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Isolation of Herbal Plants: Antifungal and Antibacterial Activities

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ABSTRACT:

Aim of this present study was to evaluate the antifungal and antibacterial activity on naturetic plants. The study was on six naturetic plants like *Sapindus emarginatus, Hibiscus rosa-sinensis*, *Mirabilis Jalapa*, *Euphorbia tirucalli L*, *Vitex negundo L, Saussurea Lappa Costus* of Methanol, Chloroform, N-Hexane and Water extracts on various *Aspergillus flavus, Candida albicans Candida glabreta,* fungal strains *Bacillus subtilis, Escherichia coli, Staphylococcus epidermidis* bacterial strains using standard methods Agar tube dilution for antifungal, Agar diffusion assay for antibacterial as per Protocol to determine the Zone of Inhibition.The different concentration of various plants extracts tested on microorganism for the Zone of inhibition. It shows that water extract of *Sapindus emarginatus* and *Mirabilis jalapa* reported the highest inhibition against the bacterial organism at 20mm- 23mm and 16mm-19mm against the fungal strains. The methanol extracts of *Hibiscus rosa-sinensis, Vitex negundo L, Saussurea lappa Costus* reported the highest inhibition against the bacterial organism at 23mm- 26mm and 17mm-20mm against the fungal strains. The n-Hexane extracts of *Euphorbia tirucalli L* report highest inhibition at 21mm- 24mm and 16mm-19mm against the fungal strains.The present study it was conclude that the highest Zone of inhibition against all different extracts of Plants but there was no response against chloroform extract.

Key words: Zone of Inhibition; Fungal Strains; Bacterial Strains; Extracts.

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INTRODUCTION:

Fungus is a eukaryotic organism that digests its food externally and absorbs the nutrient molecules into its cells. Bacteria are large group of, prokaryote microorganisms are vital in recycling nutrients, thus Fungal infections are still a significant cause of morbidity and mortality for various fungal diseases similarly Bacterial infection cause the suffer for various pathogenic diseases around the world to avoid these nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine[1]. The plant derived antimycotic are attracting the attention of botanists and mycologists because they are natural, cheaper, safer, eco friendly and within the reach of the current medical community. Therefore Natural products offer a virtually unlimited source of unique molecules and not only serve as a reservoir for new potential drugs, but also for probes of fungal and microbial biology [2]. Thus the Natural Plants are used as treatment for the fungal and bacterial base infection then compare to synthetic.

METHODS:

Preparation of crude extract

Table 1: Anti bacterial and antifungal activity of *Sapindus emarginatus* and *Mirabilis jalapa*

Zone of Inhibition (in mm)							
Sapindus emarginatus							
Plants Extract	**Conc. mg/ml**	*B. subtilis*	*E. coli*	*S. epidermidis*	*C. glabreta*	*A. flavus*	*C. albicans*
Methanol Extract	100	18	17	19	14	15	16
	50	15	14	13	12	13	14
	25	13	12	14	11	10	12
Chloroform Extract	100	17	16	18	13	11	12
	50	16	14	16	11	10	12
	25	15	12	15	09	08	11
n-Hexane Extract	100	18	16	19	12	13	10
	50	15	16	17	10	11	08
	25	12	14	15	07	09	07
Water Extract	100	21	20	22	17	17	19
	50	19	18	20	15	16	17
	25	17	16	18	14	15	15
Mirabilis jalapa							
Methanol Extract	100	17	16	18	13	11	12
	50	15	15	17	11	10	12
	25	14	15	16	09	08	11
Chloroform Extract	100	18	17	19	14	15	16
	50	16	14	17	12	13	14
	25	13	12	14	11	10	12
n-Hexane Extract	100	16	15	17	16	14	15
	50	15	14	16	14	12	13
	25	13	12	14	13	10	11
Water Extract	100	20	23	21	18	16	19
	50	18	17	19	15	14	16
	25	15	14	13	12	13	15

The fresh leaves of *Sapindus emarginatus*, *Hibiscus rosa-sinensis*, *Mirabilis jalapa, Euphorbia tirucalli L, Vitex negundo L, Saussurea lappa Costus* were collected and dried at room temperature after that extracts were taken for isolation using cold percolation method [3].

