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Screening of Selected Single Plants and its Siddha Formulation for Antibacterial and Antifungal Activity

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The study deals with antibacterial and antifungal activity of selected single plants (*Adathoda vasica, Ocimum tenuiflorum, Solanum xanthcarpum, Tylophora asthmatica*) and its Siddha formulation. The antibacterial and antifungal activity was determined using the hole-in-plate bio assay procedure. *Staphylococcus aureus*, *Escherichia Coli, Pseudomonous arrigonosa, Bacillus subtilis* (antibacterial) *Candida albicans* and *Aspergillus niger* (antifungal) were used as the test microorganisms. In this microbial study the findings shows Siddha formulation are having more antibacterial activity than antifungal activity which is determined by zone of inhibition, MIC and MBC on different bacterial and fungal strains. Further, the results of antibacterial activity of siddha formulation were compared with standard drug Amoxcillin and for anti fungal activity of siddha formulation were compared with standard drug chloramphenicol.

Key words: Siddha formulation, Antimicrobial activity, Antifungal activity, Hole-in-plate bio assay.

Siddha and Ayurveda are the traditional Indian Medicinal System practiced for thousands of years. The current accepted modern medicine has gradually developed over the years by scientific and observational efforts of scientists. However, the basis of its development remains rooted in traditional medicine and therapies (Vishunakanta and Rana,2008). There is an increased demand in usage of alternative medicines including Siddha preparation around the world for treating various diseases. There are various types of Siddha preparation which includes Chooranam or Parpam. Chooranam is basically a single or compound herb given as such to the patients. However the amount of effectiveness and the safety of the Siddha formulation are under research so this system didn't reach to community wise hence the system lacks. Herbal medicine of natives in every country forms a major part of the world heritage of the plant material (Mahasneh AM, 2002). Although active ingredients may occur in lower concentrations, plant extracts may be a better source of antimicrobials than synthetic drugs (Quave *et al.*, 2008). Synergistic effects are often to bioactivity in plant extract and some activity is usually lost during purification (Cos *et al.*, 2006).The present work is a preliminary study on antibacterial and antifungal activity of siddha formulation against bacteria and fungi.

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Antibacterial activity

The antibacterial activity was determined using the hole-in-plate bio assay procedure. The pure cultures of the microorganism were inoculated onto Muller-Hilton nutrient broth incubated at temperature of 37°C for 24hours.Using a sterile cork-borer of 5mm diameter, four holes were made into the Petri dishes seeded with bacterial culture. Concentrations of 25µg, 50µg and 100µ g/ml of individual plant powder and its Siddha formulation

(chooranam) were reconstituted in distilled water and transferred into the wells. The plates were incubated at temperature of 37°C for 24 hours. *Staphylococcus aureus*, *Escherichia Coli*, *Pseudomonous arrigonosa* and *Bacillus subtilis* were used as the test microorganisms. All bacterial cultures were maintained on nutrient agar slants at temperature of 4ºC and sub cultured onto nutrient agar broth for 24 hours prior to testing.

Antifungal activity

Holes were made into the Petri dishes containing inoculated medium. Concentrations of 25µg, 50µg and 100µg / ml of individual plant powder and its Siddha formulation (chooranam) were reconstituted in distilled water and transferred into the wells. Which is the examined against *Candida albicans* and *Aspergillus Niger*. The diameter of the clear zone around the wells (inhibition diameter) was measured at the end of the incubation period. The individual plant powder and chooranam that have high mean diameter is subjected to be maximum activity as described above. Three concentrations in wells per plate against a single microorganism were used.

Isolation and standardization of inoculum

The microbial inoculums were standardized at 0.5 McFarland. In microbiology, McFarland standards are used as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range. Original McFarland standards were made by mixing specified amounts of barium chloride and sulphuric acid together. Mixing the two compounds forms a barium sulfate precipitate, which causes turbidity in the solution. A 0.5 McFarland standard is prepared by mixing 0.05 ml of 1.175% barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$), with 9.95 ml of 1% sulfuric acid (H_2SO_4). The standard could be compared visually to a suspension of bacteria in sterile saline or nutrient broth.

