6.0 BIOLOGICAL STUDIES OF LEAVES OF *Corchorus fascicularis* L.

6.1 ESTIMATION OF ANTIOXIDANT POTENTIAL OF LEAVES EXTRACTS OF *Corchorus fascicularis* L.

Oxidative stress induced reactive oxygen species (ROS) and free radicals are believed to be major cause of physiological disorders like Alzheimer's, Parkinson's, arthritis, atherosclerosis, coronary heart diseases, emphysema, gastric ulcer, diabetes mellitus, cirrhosis, aging and cancer[1,2,3]. ROS are highly reactive molecules which include free radicals such as superoxide ions (O_2^-), hydroxyl radicals (OH^-), singlet molecular oxygen radicals and hydrogen peroxide (H_2O_2). Superoxide anion radical is one of the strongest reactive oxygen species among free radicals that are generated first after oxygen is taken into living cells[4]. Reputed therapeutic effects of many traditional medicinal plants may be recognized for the presence of these natural antioxidants, which can scavenge oxygen radicals and inhibit per oxidation[5]. Therefore, in recent years, significant attention has been paid to explore the potential of antioxidant property of plant extracts of plant origin, which may be used for human consumption. Crude extracts from plant materials rich in phenolic compound, are increasingly of interest in the food industry, because they can retard oxidative degradation of lipids and thereby improve the quality and nutritive value of food. Some studies have shown that the increased dietary intake of natural antioxidants such as flavonoids and other phenolic compounds present in most plants, may act as potent candidates in preventing diseases related to oxidative stress[6].

6.1.1 DPPH Radical Scavenging Activity:

Since the phytophenolics are powerful antioxidants equivalent to ascorbate and were search as soft antioxidant. Therefore, the different extracts of *Corchorus fascicularis* L. were further investigated for their *in vitro* antioxidant activity. The results indicated that almost the ethanolic extract had comparable radical scavenging potential than reference standard due to presence of phenolic compounds.

DPPH is one of the free radicals generally used for testing preliminary radical scavenging activity of plant extract or compound. The results of DPPH radical scavenging effects of leaves extract of *Corchorus fascicularis* L. have been tabulated in Table- 8 and shown in Fig.A-1.

Table 8: Results of DPPH radical scavenging activity

Sr. No.	Concentration (µg/ml)	%Inhibition by DPPH			
		AA	**HE**	**CE**	**EE**
1	20	44.45	38.37	32.23	34.98
2	40	45.41	42.18	38.37	41.33
3	60	50.23	49.85	46.33	47.29
4	80	55.33	52.75	50.12	56.78
5	100	60.71	57.07	57.23	59.71

AA-Ascorbic Acid, HE-*n*-hexane extract, CE-Chloroform extract, EE-Ethanol extract

In table no.9 Ethanol Extract (EE) of leaves of *Corchorus fascicularis* L. has shown comparable DPPH radical scavenging activity with ascorbic acid as standard for 20, 40, 60, 80 and 100 µg/ml concentrations.

