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A STUDY ON THE ANTIMICROBIAL EFFECTS OF CRUDE PRINCIPLES EXTRACTED FROM *PIPER BETLE* LINN. LEAF STALKS AND *OCIMUM SANCTUM* LINN. LEAVES

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ABSTRACT: The nature harbours a huge source of active principles possessing antimicrobial property. This phenomenon provokes microbiologists, ethnopharmacologist and natural product chemists to perform extensive research on phytoprinciples. The extensive use of these principles in phytotherapy is becoming an excellent alternative strategy against the occurrence of microbial resistance due to some conventional medicines. The current research article dealt with ethanolic crude extractives from the dried leaves of *Ocimum sanctum* Linn. and fresh Leaf stalks of *Piper betle* Linn. were screened for its antimicrobial activity using the agar-well diffusion method. The sample of the mentioned herbs could be easily found in large amount from the environment of north east region in India. In our present study with *Ocimum sanctum* L. (tulsi) leaf extract showed marked inhibitory effect on *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *E.coli*, *Vibrio cholerae* where a highest zone of inhibition estimated was 30 mm against *E. coli.* Tulsi extractive possess partial inhibition against *Candida albicans.* Crude ethanolic extractive of *Piper betle* Linn. (Paan) stalk remarkably inhibited. *A. niger*, *Escherichia coli* and *Candida albicans* where the highest inhibition zone was 27 mm against *Aspergillus niger*. This study has also focussed about the microbiostatic nature of the crude extracts isolated from the mentioned herbal parts. This work also emphasizes on the strategy for recycling or reusing betle stalks thrown as a waste materials beside many paan shop and tulsi leaves as thrown outside many temples after worshipping.

INTRODUCTION: The traditional system of medicine in India dates back to the early age of Rig Veda (450 - 1600BC) which provides ample proof of the various recipes of Indian Herbs in curing many maladies [1, 2]. This has interested the phytochemists and biologists to pursue intensive research on Indian medicinal plants, which exhibited extensive therapeutic values [3].

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The emergence of drug resistance in the infectious microorganisms have increased the need for antimicrobial agents that are highly effective and at the same time possess low toxicity against the host. Drug resistance in bacteria, the appearance of life-threatening viruses, and the tremendous increase in the incidence of microbial infections in the third world countries each only underscore our inadequacy to cope with these medical problems [4].

This search is driven by the development of resistance in infectious microorganisms to existing compound & by the menacing presence of naturally resistant organisms. There is an extensive research in search of antimicrobial compounds of herbal

origin, the availability of these compounds are very low. Thus the objective of this work is to exploit the naturally derived metabolites from the plant extracts of two very Common plants [5] *Piper betle* Linn. leaf stalk [6, 7, 8], *Ocimum sanctum* Linn. leaf were used as an alternative to fight against common pathogens.

MATERIALS AND METHODS:

Collection and Preparation of Herbal Samples: The *Ocimum sanctum* leaves and the *Piper betle* leaf-stalks were collected, dried under room temperature for two weeks, and cut into pieces [9]. About 21.1 g of Tulsi leaves (*Ocimum sanctum*) are obtained on drying and 100 ml ethanol is added to it and kept for three days. Nearly 100.01 g of dried betel leaf stalk (*Piper betle*) was mixed with 250 ml ethanol and rotated with constant agitation (200 rpm) overnight at 20 °C in a temperature controlled bioshaker for 72 h. The plant residue from the ethanolic extract [10, 11] was separated using sterile cheese cloth and filtered through sterile Whatman filter paper (no. 2). The filtrate containing the extractive was concentrated under rotary evaporator. After evaporation, the active principles extracted by the ethanol was left and the excess ethanol was vaporized. All the transfers were made asceptically. At present this information is not available for local specimens which was verified by Dr. N. D. Paria, Department of Botany C.U.

Preparation of Test Inoculum: Test bacterial culture of *Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Vibrio cholerae* were grown in nutrient broth for 24 h. Among fungal test cultures *Candida albicans* and *Aspergillus niger* were grown in Sabouraud broth for 48 h. The incubation temperature was maintained at 37 °C.

