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Antimicrobial Screening of *Scoparia dulcis* and *Eclipta alba* Plant Extract

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Abstract: ***Medicinal plants are an amusing reservoir of therapeutic agent and nowadays pathogenic microorganisms are becoming resistant and altering themselves into multidrug safe strains because of use of aimless antibiotic numerous medication. Because of numerous microbes resistant lot of antibiotics are not much effective in the treatment of infection. To fight against these human pathogens there is a need of introduction of new antibiotics. In this study, antimicrobial activity of all extracts was observed by Agar well diffusion method and 96 well plate-microdilution method for their minimum inhibitory concentration. In Scoparia dulcis plant chloroform, ethyl acetate, acetone and methanol extract showed good zone of inhibition against B. subtilis, E. coli, Strep. group B, S. pyogene while Eclipta alba showed good zone of inhibition for methanol against B. subtilis, chloroform against C. albicans, chloroform, acetone and methanol against S. pyogene and hexane against Streptococcus Group B. E. coli showed sensitivity against all five solvents (hexane, ethyl acetate, chloroform, acetone and methanol extract). MIC concentration indicated lowest concentration in hexane, ethyl acetate, chloroform and methanol extract of Scoparia dulcis against E. coli, ethyl acetic, chloroform and methanol against Strep. group B, methanol and methanol extract against S. pyogene, ethyl acetate against C. albicans, and Methanol against S. aureus demonstrated the indicated important significance of antibacterial activity with alternate extracts whereas Eclipta alba showed that hexane, chloroform, acetone and methanol extract against E. coli, chloroform against C. albicans, ethyl acetate, chloroform, acetone, and methanol extract against S. pyogene and methanol against B. subtilis are significant for antimicrobial activity. The current research confirms that both the plants are sensitive towards the pathogenic microorganism.***

Keywords: *Scoparia dulcis*, *Eclipta alba*, antimicrobial test, Agar well diffusion, minimum inhibitory concentration (MIC)

1. Introduction

As back old circumstances, in scan for save for their illness, the general population searched the medications in nature. In perspective of the way that at the time there was not adequate data either concerning the purposes behind the illnesses or concerning which plant and how it could be used as a cure, everything depended on understanding. In time, the explanations behind the utilization of therapeutic plants for treatment of specific illnesses were being found, hence, the medicinal plants' use bit by bit deserted the empiric system and progressed toward becoming established on explicatory actualities. The plants were selected as they have been proven to have many beneficial medicinal compounds. In this study, *Eclipta alba* and *Scoparia dulcis* plants were extracted by using solxhlet hot extraction method in five different solvents (hexane, ethyl acetate, chloroform, acetone and methanol). Further antimicrobial activity of both performed using two methods: Agar well diffusion method was done with the presence of six different pathogens and antibiotics to find out plants extract strong activity against each pathogen. Followed by minimum inhibitory concentration [MIC] was done for the extracts, which shows clear zone of inhibin by using 96 well plate micro-dilution method to see antimicrobial activity of plants extracts.

Problem definition

This study addresses the problem of multi-drug resistant bacteria, which has become a major global threat due to the increase rate of emerging new drug resistant pathogens that possess resistance against not only the first generation of antibiotics, but towards second and third generation too. This makes it quite technically challenge for the researchers and scientist to find new drugs that can be alternative treatment for such mutant bacteria. Especially with the increase risk of developing further resistant toward those new drugs. However, apart from multi drug resistant microbes, few humans showed less to sever allergies to antibiotics available in the market, which makes it difficult to treat such patients. Thus, discovery of new drugs have become a dire necessity.

2. Methodology

2.1 Collection of plant material

Entire plant of *Eclipta alba* and *Scoparia dulcis* (leaves, stem, roots, blossoms and natural product) were gathered from Kapasia, Bangladesh and the plant were distinguished and ensured and the voucher number [35897, 43794] was kept at the Bangladesh National Herbarium.

2.2 Preparation of the extracts

Both plants were washed under water to evacuate clean and afterward sun dried for 10 days until a steady weight was acquired. The dried plants were then powdered by using blender to get an extremely fine powder and kept it in fridge in a sealed shut glass compartment until further utilize. A measure of 15 grams of the powdered plants removed consecutively for extraction by using soxhlets mechanical assembly with extremity expanding solvents [1] began with n-hexane followed by ethyl acetic acetate, chloroform, acetone and methanol each with a volume of 250 mL. Plant extraction was kept at 4°C until further utilized.

