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Review Article

COMPARATIVE STUDIES ON ANTIMICROBIAL AND ANTIFUNGAL EFFICACY FROM *BIXA ORELLANA* L., *LANTANA CAMARA* L., *STACHYTARPHETA JAMAICENSIS* (L.)Vahl., *HYPTIS SUAVEOLENS* (L.) POIT. WITH TRICLOSAN

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ABSTRACT
The aim of the present study was to assess the Antimicrobial and Antifungal activities of the Phenolic leaf extracts of *Bixa orellana* L., *Lantana camara* L *and Stachytarpheta jamaicensis* (L.) Vahl. *Hyptis suaveolens* (L.) Piot. and the Triclosan, a chlorinated aromatic compound with antibacterial and antifungal properties used in common house hold and personal care products and to compare household and personal care products and to compare their effectiveness against 4 bacterial strains - 2 Gram Positive strains – *Staphylococcus aurens* and *Bacillus substitis* and 2 Gram negative strains – *Escherischia coli* and *Pseudomonas fluorescens* and 3 Fungi- *Aspergillus niger*, *Aspergillus flavus* and *Mucor* Sp., by Agar well diffusion Assay. The phenolic extracts of all the 4 plants showed Maximum (80-100%), Relative inhibition against *Pseudomonas fluorescence*, Moderate inhibition (30-70%) against *Staphylococcus aurens* and *Bacillus substilis* and least inhibition (30-47%) against *Escherischia coli*, while, the Antifungal efficacy of all the 4 Phenolic plant extracts were observed to be effective at the concentration ranging from 70-300 µg. The plant phenolic extracts for Antimicrobial and Antifungal properties were compared with Standard Triclosan, a chlorinated compound. Our studies showed that the phenolic components of plant origin for antibacterial activity were equivalent to Triclosan with the same concentration, while for antifungal activity slightly higher concentrations could be a better alternative and hence there could be a substitution for Triclosan by Plant Phenolic Extracts used in house hold and personal care products, in future days to come.

***Key Words*:** *Bixa orellana L., Lantana Camara L., Phenolics, Antimicrobial, Antifungal and Triclosan.*

INTRODUCTION

The existing plant flora on globe constitutes about 250,000 to 500,000 species of plants **(**Borris,1996). Out of which only small percentage (1 to 10%) of plants are been used as a source of food by both humans and other animal species. The recent investigations are focused more on the screening of bioactive compounds present in the plants. In this effort a wide variety of bioactive compounds are identified to be used as potential herbal medicines **(**Moerman 1996).

The pharmacological industries have developed variety of antibiotics in the last few decades. In spite of this certain microorganism gains resistance against some of the antibiotics and also the side effects exerted by these drugs. There is a need for the development of more effective and safer medicines. Herbal medicines are gaining global interest because of their cost effective and eco-friendly attributes **(**Dwivedi *et al.,* 1998). There are several major groups of secondary compounds exists in the plants includes alkaloids, phenolic compounds and terpenoids. In phenolic compounds, phytochemicals such as tannins, flavonoids and coumarins. Occasionally, these substances help the plants. These compounds are produced as a defense mechanism against microorganisms, herbivors, and insects. For instance the synthesis of antimicrobial compounds by plants that are infected by bacteria and fungi known as phytoalexins **(**Michael *et al.,* 2003). Since most of the weed and certain other plants including *Bixa orellana* L., *Lantana camara* L., *Stachytarpeta jamaicensis* (L).Vahl., *Hyptis suaveolens* (L.) Piot. are known to

