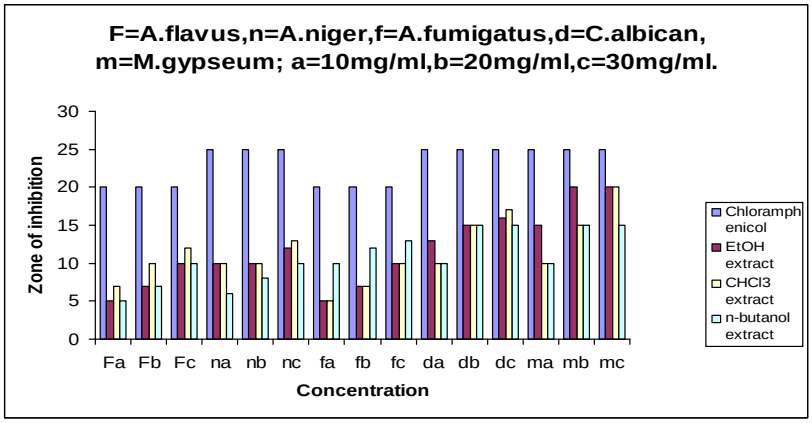

Fig. B.I. 2. Antifungal activity (zone of inhibition) of various extracts viz ethanol, chloroform and n-butanol of medicine "Sidabiyai" with control against *A.flavus, A. niger, A.fumigatus, C.albicans* and *M. gypseum.*

Table B.I.3. Antibacterial activity index (A_bI) of solvent extract (ethanol, chloroform, n – butanol) of medicine "Sidabiyai" at different concentration.

	Microorganisms	10mg/ml	20mg/ml	30mg/ml	Chloramphenicol 0.3mg/ml
EtOH extract	*E.coli*	10	12	13	14
	P.Putida	5	6	13	15
	P.auriginosa	10	16	20	15
	S.aureus	19	20	25	17
	K. pneumoniae	12	13	13	14
	(A_bI)	11.2	12.2	16.8	15
$CHCl_3$ extract	*E.coli*	10	12	13	14
	P.Putida	20	20	20	15
	P.auriginosa	12	13	13	15
	S.aureus	10	12	13	17
	K. pneumoniae	10	12	13	14
	(A_bI)	12.4	13.8	14.4	15
n-butanol extract	*E.coli*	5	6	10	14
	P.Putida	10	13	15	15
	P.auriginosa	5	6	10	15
	S.aureus	13	13	20	17
	K. pneumoniae	5	6	10	14
	(A_bI)	7.6	8.8	13	15

TableB.I. 4. Antibacterial activity index (A_bI) of solvent extract (ethanol, chloroform, n – butanol) of medicine "Sidabiyai" at different concentration.

	Microorganisms	10mg/ml	20mg/ml	30mg/ml	Chloramphenicol 0.3mg/ml
EtOH extract	*A.flavus*	5	10	12	20
	A.niger	10	10	12	25
	A.fumigatus	5	7	10	20
	C.albican	13	15	16	25
	M.gypseum	15	20	20	25
	(A_fI)	9.6	12.4	14	23
$CHCl_3$ extract	*A.flavus*	7	10	12	20
	A.niger	10	10	13	25
	A.fumigatus	5	7	10	20
	C.albican	10	15	17	25
	M.gypseum	10	15	20	25
	(A_fI)	8.4	11.4	14.4	23
n-butanol extract	*A.flavus*	5	7	10	20
	A.niger	6	8	10	25
	A.fumigatus	10	12	15	20
	C.albican	10	15	15	25
	M.gypseum	10	15	15	25
	(A_fI)	8.2	11.4	13	23

Perusal on the above table B.I.2 also demonstrate the weak or low action of n-butanol extract medicine "Sidabiyai" on test fungi of *A. flavus*, *A. niger*, *A. fumigatus*, *C. albicans* and *M. gypsum.* The finding vividly clarified the bioactive activity of n-butanol extract of medicine "Sidabiyai" have no antifungal activity on *A. flavus*, *A. niger*, *A. fumigatus*, *C. albicans* and *M. gypsum* in comparision to Chloramphenicol.

The calculated "t" (2.75) between MIZ obtained from the treatment of ethanol extract showing the highest antibacterial activity and the well known broad spectrum antibacterial Amphoterecine was to be greater than the tabulated "t" from Fishers table for $\leq$ 0.05 (for

degree of freedom n= 4), the result accounts highly significant.