2.3 Antibacterial screening

The effect of various plant extracts on different bacterial strains were screened by Agar well diffusion method. The minimum inhibitory concentrations of the plant extracts opposite of microorganisms were also determined by micro-dilution method using plant fractions serially diluted in sterile nutrient broth.

2.4 Selection of test microorganisms

The six bacterial strains used in the present study were the microorganism obtained from ATCC culture, Medigene, Germany. The bacteria used were *Escherichia coli, Staphylococcus aureus, Staphylococcus pyrogens, Basilus subtilis, Candida albicans* and *Staphylococcus Group B.* All the six bacterial strains are pathogenic and reported as multi-drug resistant. These bacteria will be maintained in 20 % glycerol stock and stored at -80°C freezer [2].

2.5 Agar- well diffusion method

The antibacterial activity was screened using agar well diffusion method at a concentration of 50 mg/mL for n-hexane, ethyl acetate, chloroform, acetone and methanol extracts. A number of 12 mm diameter holes were punched in a solidified Mueller Hinton agar and 24 hours old culture suspension of *Escherichia coli, Staphylococcus aureus, Staphylococcus pyrogens, Bacillus subtilis, Candida albicans* and *Staphylococcus Group B* were spread plated before making the wells. The plates for each bacterium were made in triplicates and incubated at 37 °C and the areas of inhibition were measured after 24 hours respectively [3]. Those bacterial strains that will be sensitive against *Eclipta alba* and *Scoparia dulcis* crude extracts and show zone of inhibition were tested for MIC. Tetracycline and Erythromycin (50 µg/mL each) were used as positive controls while ultrapure water was used as the negative control. The antibiotics that have been used are to compare with results reading [4].

2.6 Minimum Inhibition Concentration (MIC) - Micro-dilution method using 96 well plate

The plant extract that showed sensitivity against each bacterial strains, were used for MIC determination. The 96-well plates were prepared by dispensing 100 µL of Mueller Hinton broth into each well. A 100 µL of tested extracts of concentration of 50 mg/ml were added into the first nine wells of the plate. This was followed by twofold serial dilutions performed using a micropipette to obtain a concentration range from 50 to 0.390625 mg/ml. Tetracycline and erythromycin were used as positive controls with a concentration of 50 µg/ mL each with ultrapure water as the negative control. A 10 µL of 24 hour old culture inoculum was added to each well and the plates were incubated at 37 °C for 16-18 hrs [3]. The MIC values were recorded using a Microplate reader [BioTek EON, USA] [5].

2.7 Data Analysis

One-Way ANOVA was performed by using SPSS version 22 software to know the significance value.

3. Results & Discussion

Plant extract of *Scoparia dulcis* and *Eclipta alba* uncovered an antimicrobial movement against *Bacillus subtilis, Candida albicans, Escherichia coli, Staphylococcus aureus, Streptococcus pyogene* and *Strep Group B*. It has been observed that for enterobactoriacea to be viewed as touchy to any antimicrobial operator, it must create a zone of inhibion. All results of zone of inhibition are presented on table 1.

Our results show hexane extract activity of *Eclipta alba* against *E. coli* and *Strep. group B* with 8mm and 11.5 mm of zone of inhibition. But in M.K. kumar [6] study indicates hexane extracts activity as 11.5mm only for *E. coli*. Chloroform extract gives result against *E. coli* with diameter 14 mm whereas V. Lunavath and E. Mamidala, [7] reported that zone of inhibition was found 12.5mm. Chloroform extract gives result against *C. albicans* (10 mm) and *S. pyogene* (12mm) in our study. Our results of ethyl acetate give result against *E. coli, S. aureus* and *Strep. Group B* and their diameter was 9 mm, 10.5 mm and 15 mm. Similar results were reported by M. sarma and S. sarma, [8] study that *C. albicans, E. coli, S. aureus* diameters of zone of inhibition were 16mm, 14mm and 17mm. Acetone shows its activity against *E. coli* and *S. pyogene* with 9.5 mm and 8 mm. But in M.K. Kumar study, *E. coli* (6.9mm) and *S. pyogene* (6mm) both are sensitive to the acetone extract of *Eclipta alba*. It proves that acetone extract in this experiment gave good result compared. Methanol shows result against *B. subtilis, E. coli, Strep. group B* and *S. pyogene* with 12 mm, 9 mm, 12 mm and 13 mm. According to J. Bakht [9] studies reported methanol extracts showed the good activity against bacteria compared with other solvents extract. Similar results were also reported by Phongpaichit [10]. MIC result was observed from this study observed that hexane (*E. coli, S. pyogene*), ethyl acetate (*S. pyogene*), chloroform (*E. coli, C. albican, S. pyogene*), acetone (*E. coli, S. pyogene*) showed significant value < 0.05. But the highest significant among all solvents was observed in hexane extract tested against *E. coli* and *S. pyogene*, acetone extract against *S. pyogene* and methanol extract against *S. pyogene*.