Preparation of the Concentrated Extracts: Weight of the *Ocimum sanctum* leaf extract (ethanolic) obtained was found to be 4.63 g and dissolved in 20 ml absolute alcohol. Therefore, the concentration of crude extract became 0.2315 g/ml. Weight of the *Piper betle* leaf stalk ethanolic extractive obtained was found to be 3.27 g. Similarly the concentration of crude *Piper betle* leaf stalk ethanolic extractive was 0.218 g/ml.

Study of Antimicrobial Activity of *Piper betle* Linn and *Ocimum sanctum* Extracts: The antimicrobial activity of the crude extractives [12] which contain phytochemicals and the essential oil was evaluated against the microbial sample. Agar well diffusion method was adopted. 0.1 ml of test organisms were inoculated on solid media. Test solution/extracts added in the peripheral wells with varying volume and ethanol (solvent of extraction) was added in the central well as control. These plates were incubated at 37 ºC undisturbed for 24 h for Bacteria and 48 h for Fungus. Observations were taken by measuring the diameter of the zones of inhibitions after the incubation period. Actual (net) diameter of the zones were noted, considering the inhibition zone by ethanol in the control well **Table 1** and **2**.

TABLE 1: OBSERVATION TABLE FOR THE CLEAR ZONE OF INHIBITIONS OF ETHANOLIC EXTRACT OF *OCIMUM SANCTUM* LINN. (TULSI) LEAVES

Microorganisms	Extract (µl)	Mean zone diameter of the inhibition for test extract (t mm)	Diameter of the inhibition zone for solvent of extraction (c mm)	Actual diameter of inhibition zone (t-c mm)
K. pneumoniae.	60	12	–	12
	80	17		17
	100	20		20
P. aeruginosa	40	12	–	12
	50	15		15
	60	17		17
E. coli	40	25		25
	50	28	Less than	28
	60	30	5*	30
V. cholerae	80	15	Less than	15
	100	20	5*	20
C. albicans	40	12	–	12
	50	15		15
	60	19		19
A. niger	40	–	–	–
	50			
	60			

*Less than 5 mm was considered as negligible

RESULTS: The zone of inhibition suggests the efficacy of antimicrobial activity of the test extract (ethanolic extract of *Ocimum sanctum* Linn. (Tulsi) leaves and *Piper betle* Linn. (Pan) stalk. It was found that among the test organisms, *K. pneumoniae*, *P. aeruginosa, E. coli, V. cholerae* were inhibited by Tulsi extract **Fig. 1**. But *C. albicans* is partially inhibited and *A. niger* is not at all inhibited at this concentration. On the other hand it was observed that among our test organisms, *A. niger, E. coli* and *C. albicans*, were inhibited by *Piper betle* Linn. (Paan) stalk extract. But *K. pneumoniae, P. aeruginosa, V. cholerae* were not inhibited at this concentration **Fig. 2**.