Determination of minimum inhibitory concentration (mic) & minimum bactericidal concentration (MBC)

MIC is determined by dilution of the individual plant powder and its Siddha formulation are subjected to various concentrations range .0.9–15 mg/ml. Equal volume of each plant powder and

Table 1. Antibacterial activity of herbal powders and its Chooranam

S.No	Sample		*E.coli*	*Pseudomonas arrigonosa*	*Staphylococcus aureus*	*Bacillus subtilis*
1	AV Powder	25 µg/ml	6±1.34	6±0.43	6±0.34	6±1.34
		50 µg/ml	9±0.43	8±0.34	8±0.43	8±0.99
		100 µg/ml	11±0.86	12±0.65	10±0.53	10±0.47
		Amox – 10mg/ml	18±1.09	16±0.45	16±0.86	16±0.76
2	SX Powder	25 µg/ml	7±0.77	6±0.88	6±0.65	7±0.84
		50 µg/ml	9±0.54	9±0.34	8±0.76	9±0.72
		100 µg/ml	13±0.84	12±0.34	10±0.65	10±0.65
		Amox –10 mg /ml	18±0.86	16±0.12	16±0.86	16±0.56
3	TA Powder	25 µg/ml	6±0.88	6±0.24	6±0.65	6±0.87
		50 µg/ml	8±0.67	8±0.45	8±0.65	9±0.86
		100 µg/ml	13±0.76	12±0.65	11±0.88	11±0.98
		Amox – 10 mg/ml	18±0.83	16±0.41	16±0.65	16±0.76
4	OT Powder	25 µg/ml	7±0.12	6±0.76	6±0.67	7±0.49
		50 µg/ml	8±0.97	7±0.34	7±0.76	7±0.35
		100 µg/ml	11±0.34	10±0.34	9±0.78	9±0.48
		Amox – 10 mg/ml	18±1.34	16±0.45	16±0.67	16±0.42
5	SF Powder	25 µg/ml	7±0.12	7±0.56	7±0.87	7±0.54
		50 µg/ml	10±0.34	9±0.56	10±0.86	9±0.72
		100 µg/ml	15±0.43	14±1.34	13±0.94	13±0.54
		Amox – 10 mg/ml	18±0.88	16±0.76	16±0.34	16±1.34

AV- *Adhatoda vasica,* SX - *Solanum xanthocarpum,* TA- *Tylophora asthamtica,* OT- *Ocimum tenuiflorum,* SF - Siddha formulation

nutrient broth were mixed in a test tube. Specifically 0.1 ml of standardized inoculum (1–2 × 10^7 cfu/ml) was added to each tube. The tubes were incubated aerobically at 37°C for 18–24 hr. Two control tubes were maintained for each test batch. These included antibiotic control (tube containing extract and the growth medium without inoculum) and organism control (the tube containing the growth medium, physiological saline and the inoculum). The lowest concentration (highest dilution) of the extract that produced no visible bacterial growth (no turbidity) when compared with the control tubes were regarded as MIC. However, the MBC was determined by sub culturing the test dilution on to a fresh drug-free solid medium and incubated further for 18–24 hr. The highest dilution that yielded no single bacterial colony on a solid medium was taken as MBC.

The results of anti-microbial and antifungal activity and MIC AND MBC were expressed in Tables 1-3 and in Figs 1-5.

Table 2. Anti Fungal activity of herbal powders and its Chooranam

S.No	Sample		*Aspergillus niger*	*Candida albicans*
1	AV – Powder	25 µg/ml	6±0.83	6±0.45
		50 µg/ml	7±0.72	7±1.03
		100 µg/ml	8±0.94	8±0.93
		Chlor– 10 mg/ml	16±0.45	17±1.04
2	SX – Powder	25 µg/ml	6±0.59	6±0.44
		50 µg/ml	8±0.83	8±0.84
		100 µg/ml	10±0.74	10±0.43
		Chlor – 10 mg/ml	16±0.83	17±0.56
3	TA – Powder	25 µg/ml	6±0.44	6±0.64
		50 µg/ml	7±0.67	7±0.46
		100 µg/ml	9±0.45	8±0.57
		Chlor – 10 mg/ml	16±0.56	17±0.83
4	OT – Powder	25 µg/ml	6±0.54	6±0.67
		50 µg/ml	7±0.98	8±0.45
		100 µg/ml	11±0.56	9±0.43
		Chlor – 10 mg/ml	16±0.54	17±0.93
5	SF – Powder	25 µg/ml	7±0.23	7±0.78
		50 µg/ml	9±0.24	9±0.74
		100 µg/ml	11±0.65	12±0.56
		Chlor – 10 mg/ml	16±0.67	17±0.74

AV- *Adhatoda vasica,* SX - *Solanum xanthocarpum,* TA- *Tylophora asthamtica,* OT- *Ocimum tenuiflorum,* SF - Siddha formulation, Chlor – chloramphenicol.