Study showed a methanol extract displayed better MIC compared to aqueous extract against gram-positive and gram-negative bacteria and fungus. The all five-extract showed good inhibitory activity on almost all the microbes test. But antimicrobial activity of hexane extract against *E. coli* and *S. pyogene*, acetone and methanol extract against *S. pyogene* were showed highest inhibitory activity by Agar well diffusion method.

The hexane extract of *Scoparia dulcis* shows *B. subtilis, E. coli, S. aureus* and *S. pyogene* in diameter of 8 mm, 10 mm, 11 mm and 21 mm zone of inhibition. Chloroform extract shows positive results 13 mm for both the strains *S. aureus* and *E. coli*. In Prabavathy D and Niveditha R., 2015 study

shows chloroform extract with 8 mm for both the strains *S. aureus* and *E. coli.* This shows that the Chloroform extract in our results has performed promising result. Ethyl acetate extract shows positive result against *C. albicans* with 8mm in A. Adebiyi [11] study showed their result for ethyl acetate extract shows results for *B. subtilis, E. coli, S. aureus, Strep. group B.* and their diameters are 6.5 mm, 10.5 mm, 8.5 mm and 12 mm. Acetone extract with *B. subtilis*, *Strep. group B* and *S. pyogene* gives result 14 mm, 11 mm and 9.5 mm. But in Prabavathy D and Niveditha R., 2015 study shows their activity for *E. coli* and *S. aureus* strains with 12 mm and 11mm respectively. Methanol extract shows result against *B. subtilis*, *E. coli, S. aureus*, *Strep. group B, S. pyogene* by giving 12 mm, 13 mm, 12 mm, 16.5 mm and 13 mm.

Previous few studies of *S. dulcis* plant extracts in W. Wankupar *et al*., 2015, Zulfiker [12] reported antimicrobial activity against a wide range of human pathogenic microorganisms, including Gram-positive, Gram-negative bacteria. These findings agree with Latha [13] and Jonathan 2009 [14] who also reported that chloroform/methanol fractions of *S. dulcis* exhibited effective activity in controlling the growth against human pathogenic bacteria and fungi. In this study MIC result was observed that hexane (*E. coli*), ethyl acetate (*Strep Group B, C. albicans, E. coli)*, chloroform (*Strep Group B and E. coli*), acetone [*S. pyogene* and *E. coli*), methanol (*S. aureus, S. pyogene, Streptococcus group B* and *E. coli*), showed significant value < 0.05. But the highest significant among all was observed in hexane extract tested against *E. coli*, ethyl acetate extract tested against *C. albicans* and *E. coli*, chloroform extract against *Strep Group B* and *E. coli*, acetone extract against *S. pyogene* and *E. coli*, methanol extract against *S. pyogene, Strep Group B* and *E. coli.* The sensitivity of *E. coli* and *S. aureus* to some of the extracts implies that chemical compounds in the extracts can be further developed to fight against this microorganism and the use of the plant for the treatment many of the diseases [15-16].

Scoparia dulcis is used mainly for the treatment of diabetes [17-18]. With a rising mortality rate each year due to different infectious disease, phytotherapeutics agents that show resistance to the causative microbial agents such as *Staphylococcus aureus*, streptococcus pyogene, *Bacillus subtilis*, enteric bacteria (*Enterococcus*, *Pseudomonas, Escherichia coli*) serves as a new frontier regarding the treatment as the latter is most often the common route to outbreaks in developing countries.