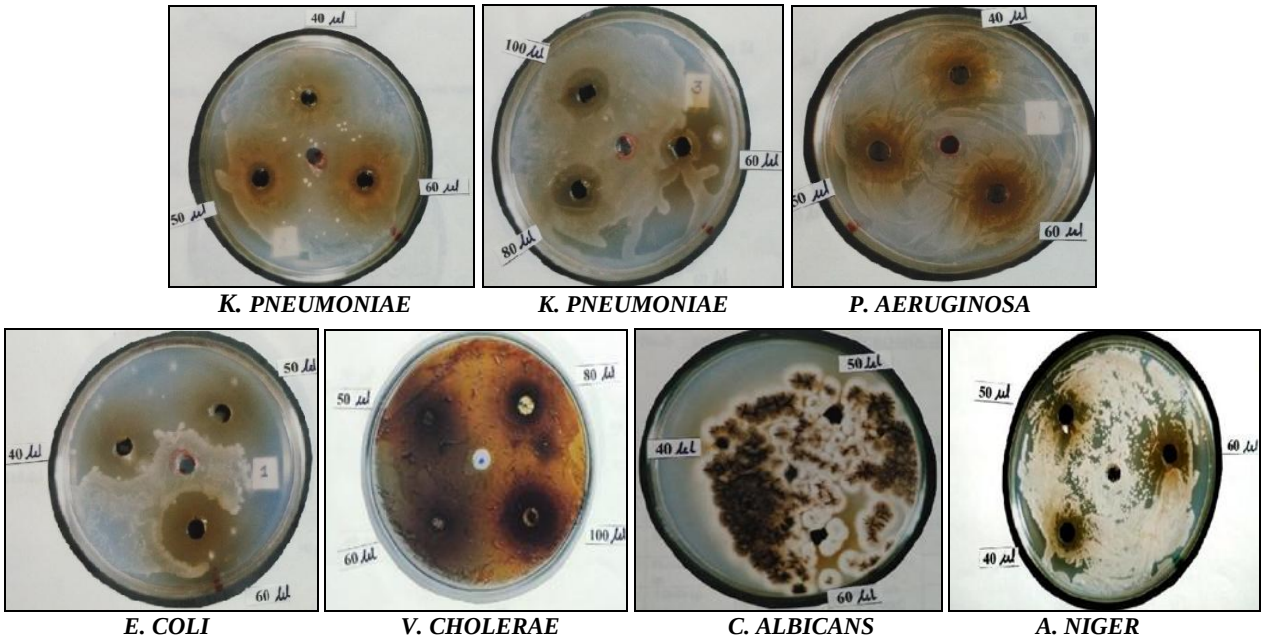


FIG. 1: ZONE OF INHIBITIONS OF ETHANOLIC EXTRACT OF *OCIMUM SANCTUM* LINN. (TULSI) LEAVES

TABLE 2: DETERMINATION OF MICROBIOCIDAL OR MICROBIOSTATIC ACTIVITY

Test Tube	Broth	Loopful Culture Inoculated		Tulsi Extract added(µl)		Growth (turbidity)		Growth In fresh media
1	Nutrient Broth	*E. coli*	Incubation	100	Incubation for	-		+
2	Nutrient Broth	*K. pneumoniae*	for 4 h at 37 ºC	100	24 h (bacteria)	-		+
3	Nutrient Broth	*P. aeruginosa*		100	and 48 h (fungi)	-	#	+
4	Nutrient Broth	*V. cholerae*		100	at 37 ºC	-		+
5	Sabouraud Broth	*C. albicans*		100		-		+
6	Sabouraud Broth	*A. niger*		100		-		+

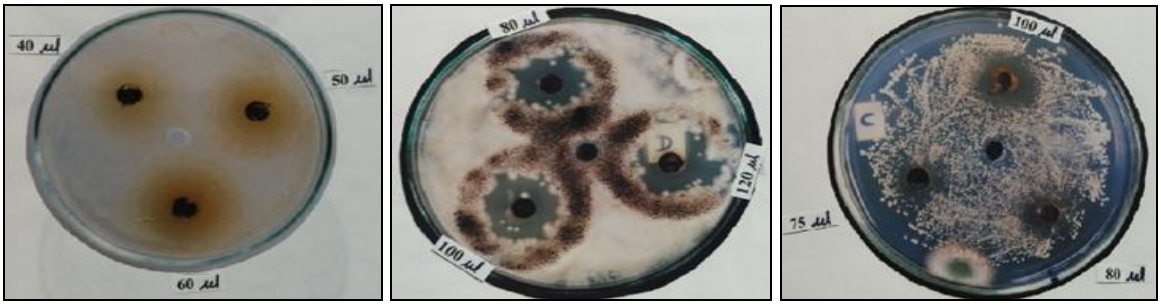
Loopful culture from the negative growth test tube were reinoculated in 5 ml fresh culture media and incubated again without adding the extract. Turbidity or growth in the fresh media was observed.