Table.B.I.3 illustrate the highest antibacterial index A_bI, 16.8 mm in average was accorded in ethanol extract at 30 mg/ml which was followed by A_bI 12.2 mm at 20 mg/ml and A_bI 11.2 mm at 10 mg/ml while A_bI 15 mm of control. In chloroform extract the highest A_bI 14.4 mm at 30 mg/ml followed by A_bI 13.8 mm at 20 mg/ml and A_bI 12.4 mm at 10 mg/ml against A_bI 15 mm of control. For n- butanol extract the highest A_bI 13 mm at 30 mg/ml followed by A_bI 8.8 mm at 20 mg/ml, and A_bI 7.6 mm at 10 mg/ml against A_bI 15 mm of control. The present investigation clearly showed that the ethanol extract of "Sidabiyai" at 30 mg/ml has higher potent than Amphoterecine however lower concentration 20 mg/ml, 10 mg/ml and other extracts of chloroform and n- butanol at all concentration are not effective upto Amphoterecine.

Table B.I.4 delineated in ethanol extract the highest antifungal index, A_fI, 14 mm at 30 mg/ml followed by 12.4 mm at 20 mg/ml, A_fI 9.6 mm at 10 mg/ml when the control accord 23 mm. For chloroform extract the highest i.e. A_fI 14.4 mm accord at 30 mg/ml which was followed by A_fI 11.4 mm at 20 mg/ml, A_fI 8.4 mm at 10 mg/ml against A_fI 23 mm in control. In n- butanol extract the highest A_fI 13mm accord at 30 mg/ml, followed by A_fI 11.4 mm at 20 mg/ml, A_fI 8.2 mm at 10 mg/ml against control A_fI 23mm. The finding evident that antifungal index A_fI of ethanol extract, chloroform extract and n- butanol extract at all concentration i.e. 10, 20, 30 mg/ml are less effective than Chloramphenicol.

Fig. B.1.2 demonstrate the antifungal activity of test extracts of ethanol, chloroform and n- butanol of medicine "Sidabiyai" to the test fungi viz. *A. flavus, A. niger, A. fumigatus, C. albicans* and *M. gypsum* by bar of zone of inhibition. The figure vividly exemplify the three extracts of ethanol, chloroform, n- butanol extract of medicine "Sidabiyai" remain lagging far behind the broad spectrum fungicide Chloramphenicol. In other words the bioactive present in the extract of different solvent have very low antifungal activity with relation to Chloramphenicol.

Regarding Phytochemical screening, **t**he $CHCl_3$ extract subjected to column chromatography at different proportion of n-hexane and chloroform yielded fractions, then evaporated and scrutinized on TLC eventually created the unequivocal fractions. The homogenous fractions thus pooled give 10 major fractions F_1 to F_{10}. Out of these ten fractions, fraction fifth i.e. F_5 (0.78g) separated on a 1 x 20cm silica gel 100-200 mesh column eluted with (80:1) $CHCl_3$ – MeOH to yield 7mg of compound with R_f value 0.8 ($CHCl_3$: MeOH: H_2O). The compound thus isolated and purified is assigned to compound 1.

Compound 1, from medicine "Sidabiyai" is characterized by a dark yellow crystalline. The IR spectrum showed important absorption attributable to ν_{max} (KBr, cm^{-1}) 3348.1(Hydroxyl group); 2922.92 (C-H), 2852.52(C-H); 1725.21(C-O saturated five member ring) Dyer, [15]. The high resolution Mass spectrometer showed the [M+] at m/z 130.102 and gave a molecular formula $C_7H_{14}O_2$ this structural type was supported by ^{1}H-NMR ($CDCl_3$) (δ, ppm) 5.48, 3.76, 2.08, 2.13, 1.93, 1.08, 2.0 ^{13}C- NMR) (δ, ppm) spectra showed 104.5, 76.31, 30.9, 28.6, 28.3, 13.1. The probable structure is 2-hydroxyl 5-isopropyl tetrahydrofuran.

The common properties of tetrahydro furan is their toxicity against cell of foreign organisms. These activities have been widely studied for their potential use antiinflamatory in exhibiting anti- angionic, antibacterial, antioxidant etc. in the rlimination and reduction of serious human diseases like cancer cell lines[16, 17] .

The presence of tetrahydrofuran in the traditional herbal medicine corroborates the antibacterial activities eventually it is clear that Sidabiyai could be potent antimicrobial agents particularly to specific bacterial strains.

CONCLUSION

The antibacterial index reflect the superior activity of ethanol extract at higher concentration irrespective to test bacterial strains and lesser action with chloroform and n- butanol extracts, than Amphoterecine whereas antifungal index rectify the inferiority of all test extract in all fungal types in comparison to Chloramphenicol.

Investigation on isolation and identification of compound, Sidabiyai the traditional medicine revealed 2- hydroxyl 5-isopropyl tetrahydrofuran. The Mass, IR, NMR analysis confirmed the chemical structure of the compound. The results of the present research work strengthen the spectacular knowledge of therapeutic and phyto-chemistry of prestigious; Indigenous health care system of the aged old traditional technological values of health care.

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