Table 1: Observation of Zone of inhibition of Eclipta alba and Scoparia dulcis

Bacteria	Hexane		Ethyl Acetate		Chloroform		Acetone		Methanol		Tetracycline		Erythromycin	
	SD	EA	SD	EA	SD	EA	SD	EA	SD	EA	SD	EA	SD	EA
Strep Group B		15	12	15	12		10		18		18	24	14	
					12		12		15	12	16	22	14	25
Staphylococcus aureus	21.5		9	15					12		27	32	40	25
			8	6							32	29	38	30
Staphylococcus pyrogen					11	12	10	20	16	13	19	34	34	25
					14		9	14	10		17.5		34	
Candida albicans			8	24							2	26	16	24
						1						30		39
Bacillus subtilis			6		12		14		14		35	30	34	34
			7		9		14		10	12		32	36	35
Escherichia coli		8	9	9	14	14	19	9	12	8	22	22	24	19
		8	12	9	12	15	18	10	14	10	22	25	26	20

*EA - *Eclipta alba* and SD - *Scoparia dulcis*

Minimum Inhibitory Concentration of *Eclipta alba*

Methanol extract was compared against standard antibiotics (erythromycin) in *B. subtilis*, showed the minimal inhibitory concentration at dilution of 1:320. In this method, tetracycline was also used as second standard antibiotic, however was not considered for the comparison with plant extract as after performing one- way Anova did not showed any significance value. Thus, erythromycin was selected as it showed significance value $p < 0.05$ (table 2).

For the bacterium *E. coli*, the acetone extract showed similar result at dilution factor of 1:320 when compared with the standard antibiotics. But hexane, ethyl acetate, and chloroform extract showed negative result. The minimal inhibitory concentration of hexane, ethyl acetate, and chloroform extract is at 1:320 dilution while for acetone and methanol the dilution is 1:160.

Hexane extract when test against *C. albicans* and *S. pyrogen* showed more reading compared to the standard antibiotics. While ethyl acetate, chloroform, acetone and methanol extract when test on bacteria *S. pyogene* showed lesser reading compared to the standard antibiotics at the last dilution factor of 1:320.

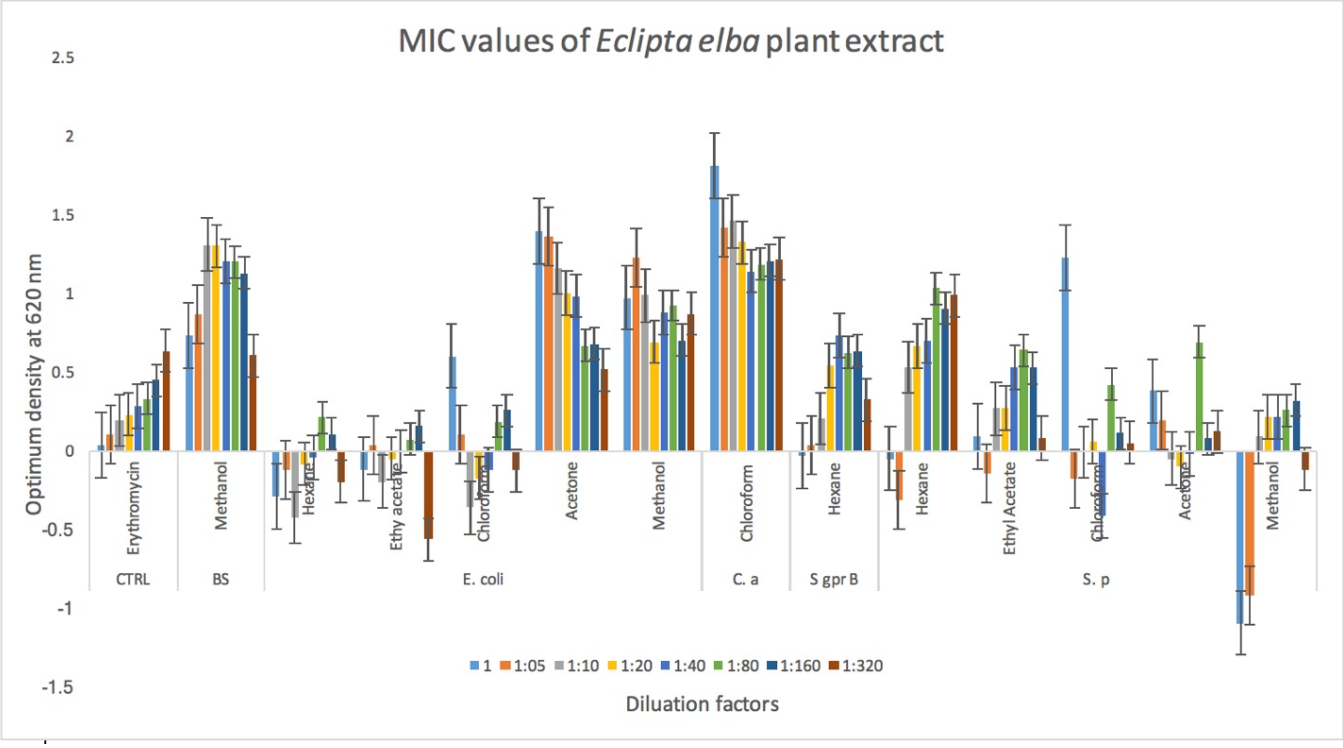
Thus, after performing one- way Anova, it was observed that hexane (*E. coli, S. pyogene*), ethyl acetate (*S. pyogene*), chloroform (*E. coli, C. albican, S. pyogene*), acetone (*E. coli, s. pyogene*) showed significant value < 0.05. But the highest significant among all solvents was observed in hexane extract tested against *E. coli* & *S. pyogene*, acetone extract against *S. pyogene* and methanol extract against *S. pyogene* (figure 2).