Therefore, the extractive at this concentration is acting as Microbiostatic agent.

TABLE 3: OBSERVATION TABLE FOR THE CLEAR ZONE OF INHIBITIONS FOR ETHANOLIC EXTRACT OF *PIPER BETLE* LINN. (PAAN) LEAVES

Microorganisms	Extract added(µl)	Mean diameter of the inhibition zone for test extract(t mm)	Diameter of the inhibition zone for solvent of extraction(c mm)	Actual diameter of inhibition zone (t-c mm)
K. pneumoniae	40	–	–	–
	50			
	60			
P. aeruginosa	40	–	–	–
	50			
	60			
E. coli	40	15	Less than 5*	15
	50	20		20
	60	24		24
V. cholerae	80	–	Less than 5*	–
	100			
C. albicans	75	12	Less than 5*	12
	80	15		15
	100	20		20
A. niger	80	15	Less than 5*	15
	100	20		20
	120	27		27

*Less than 5 mm is considered negligible



E. COLI ***A. NIGER*** ***C. ALBICANS***

FIG. 2: ZONE OF INHIBITIONS FOR ETHANOLIC EXTRACT OF *PIPER BETLE* LINN. (PAAN) LEAVES

TABLE 4: DETERMINATION OF MICROBIOCIDAL OR MICROBIOSTATIC ACTIVITY

Test Tube	Broth	Loopful Culture Inoculated		Betle Extract added (µl)		Growth turbidity		Growth in fresh media
1	Nutrient Broth	*E. coli*	Incubation	100	Incubation for	-		+
2	Nutrient Broth	*K. pneumoniae*	For 4 h at	100	24 h (Bacteria)	-		+
3	Nutrient Broth	*P. aeruginosa*	37ºC	100	and 48 h (Fungi)	-		+
4	Nutrient Broth	*V. cholerae*		100	at 37 ºC	-	*	+
5	Sabouraud Broth	*C. albicans*		100		-		+
6	Sabouraud Broth	*A. niger*		100		-		+

* Loopful culture from the negative growth test tube were reinoculated in 5 ml fresh culture media and incubated again without adding the extract.

Turbidity or growth in the fresh media was observed. Therefore, the extractive at this concentration is acting as microbiostatic agent.

DISCUSSION: In the present study, it was clearly found that highly active principles are present in the ethanolic extract of *Ocimum sanctum* Linn. (Tulsi) leaves, which have antibacterial and antifungal properties. The active principles inhibited the growth of the test organism like *K. pneumoniae, P. aeruginosa, E. coli, V. cholerae,* and *C. albicans*. At different doses of the extractive, the zone of inhibitions varied remarkably [13]. Beside these findings, the extended study revealed that the extract possessed microbiostatic property at low concentration of the extractive. The ethanolic extract of *Piper betle* Linn. leaf stalk, also revealed that some active compounds arrests the growth of the test organism like *E. coli, A. niger* and *C. albicans*. Inhibition zone varied remarkably with variable concentration of the extractive. The ethanolic extract possesses Microbiostatic [14] effect over all test microbial samples as well as successfully helped in the field of environmental science by recycling or reuse betle stalks and tulsi leaves thrown as a waste materials.

CONCLUSION: Phytomedicines are emerging in the present world very rapidly. Historically plants have provided a source of inspiration for novel drug compounds. Their role is twofold in the development of new drugs: They may become the base for the development of a medicine, a natural blue print and relatively safer than the synthetic ones.

Some other benefits are:

a) They are effective and gentle.
b) Consumer believes that natural products are superior and overcome the dissatisfaction with conventional medicine and devoid of many of the side effects that are often associated with synthetic antimicrobials.

Our present study revealed the efficacy of well-known plant extractives acts as a microbiostatic agent against a wide range of common pathogens. The active principles can be isolated by solvent fractionation, chromatography and NMR spectroscopy. This results is inspiring to continue further work to find a potential herbal remedies for common microbial species mentioned in this article.

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CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

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