contain high phenolic phytochemicals. Due to which they exhibit high resistance against microbial and fungal infections. Hence, Current work accounts for the comparative study of antimicrobial and antifungal property of the phenolic leaf extracts from *Bixa orellana* L., *Lantana camara* L and *Stachytarpeta jamaicensis* (L).Vahl., *Hyptis suaveolens* (L.) Piot. The antimicrobial and antifungal activity of the phenolic extract from these plants (leaves) was also compared with the Triclosan. Triclosan is a chlorinated aromatic compound with antibacterial, antifungal and antiviral properties. The Triclosan is used in a variety of common household and personal care products, including soaps, mouthwashes, deodorants, toothpastes, dish detergents, acne creams and hand sanitizers (Daughton *et al.*, 1999). It reacts with the free chlorine in tap water to produce chloroform gas which is potential carcinogen. Thus, the adequate use of triclosan leads to the contamination of surface waters, as they undergo slow biodegradation they tend to persist in the environment for a long time **(**Rule *et al.*, 2005). Apart from this the environmental toxicity of the triclosan is mainly due to the fact that it can undergo ring closure to form chlorinated dioxins upon exposure to heat or radiation (Okumura *et al.*, 1996). Although small amounts of dioxins are produced, some dioxins are extremely toxic and are very potent endocrine disruptors. Since the naturally occurring phenolic phytochemicals also exhibit broad spectrum of antimicrobial and antifungal property similar to triclosan. The substitution of triclosan by plant phenolics in the house hold products will be beneficial as well as eco-friendly. In this direction current work is focused on comparative analysis of antimicrobial and antifungal properties of the phenolic phytochemicals (*Bixa orellana* L., *Lantana camara* L., *Stachytarpeta jamaicensis* (L.) Vahl., *Hyptis suaveolens* (L.) Piot and trilosan.

MATERIALS AND METHODS

Plant Materials

Fresh leaves of *Bixa orellana* L., *Lantana camara* L., *Stachytarpeta jamaicensis* (L.) Vahl., *Hyptis* suaveolens (L.) Piot. were collected from Bangalore University, Jnana bharathi campus, Bangalore, Karnataka, India. They were dried in the shade, powdered and stored in an air tight container for analyses.

Microorganisms used

Bacterial and fungal cultures used were procured from Biotechnology and Microbiology Department, Bangalore University, Bangalore, Karnataka, India. Two gram negative bacterial strains used in our study were *Escherichia coli and Pseudomonas florescence.* The two gram positive bacterial strains used were *Staphylococcus aureus and Bacillus subtilis*. The fungal strains used were *Aspergillus Niger, Aspergillus flavus* and *Mucor sp.*

Phytochemical analysis

The phytochemical screening of the phenolic leaf extracts of *Bixa orellana* L., *Lantana camara* L and *Stachytarpeta jamaicensis* (L.) Vahl., *Hyptis suaveolens* (L.) Piot. was carried out using standard procedures **(**Trease *et al.*, 1983 ;1989,Harborne 1991 ;1998, Edeoga *et al.*, 2005). The extract was tested for the presence of phenolic compounds, saponins, coumarins, terpenoids, flavonoids, phlobatannins, and tannins. The Total phenolics were extracted and estimated according to **(**AOAC, 1960).

Antimicrobial activities of the phenolic extract

Media

The medium employed was nutrient agar (Himedia) to culture bacteria and potato dextrose agar (Himedia) to culture fungus. As methanol was used as solvent in the extraction procedures and thus it is used to develop negative controls in the assays. The Antimicrobial agent such as Triclosan (40 and 80 µg) was used as standard reference in the study.

Agar diffusion-pour plate method (Bacteria)

The phenolic leaf extract was screened for antimicrobial activity using the Agar-Well diffusion method (Barry *et al.*, 1979, Abolhassani, 2004). Briefly, an overnight culture of each organism was prepared appropriately from its stock and inoculated each into the sterile nutrient broth of 5ml, each incubated for

18-24hrs at 37^0C. From this culture 0.5ml was added to 20 ml of sterile nutrient agar cooled to about 40-45^0C, then poured into sterile Petri dishes and allowed to solidify for about 45-60mins. Using a sterile cork-borer of 6mm diameter, the wells were made according to the number of the test tubes for the experiment. The graded concentrations of the extracts were put into the wells accordingly including the controls. The studies were done in three triplicates to ascertain the results obtained. The plates were left on the bench for about 2hrs to allow the extract diffuse properly into the nutrient agar i.e. pre-diffusion. The plates were incubated for 18-24hrs at 37^0C.