Table 3. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of herbal powders and its Chooranam

S. No	Concentration	MIC (mg/ml)	MBC (mg/ml)
1.	AV Powder	5.30	5.60
2.	SX Powder	3.20	4.40
3.	TA Powder	3.60	3.90
4.	OT Powder	4.10	5.10
5.	Siddha Formulation	3.00	3.60

AV- *Adhatoda vasica,* SX - *Solanum xanthocarpum,* TA- *Tylophora asthamtica,* OT- *Ocimum tenuiflorum*

RESULTS AND DISCUSSION

Anti bacterial activity

The table shows the zone of inhibition of different bacterial strains against *Adathoda vasica, Ocimum tenuiflorum, Solanum xanthcarpum, Tylophora asthmatica* and Siddha formulation with different concentration compared with standard, in which comparing zone diameter with standard all the plant powder shows better antibacterial activity. Among Siddha formulation shows more activity than other individual plant powder.

Anti fungal activity

The table shows the zone of inhibition of different bacterial strains against *Adathoda vasica, Ocimum tenuiflorum, Solanum xanthcarpum, Tylophora asthmatica* and Siddha formulation with different concentration compared with standard, in which comparing zone diameter with standard all the plant powder shows better antifungal activity. Among Siddha formulation shows more activity than other individual plant powder but comparatively all the plant powders are having more antibacterial activity than antifungal activity.

Determination of minimum inhibitory concentration (mic) and minimum bactericidal concentration (MBC)

The MIC of *Solanum xanthocarpum* - 3.20, *Ocimum tenuiflorum* - 4.10, *Tylophora asthmatica* - 3.60, *Adathoda vasica* - 5.30 and Siddha formulation - 3.00 mg/ml. The MBC of *Solanum xanthocarpum* - 4.40, *Ocimum tenuiflorum* - 5.10, *Tylophora asthmatica* - 3.90, *Adathoda vasica* - 5.60 and Siddha formulation - 3.60mg/ml.

Fig. 1. Antibacterial activity of individual herbal powders and its chooranam with standard standard amoxicillin