Preparation of test samples

The dried methanolic extracts, n-Hexane extracts, and chloroform extract were respectively dissolved in sterile dimethylsulfoxide (DMSO) and sterile water at selected concentrations

Microorganisms tested and culture media

The strains of bacteria and fungi were grown in Nutrient Agar Media and Nutrient Agar Broth and the species of different fungi were grown in Sabouraud Dextrose Agar and Sabouraud Dextrose Broth. The concentration of bacterial suspension was adjusted to 10^8 cells /ml, and that fungal suspension was adjusted to 10^7 cells /ml [4]

Bacterial screening by Agar Diffusion assay

The antibacterial activity and antibiotic sensitivity were tested by Agar Diffusion Method on 10 ml aliquots of nutrient broth was inoculated with the test organisms *Staphylococcus epidermidis* , *Bacillus subtilis* and *Escherichia coli* incubated at 37°C for 24 hr[5,6].Then using a sterile Pipette, 0.6 ml of Broth culture of the test organisms was added to 60 ml molten agar which had been cooled to 45°C,mixed well and poured into a sterile Petri plate and allowed to set and harden and the required number of cavities are cut using a sterile cork borer should be used to different organisms. Then using a 0.1 ml pipette,100µl of the test samples dissolved in an appropriate solvent was poured into appropriately labeled cups.The same concentration of the standard antimicrobial agents(streptomycin 1mg/ml and ampicillin 10µg/ml) and the solvent (as control) are used. After that plate are left at room temperature for 2hr to allow diffusion of the sample and incubated face upwards at 37°C for 24 hr. then the diameter of the Zones of inhibition is measured to the nearest mm [7, 8]

Fungal screening by Agar tube dilution assay

The antifungal activity and antifungal sensitivity were tested by Agar tube dilution Method the test sample was dissolved in sterile DMSO to serve as stock solution. Then sabouraud dextrose agar was prepared by mixing sabouraud 4% glucose agar and agar agar in distilled water [9, 10]. It was then stirred with a magnetic stirrer to dissolve it and a known amount was

Table 2: Anti bacterial and antifungal activity of *Hibiscus rosa-sinensis, Vitex negundo L, Saussurea lappa Costus*

Plants Extract	Conc. mg/ml	*B. subtilis*	*E. coli*	*S. epidermidis*	*C. glabreta*	*A. flavus*	*C. albicans*
		Zone of Inhibition (in mm)					
		Hibiscus rosa-sinensis					
Methanol Extract	100	26	24	23	19	17	20
	50	22	20	21	17	15	18
	25	20	17	19	15	14	15
Chloroform Extract	100	17	17	19	16	13	16
	50	15	14	13	14	13	14
	25	13	12	11	11	10	12
n-Hexane Extract	100	18	17	19	14	15	16
	50	16	14	17	12	13	14
	25	13	12	14	11	10	12
Water Extract	100	16	17	18	13	14	16
	50	16	15	17	11	13	14
	25	13	12	14	10	11	12
		Vitex negundo L					
Methanol Extract	100	23	25	24	18	17	19
	50	21	20	21	16	15	18
	25	20	18	19	15	14	16
Chloroform Extract	100	18	19	17	16	14	16
	50	17	16	15	14	13	14
	25	13	12	11	11	10	12
n-Hexane Extract	100	18	17	19	14	15	16
	50	16	14	17	12	13	14
	25	13	12	14	11	10	12
Water Extract	100	19	16	18	14	14	16
	50	16	15	17	11	13	14
	25	13	12	14	10	11	12
		Saussurea lappa Costus					
Methanol Extract	100	24	25	24	19	17	17
	50	21	22	20	17	15	15
	25	20	18	19	15	13	14
Chloroform Extract	100	19	19	17	17	14	16
	50	17	16	15	14	13	14
	25	15	15	13	11	10	12
n-Hexane Extract	100	19	17	19	14	15	16
	50	16	14	17	13	13	14
	25	13	12	14	11	10	12
Water Extract	100	15	16	18	14	14	16
	50	16	15	17	11	13	14
	25	13	12	15	10	11	12