According to J. Bakht [9] studies reported methanol extracts showed the good activity against bacteria compared with other solvents extract. Similar results were also reported by Phongpaichit [10]. Study showed a methanol extract displayed better MIC compared to aqueous extract against gram-positive and gram-negative bacteria and fungus. Hexane, chloroform, ethyl acetate, acetone and methanol extract of *Eclipta alba* were tested against some gram positive and gram negative bacteria and it showed good result by increasing effect on microbial growth

inhibition with minimum concentration of the extract. As reported by Estari [19], antimicrobial activity (MIC value) of *Eclipta alba* leaf extracts was performed, which high activity against E. coli and S. aureus bacteria against n-hexane and carbon tetra chloride fraction. Traditionally, *E. alba* has been reported as an herbal plant used for the for treatment of stomach and digestion disorders, skin diseases and conjunctivitis [20]. Since the selected bacterium (*S. aureus*, *E. coli*, *S. phyogen*, *C. albican* and *S. group B*) are reported to be human pathogens causing various skin, stomach disorder and other diseases [20-22].

Table 2: Minimum inhibitory concentration of Eclipta alba plant extract

Bacteria			*BS*	*E. coli*					*C. albican*	*Strpto coccus group B*	*Staphylococcus Pyogene*				
Solvents	Tetracyclin	Erythromycin	Methanol	Hexane	Ethyl acetate	Chloroform	Acetone	Methanol	Chloroform	Hexane	Hexane	Ethyl Acetate	Chloroform	Acetone	Methanol
1	0.2547±.02	0.0367±.12	0.729±0 66*	-0.2933±.12*	-0.1167±.18	0.6017±.48*	1.3957±.00*	0.9733±.54	1.8093±.26*	-0.036±.20	-0.0493±.34*	0.0903±.61*	1.2257±.38*	0.3783±.21*	-0.9167±.17*
2	0.1783±.07	0.0987±.07	0.8703±.02*	-0.118±.10*	0.0333±.08	0.0987±.15*	1.3657±.02*	1.2243±.04	1.417±.08*	0.0383±.51	-0.3113±.32*	-0.1447±.10*	-0.1797±.12*	0.193±.02*	-0.9167±.01*
3	0.4703±.30	0.1917±.07	1.311±.08*	-0.4273±.05*	-0.1987±.07	-0.3637±.16*	1.1603±.14*	0.9893±.19	1.4583±.12*	0.2037±.46	0.5313±.10*	0.268±.23*	-0.0093±.16*	-0.053±.11*	0.087±.01*
4	0.3173±.25	0.2303±.06	1.3037±.14*	-0.0833±.11*	-0.052±.07	-0.176±.15*	1.0023±.08*	0.6923±.07	1.325±.10*	0.5407±.03	0.663±.00*	0.271±.13*	0.0593±.10*	-0.104±.03*	0.2137±.04*
5	0.1913±.09	0.2803±.05	1.204±.02*	-0.0467±.06*	-0.0037±.05	-0.1217±.12*	0.9837±.09*	0.8767±.07	1.1413±.11*	0.7323±.15	0.6987±.16*	0.5323±.15*	-0.4123±.608	-0.0223±.078	0.2137±.05*
6	0.1853±.06	0.333±.03	1.2037±.20*	0.2133±.05*	0.0713±.12	0.1863±.06*	0.666±.57*	0.924±.14	1.1877±.11*	0.6207±.16	1.0343±.09*	0.6403±.26*	0.4217±.678	0.6927±.24*	0.2547±.04*
7	0.2447±.12	0.4467±.12	1.1313±.14*	0.1057±.02*	0.1543±.03	0.2563±.12*	0.678±.59*	0.7037±.06	1.2103±.13*	0.635±.71	0.9037±.04*	0.5283±.16*	0.109±.068	0.0753±.07*	0.3173±.05*
8	0.2977±.11	0.6327±.33	0.606±.54*	-0.1967±.16*	-0.5653±.55	-0.1253±.23*	0.5147±.38*	0.87±.75	1.2177±.17*	0.3233±.42	0.987±.52*	0.0813±.05*	0.0493±.10*	0.1227±.21*	-0.12±.42*