Agar diffusion-surface plate method (Fungi)

A sterile potato dextrose agar was prepared accordingly and aseptically poured into the sterile plates in duplicates and solidified properly. 0.5ml of the organism was spread on the surface of the agar using a sterile cotton swab. Four wells were bored using a sterile cork-borer of 6mm diameter. The graded concentrations of the extracts were put into the wells accordingly including the controls. All the plates were left on the bench for 2hrs to allow the extract diffuse properly into the agar i.e. prediffusion. The Plates were incubated at 25^0C for 72hrs (Ayfer Atep *et al.*, 2003).

RESULTS AND DISCUSSION

The results of the phytochemical screening of phenolic extract from *Bixa orellana* L., *Lantana camara* L., *Stachytarpeta jamaicensis* L. and *Hyptis suaveolens.* L. leaves revealed the presence of common types of phytochemical ingredients that includes phenolic compounds, terpenoids and flavonoids. However the tannins, saponins and coumarins were absent in all these extracts (Table 1)

Table 1: Phytochemical screening of Phenolic extracts from leaves of *Bixa orellana* L., *Lantana camara* L., *Stachytarpeta jamaicensis* L., *Hyptis suaveolens.* L.

Phytochemical Tests	*Bixa orellana* L.	*Lantana camara* L.	*Stachytarpeta jamaicensis* L.	*Hyptis suaveolens.* L.
Test for the Detection of phenolic compounds	++ve	++ve	++ve	++ve
Flavonoids	++ve	++ve	++ve	++ve
Tannin	- ve	- ve	- ve	- ve
Coumarian	- ve	- ve	- ve	- ve
Terpenoids	++ve	++ve	++ve	++ve
Phodatannins	- ve	- ve	- ve	- ve
Saponins	- ve	- ve	- ve	- ve

Note: ++ positive, -ve Negative

The antibacterial activity of the phenolic extracts from Bixa *orellana* L., *Lantana camara* L., *Stachytarpeta jamaicensis* L., and *Hyptis suaveolens* L. leaves demonstrated slightly lesser inhibition zone in comparison to triclosan at similar concentration (80µg). The percentage of relative inhibitory zone (%RIZ) was calculated by applying the expression (Jhon Rojas *et al.*, 2006).

$$RIZ = \frac{IZDsample - IZD\ negative\ control}{IZDstandard} \times 100$$

Where RIZ is the percentage of relative inhibition zone diameter and IZD is the inhibition zone diameter (mm). Compensates the possible effect of the solvent (blank) other than water on the IZD. The resulting IZD of the samples were either higher than or equal to the IZD of the blanks. The phenolic extracts from

Bixa orellana L., *Lantana camara* L., *Stachytarpeta jamaicensis* L., *Hyptis suaveolens*. L. leaves exhibited an average of 30-100% relative inhibitory zone in comparison with triclosan (Table 2, Figures 1 and 3).