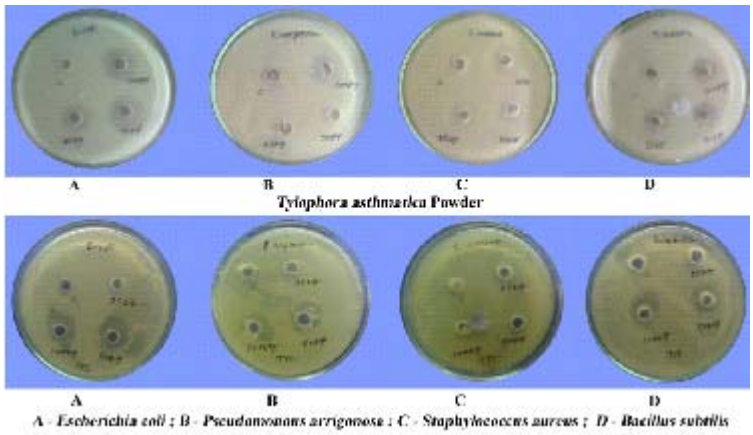

Fig. 2. Antibacterial activity

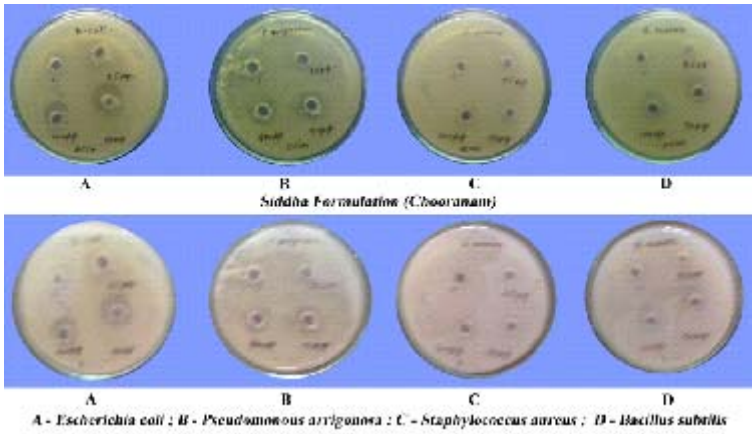

Fig. 3. Antibacterial activity *Ocimum tenuiflorum* powder

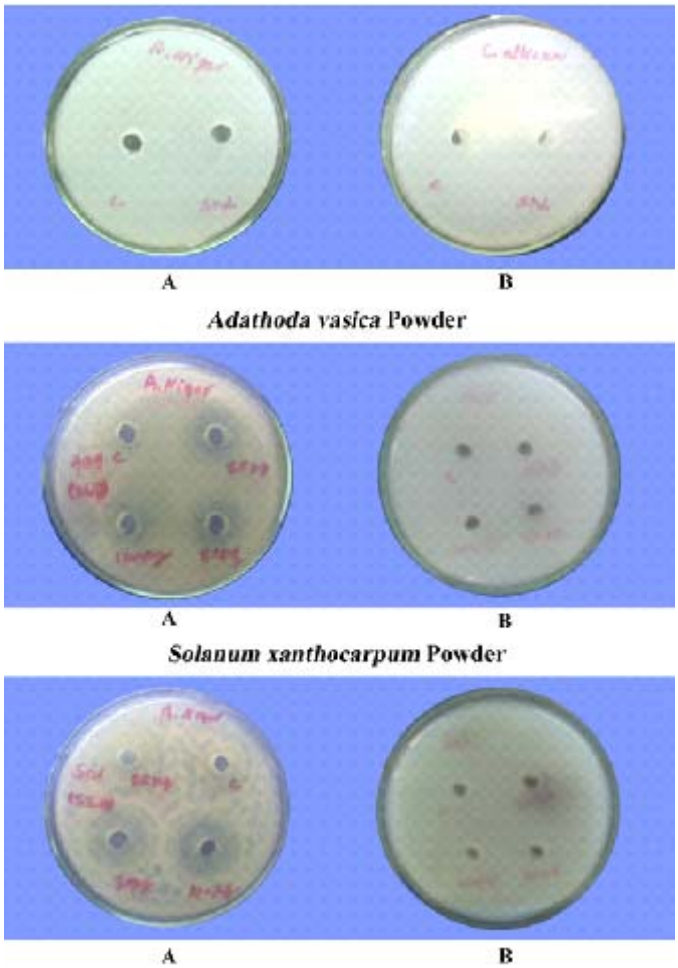

Fig. 4. Antifungal activity of individual herbal powders and its chooranam with standard Standard Chloramphenicol

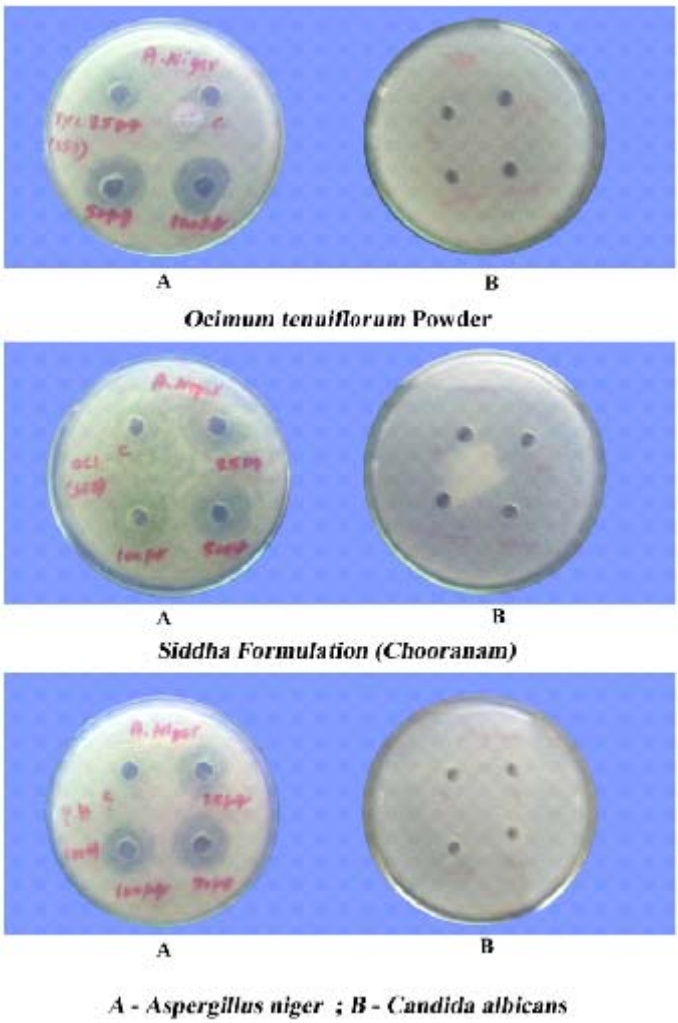

Fig. 5. Antifungal activity *Tylophora asthmatica* powder

CONCLUSION

Single and polyherbal preparations (combination of two or more herbs) have been used in siddha system of medicine in ancient times. In this microbial study the findings shows siddha formulation are having more antibacterial activity when compared to individual herbal plant powders. This siddha formulation is having more antibacterial activity than antifungal activity which is determined by zone of inhibition, MIC and MBC on different bacterial and fungal strains.

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