*CTRL-control, BS- Bacillus subtilis,C.a. –Candida albicans, S gpr B.-Streptococcus group B, S.P.- Staphylococcus pyogene

Figure 1: Minimum Inhibitory Concentration analysis of Eclipta alba

Minimum Inhibitory Concentration of *Scoparia dulcis*

Acetone extract showed the minimum concentration of dilution at 1:320 compared with standard antibiotics (erythromycin) when tested against *B. subtilis.* For the bacterium *S. aureus*, methanol extract showed more reading compared to the standard antibiotics at dilution of 1:320. For the bacterium *S. pyogen*, *Streptococcus group B* and *C. albican* showed negative result compared with the standard

antibiotics. Chloroform, acetone and methanol extract showed results against *E. coli* at concentration of 8th dilution (1:320) when compared with the standard antibiotic. Other extract of hexane and ethyl acetate didn't show any result (negative) (table 3)

Thus, after performing one- way Anova it was observed that hexane (*E. coli*), ethyl acetate (*Streptococcus group B, C. albican, E. coli*), chloroform (*Streptococcus group B and E. coli*), acetone (*S. pyogene* and *E.coli*), methanol (*S. aureus, S. pyogene, Streptococcus group B* and *E.coli,*), showed significant value < 0.05. But the highest significant among all was observed in hexane extract tested against *E. coli*, ethyl acetate extract tested against *C. albican* and *E. coli*, chloroform extract against *Streptococcus group B* and *E. coli*, acetone extract against *S. pyogene* and *E. coli*, methanol extract against *S. pyogene, Streptococcus group B* and *E. coli* (figure 2).

Previous study of *S. dulcis* plant extracts displayed antimicrobial activity against both Gram negative and positive bacteria by broth dilution method [12]. To support this results Zulfiker [23] reported antimicrobial activity of *S. dulcis* against a wide range of human pathogenic microorganisms, including Gram-positive, Gram-negative bacteria. These findings agree with Latha and Jonathan [13-14] who also reported that chloroform/methanol fractions of *S. dulcis* exhibited effective activity in controlling the growth against human pathogenic bacteria and fungi.

This evidence of antimicrobial activity against wide range of pathogenic organism may be the analytical presence of wide range therapeutic agents necessary to fight resistance bacterial strains [24]. These extracted bioactive compound plays an essential role in determining the therapeutic efficacy of plant. Whereby other reports have shown that bioactive compounds of flavonoid and tannin are both active against *Staphylococcus aureus, Escherichia coli* and different strains of mycobacterium [25-26]. Other studies have implicated the compounds obtained from the saponin, terpenoid, flavonoid and tannin fractions as the mainspring behind the reported activity against infectious disease causing agents like *Staphylococcus aureus* and *Streptococcus pyogenes.* Distorting the enzyme activity within bacteria is generally detected in the presence of phyto-tannins [27].