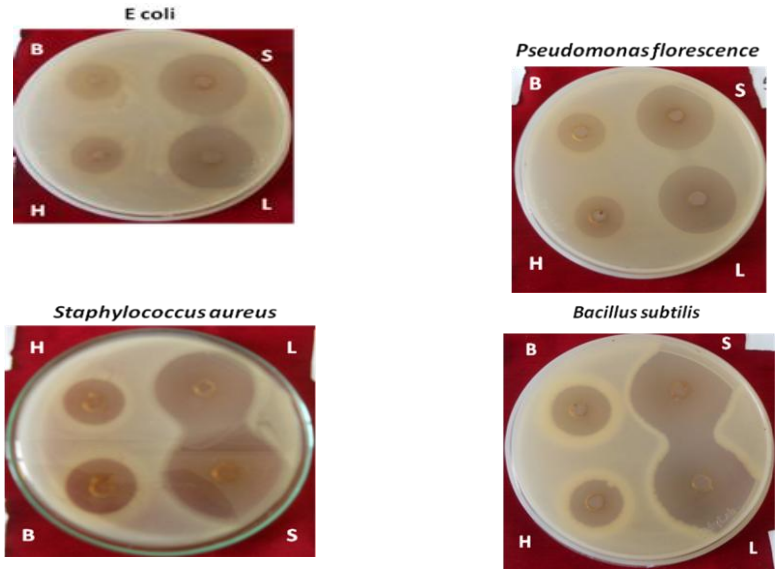

Figure1: Antibacterial activity of phenolic extracts (80ug) from B-*Bixa orellana* L., L- *Lantana camara* L., S-*Stachytarpeta jamaicensis* L., H-*Hyptis suaveolens*. L. against Bacterial Species.

The phenolic extracts of all the four plants showed maximum inhibition against *Pseudomonas florescence* and it is accessed to be 80-100% RIZ, The moderate inhibition was observed against *Staphylococcus aureus and Bacillus subtilis* (30-70% RIZ) and least inhibition against *E.coli* (30-47% RIZ). The less susceptibility to the plant phenolics in *E.coli* is due to the fact that they exert resistance to these substances due to the presence of membrane transport protein that transport these toxic substance out of the cell. The results of antifungal efficacy of the phenolic extract from leaves of *Bixa orellana* L., *Lantana camara L.,Stachytarpeta jamaicensis L.,and Hyptis suveolensL.*,(Table 3 ; Figure 2). The phenolic (leaves) extracts from individual plant exhibited varied antifungal activity against different fungal strains that are used in our study. The phenolic extract from *Bixa orellana* L., leaves containing 70µg of phenolic substance was found to have more inhibitory effect on *Aspergillus niger* and *Mucor*, whereas slightly higher concentration was required to inhibit the growth of *Aspergillus flavus*. The phenolic extract from *Hyptis suaveolens* (L.) Piot. leaves containing 300µg of phenolic substance was effect on all the three fungal strains.

The *Mucor* when compared to other two fungal strains was found to be susceptible to the phenolic extract prepared from the leaves of lantana camara L., and *Stachytarpheta jamaicensis* (L.)Vahl. Over all the antifungal efficacy of all the four plant extracts observed to be effective at the phenolic concentration ranging from 70-300µg.

The antimicrobial and antifungal properties of the plant extract are mainly due to the presence of wide variety of phenolic and hydroxyl derivatives of phenolic compounds. Among which Cinnamic and caffeic acids are common representatives of a wide group of phenyl propane-derived compounds. The common herbs tarragon and thyme both contain caffeic acid, which is effective against viruses **(**Wild,1994), bacteria **(**Brantner *et al.*,,1996, Thomson 1978) and fungi (Duke 1985). Catechol and pyrogallol both are hydroxylated phenols shown to be toxic to microorganisms. Catechol has two hydroxyl groups, and pyrogallol has three.

Table 2: Antibacterial activity of phenolic extracts (80ug) from *Bixa orellana* L., *Lantana camara* L., *Stachytarpeta jamaicensis* L., *Hyptis suaveolens.* L. against Bacterial Species.