dispensed into screw capped test tubes. Then test tube containing media are autoclaved at 121 °C fro 15 minutes .The tubes are allowed to cool to 50°C and the test sample of desired concentrations pipette from the stock solution into the non-solidified Sabouraud agar media. The tubes are then allowed to solidify in a slanting position at room temperature. Each tube was inoculated with a 4 mm diameter piece of inoculums removed from a seven day old culture of fungi. All culture containing tubes are inoculated at optimum temperature of 28-30°C fro growth for 7 -10days.Humidity is controlled by placing an open pan of water in the incubator. Cultures are examined at least twice weekly in the incubation. After the incubation for 7-10 days, the test tubes with no visible growth microorganism was taken to represent the minimum inhibitory concentration (MIC) of the test sample which was expressed in µg/ml [11, 12]

RESULT:

The water extract of *Sapindus emarginatus* and *Mirabilis jalapa* showed the highest Zone of inhibition with the concentration of 100mg/ml against the bacterial test organism

Table 3: Anti bacterial and antifungal activity of *Euphorbia tirucalli L*

Zone of Inhibition (in mm) *Euphorbia tirucalli L*							
Plants Extract	**Conc. mg/ml**	*B. subtilis*	*E. coli*	*S. epidermidis*	*C. glabreta*	*A. flavus*	*C. albicans*
Methanol Extract	100	17	20	18	15	14	15
	50	15	18	16	14	13	13
	25	13	16	15	14	10	11
Chloroform Extract	100	19	16	18	14	14	16
	50	16	15	17	11	13	14
	25	13	12	14	10	11	12
n-Hexane Extract	100	23	22	24	18	16	19
	50	21	20	21	16	15	18
	25	20	18	19	15	14	16
Water Extract	100	18	17	19	14	15	16
	50	16	14	17	12	13	14
	25	13	12	14	11	10	12

Table 4: Anti bacterial and Anti fungal activity of Standard Drugs against bacterial and fungal test organism.

Zone of Inhibition (in mm)							
Drug	**Conc. mg/ml**	*B. subtilis*	*E. coli*	*S. epidermidis*	*C. glabreta*	*A. flavus*	*C. albicans*
Ampicillin	100	31	33	30	NT	NT	NT
	50	27	28	27	NT	NT	NT
	25	25	26	24	NT	NT	NT
Streptomycin	100	33	35	32	NT	NT	NT
	50	29	31	28	NT	NT	NT
	25	25	27	26	NT	NT	NT
ketoconozole	100	NT	NT	NT	28	29	26
	50	NT	NT	NT	26	27	24
	25	NT	NT	NT	24	25	22
Amphotericin B	100	NT	NT	NT	26	27	25
	50	NT	NT	NT	23	24	21
	25	NT	NT	NT	20	22	19

NT: not tested

with the diameter of 20mm- 23mm and with the diameter of 16mm-19mm against the fungal strains other than that of Methanol, Chloroform and n-Hexane extracts with lower diameter Zone of inhibition shown in the table 1

The methanol extracts of *Hibiscus rosa-sinensis, Vitex negundo L, Saussurea lappa Costus* shows highest Zone of inhibition with the concentration of 100mg/ml against the bacterial test organism with the diameter of 23mm- 26mm and with the diameter of 17mm-20mm against the fungal strains other than that of Water, Chloroform and n-Hexane extracts with lower diameter Zone of inhibition shown in the table 2

The n-Hexane extracts of *Euphorbia tirucalli L* shows highest Zone of inhibition with the concentration of 100mg/ml against the bacterial test organism with the diameter of 21mm- 24mm and with the diameter of 16mm-19mm against the fungal strains other than that of Water, Chloroform and Methanol extracts with lower diameter Zone of inhibition shown in the table 3

CONCLUSION: The data obtained by the present study suggest that the highest Zone of inhibition against all different extracts of Plants but there was no response against chloroform extract.

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