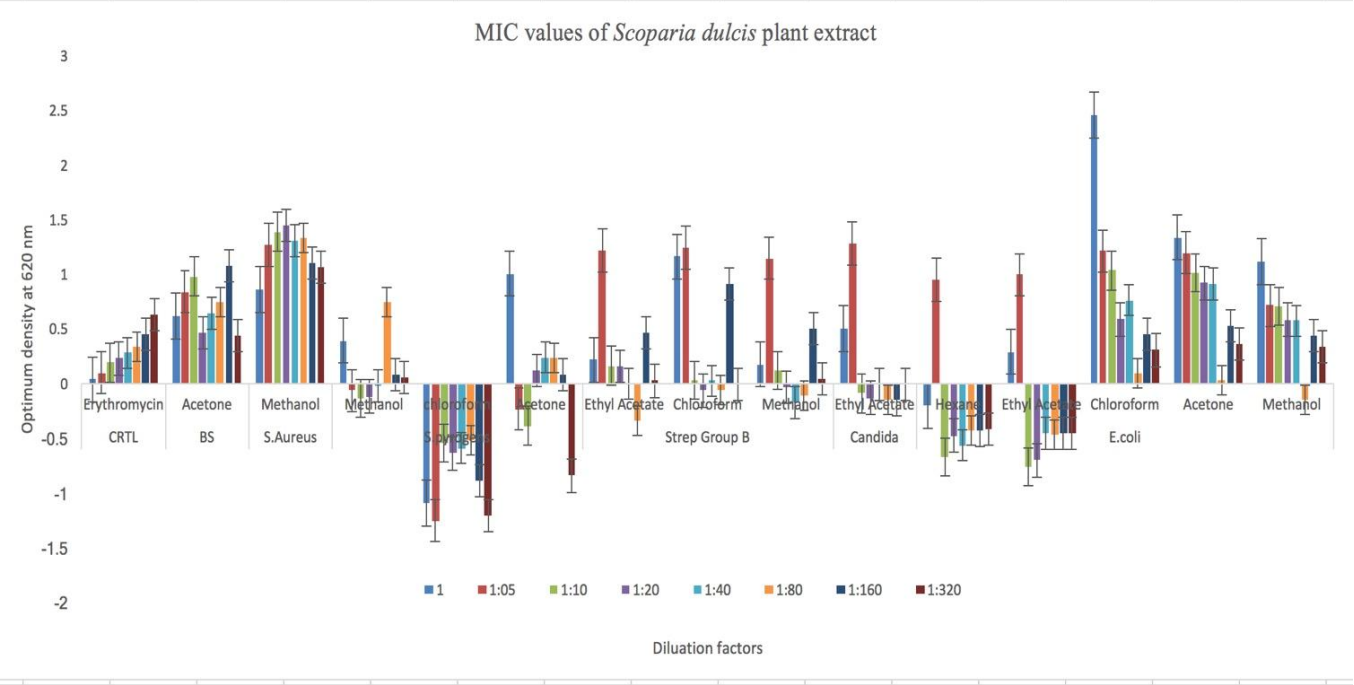
The sensitivity of *E. coli* and *S. aureus* to some of the extracts implies that chemical compounds in the extracts can be further developed to fight against this microorganism and the use of the plant for the treatment many of the diseases [15-16]. Therefore, all the extracts could serve as source of compounds that may be effective in the management of the illnesses. *Scoparia dulcis* is used mainly for the treatment of diabetes [17-18]. With a rising mortality rate each year due to different infectious disease, phytotherapeutics agents that show resistance to the causative microbial agents such as *Staphylococcus aureus*, streptococcus pyogene, bacillus subtilis, enteric bacteria (*Enterococcus, Pseudomonas, Escherichia coli*) serves as a new frontier regarding the treatment as the latter is most often the common route to outbreaks in developing countries.
The results from the current studies revealed that the bioactive extract from both the plants could be the main constituent responsible for the antibacterial activity. Thus, these data can be used for the treatment of various diseases caused by the studied bacterial colony as it exhibited good activity against them.

4. Conclusion

It can be concluded that both plants *Scoparia dulcis* and *Eclipta alba* showed antimicrobial activity against almost all the pathogens. It indicates that the crude extracts have the potential as antimicrobial compounds against microorganisms and might be used in the treatment of infectious diseases caused by multidrug resistant organisms. They can also be a source of the development of novel anticancer drug leads. The present research consequently offers a scientific basis for traditional use of *Scoparia dulcis* and *Eclipta alba*. Additional evaluation of the antibacterial and antifungal properties of the plant extracts against different microbial agents will be beneficial and accessible to the society.