Organisms used	**Zone of Inhibition (mm)**					**Percentage Relative inhibition zone**			
	Bixa orellana L.	*Lantana camara* L.	*Stachytarpeta jamaicensis* L.	*Hyptis suaveolens.* L.	Triclosan	*Bixa orellana* L.	*Lantana camara* L.	*Stachytarpeta jamaicensis* L.	*Hyptis suaveolens.* L.
Bacillus substilis	17	12	12	13	17	76	70	70	47
Escherischia coli,	11	10	11	07	23	47	43	47	30
Pseudomonas fluorescence	22	22	20	18	22	100	100	90	81
Staphylococcus aurens	11	08	11	07	22	50	36	50	31

The site of occurrence and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity **(**Geissman1963). More over phytochemicals are of natural origin and they undergo biodegradation at much faster rate compared to triclosan which is synthetic chlorinated compound and it is not easily biodegradable. Therefore the plant phenolic substances are of great advantage over synthetic non biodegradable compounds. Since, the phytochemicals are of natural origin and they undergo biodegradation at much faster rate compared to triclosan which is synthetic chlorinated compound and it is not easily biodegradable.

Hence, it persists in nature for long period during which it can undergo chemical reaction to form undesirable and much more toxic products and thus it may also exert toxic effect on live organism both aquatic and higher animals including humans. The chlorinated dioxins that are formed by triclosan in surface water and is chemically stable, these are eliminated from the body slowly leading to bioaccumulation.

Table 3: Antifungal efficacy of the different phenolic extracts from the leaves of *Bixa orellana* L., *Lantana camara* L., *Stachytarpeta jamaicensis* L., *Hyptis suaveolens.* L. against fungal species

Extract	Extract Concentration (µg)	Zone of Inhibition (mm)		
		Aspergillus niger	*Aspergillus flavus*	*Mucor Sp.*
Bixa orellana L.	70(B1)	02	-	06
	140(B2)	10	03	12
	280(B3)	15	12	24
Lantana camara L.	108(L1)	-	01	03
	217(L2)	01	04	08
	434(L3)	07	11	12
Stachytarpeta jamaicensis L.	89(S1)	-	-	06
	178(S2)	04	05	10
	356(S3)	11	11	24
Hyptis suaveolens. L.	159(H1)	-	-	06
	318(H2)	02	02	10
	636(H3)	10	08	14
Triclosan	40(T1)	10	13	07
	80(T2)	11	17	11

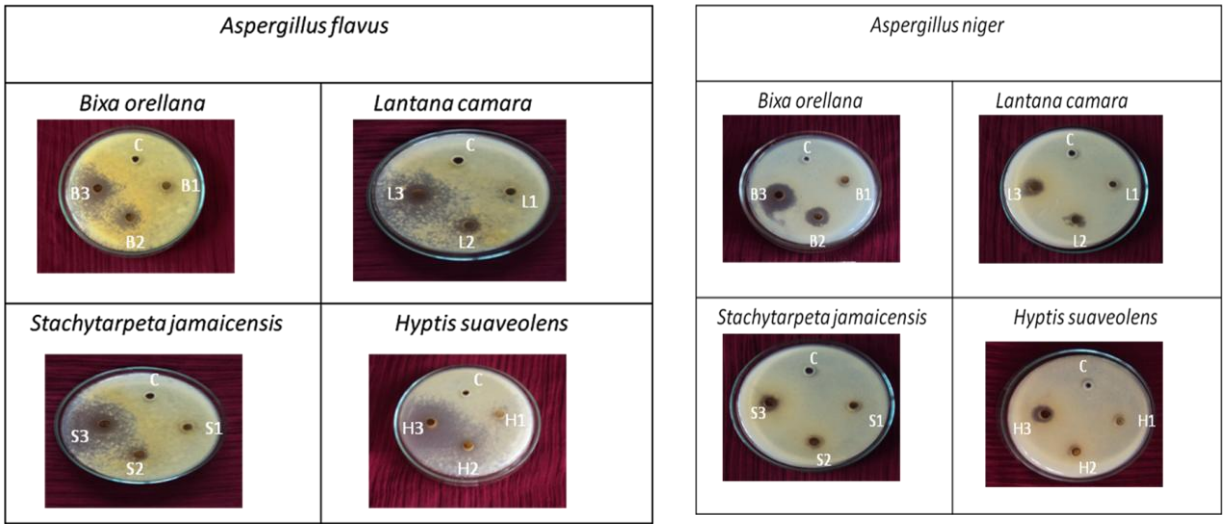

Figure 1: Antifungal efficacy of the different phenolic extracts from the leaves of *Bixa orellana* L., *Lantana camara* L., *Stachytarpeta jamaicensis* L., *Hyptis suaveolens.* L. against fungal species