Table 3: Minimum inhibitory concentration of Scoparia dulcis plant extract

Bacteria				*S. aureus*	*S. pyrogens*			*Strep Group B*			*Candida*	*E. coli*				
Solvents	Tetracyclin	Erythromycin	Acetone	Methanol	Methanol	chloroform	Acetone	Ethyl Acetate	Chloroform	Methanol	Ethyl Acetate	Hexane	Ethyl Acetate	Chloroform	Acetone	Methanol
1	0.2547 ±.02	0.0367±.12	0.62±.07	0.8583 ±.24*	0.394±.06*	-1.0933 ±.02	1.0067 ±.26*	0.2157 ±.39*	1.1613 ±.26*	0.1767 ±.21*	0.501±.19*	-0.2057 ±.02*	0.2917 ±.02*	2.46±.33*	1.3383 ±.10*	1.12±.13*
2	0.1783 ±.07	0.0987±.07	0.841 ±.06	1.2693 ±.11*	-0.063±.54*	-1.259±.18	-0.2333 ±.27*	1.2187 ±.08*	1.2443 ±.31*	1.145±.14*	1.2823 ±.10*	0.95±.26*	0.9973 ±.46*	1.213±.38*	1.1963 ±.13*	0.7163 ±.31*
3	0.4703 ±.30	0.1917±.07	0.979 ±.11	1.3887 ±.15*	-0.138±.05*	-0.5487 ±.46	-0.386±.66*	0.163±.03*	0.0273 ±.22*	0.119±.03*	-0.089±.17*	-0.676±.23*	-0.7613 ±.41*	1.0347 ±.19*	1.0143 ±.15*	0.7097 ±.12*
4	0.3173 ±.25	0.2303±.06	0.4663 ±.62	1.4513 ±.11*	-0.1203 ±.05*	-0.6403 ±.61	0.1217 ±.02*	0.1593 ±.07*	-0.0633 ±.06*	-0.0327 ±.06*	-0.1323 ±.11*	-0.477±.24*	-0.7027 ±.12*	0.5883 ±.05*	0.9207 ±.06*	0.584±.33*
5	0.1913 ±.09	0.2803±.05	0.642 ±.51	1.3067 ±.14*	-0.02±.05*	-0.5907 ±.60	0.2377 ±.04*	-0.0003 ±.09*	0.0257 ±.14*	-0.173±.02*	-0.0097 ±.11*	-0.566±.15*	-0.456±.14*	0.7633 ±.10*	0.9133 ±.05*	0.5757 ±.12*
6	0.1853 ±.06	0.333±.03	0.742 ±.58	1.3307 ±.17*	0.7447 ±.03*	-0.5193 ±.60	0.2347 ±.03*	-0.3453 ±.51*	-0.0567 ±.11*	-0.114±.04*	-0.1457 ±.23*	-0.4257 ±.06*	-0.4683 ±.02*	0.0933 ±.21*	0.031±.05*	-0.152±.10*
7	0.2447 ±.12	0.4467±.12	1.0777 ±.06	1.1027 ±.02*	0.0787 ±.01*	-0.8907 ±.34	0.077±.30*	0.46±.632*	0.9097 ±.12*	0.5053 ±.38*	-0.1473 ±.04*	-0.4253 ±.03*	-0.46±.04*	0.4537 ±.17*	0.5267 ±.18*	0.4377 ±.11*
8	0.2977 ±.11	0.6327±.33	0.4347 ±.08	1.0643 ±.12*	0.0543 ±.10*	-1.2123 ±.05	-0.8447 ±.38*	0.024±.06*	-0.0123 ±.12*	0.0453 ±.01*	-0.0057 ±.09*	-0.4163 ±.26*	-0.4533 ±.14*	0.305±.09*	0.366±.11*	0.3317 ±.12*



*CTRL-control, BS- Bacillus subtilis,C.a. –Candida albicans, S gpr B.-Streptococcus group B, S.P.- Staphylococcus pyogene
Figure 2: Minimum Inhibitory Concentration analysis of Scoparia dulcis

5. Future Scope

Further investigation on the antibiotic susceptibility, phytochemical and biological activity studies and selective cytotoxicity studies of crude extract of *Scoparia dulcis and Eclipta alba* is needed in comparison with antibiotics. To increase accessibility and affordability of medicine, it is recommended that more active compounds would be isolated from these species in order to develop active antimicrobial agents. Due to prospective constraints on time some research components may not be explored. That are ccomparison studies using other Bacterial strains, efficiencies of various antibiotics and *in vivo* antimicrobial screening.

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