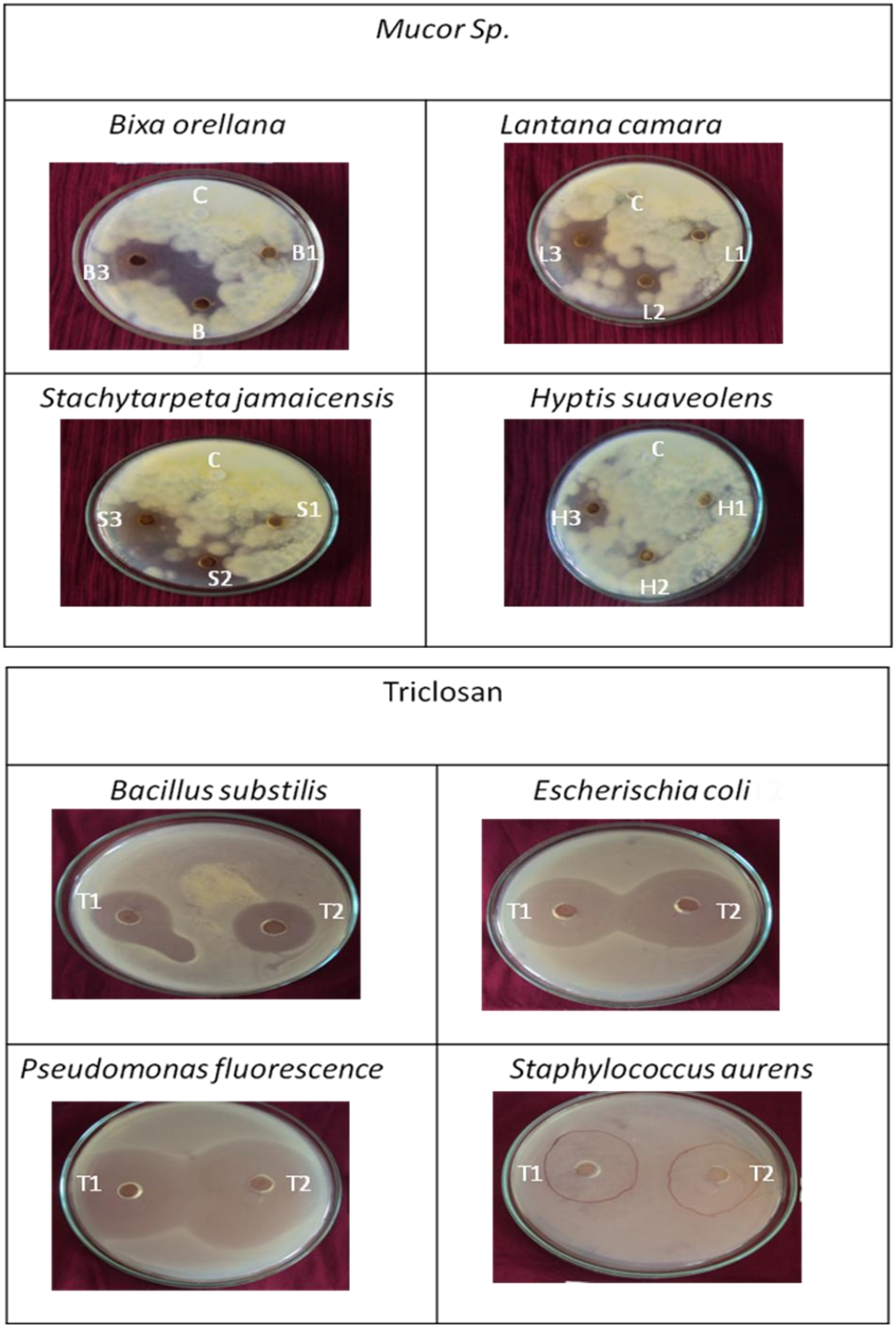

Figure 2: Antifungal efficacy of the different phenolic extracts from the leaves of *Bixa orellana* L., *Lantana camara* L., *Stachytarpeta jamaicensis* L., *Hyptis suaveolens.* L. against fungal species.

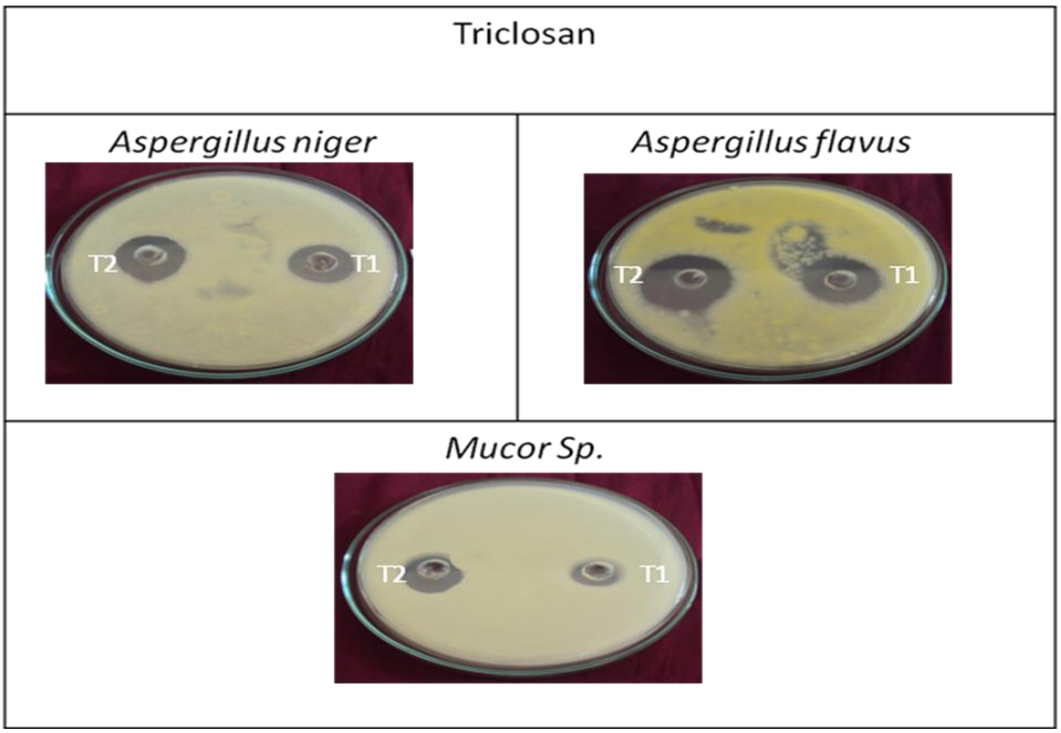

Figure 3: Antimicrobial and antifungal properties of Triclosan.

Therefore the plant phenolics being an eco-friendly could of great advantage and it can even substitute the triclosan which is been currently used as an antimicrobial agent in wide variety of household and personal care products, their by the use of pollutant such as trilosan can be completely avoided.The preliminary screening of antimicrobial and antifungal efficacy of *Bixa orellana* L., *Lantana camara* L., *Stachytarpeta jamaicensis* L., *Hyptis suaveolens.* L. plants was demonstrated in the current studies.

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