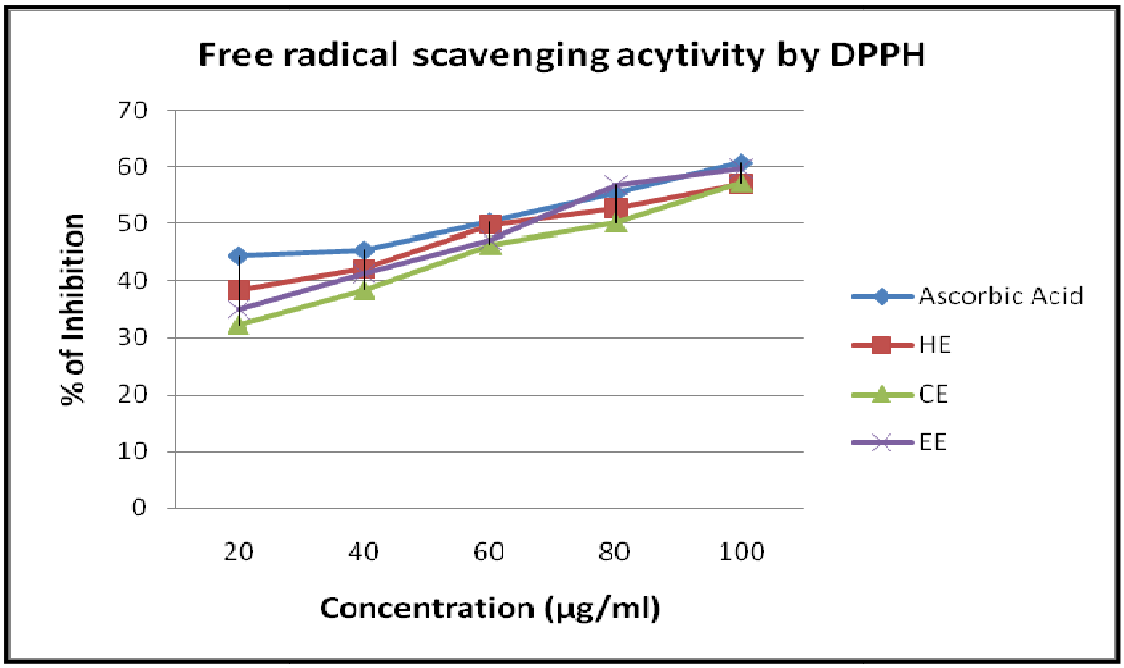


Fig.A-1: Free radical scavenging activity by DPPH

6.1.2 Total Antioxidant Capacity:

Total antioxidant activities of different extracts of *C. fascicularis* L. were tabulated in Table-9 and shown in Fig. A-2 All the extracts were found to having significant antioxidant capacity but ethanol extract showed more activity than *n*-hexane extract and chloroform extract. Therefore in ethanol extract phenolic compounds presents.

Table 9 : Result of Total antioxidant capacity

Sr. No.	Concentration (µg/ml)	HE	CE	EE
1	100	12.33	22.83	27.13
2	500	25.16	47.16	56.66
3	1000	48.56	87.33	113.33

HE- *n*-hexane Extract, CE- Chloroform Extract, EE- Ethanol Extract

Table 10: Antioxidant effect (IC_{50}) on free DPPH radicals and total antioxidant capacity of leaves of *Corchorus fascicularis* L. extracts.

Plant Extract	IC_{50} ((µg/ml) Scavenging ability on DPPH radicals	Total Antioxidant Capacity (AAE)
n-hexane	75.83 ± 0.208	49.4 ± 0.509
Chloroform	79.39 ± 0.210	90.9 ± 2.028
Ethanol	70.82 ± 0.246	110.2 ± 1.603
Ascorbic acid	59.67 ± 0.341	_

Values are the mean ± SEM, $n = 3$
AAE ascorbic acid equivalent

The percentage inhibition was calculated with respect to control. Ascorbic acid was used as standard compounds in the DPPH assay and the total antioxidant capacity of the extracts was expressed as the ascorbic acid equivalent (AAE) are interpreted in Table-10.The extracts exhibited significant antioxidant activity in the DPPH assays. The IC_{50} values obtained for DPPH scavenging of the ethanol extract were 70.82 ± 0.24 g/ml, which was found to be the smallest among all the extracts and comparable to reference standard ascorbic acid. The lower value of IC_{50} means more potent towards DPPH scavenging activity.

The total antioxidant capacity of the ethanol extract (equivalent to ascorbic acid) was found to be the highest of all the extracts (110.2 ± 1.603 g/ml), and was concentration-dependent. As concentration increases the total antioxidant capacity increases. Higher value of AAE means more potent toward total antioxidant capacity.

The free radical scavenging activity of the crude drug extracts was evaluated by the DPPH assay. The DPPH radical scavenging activity of the ethanol extract revealed high antioxidant activity, considering the fact that quenching properties were obtained only from the crude extract and were comparable with ascorbic acid. Scavenging of the DPPH radical is related to the inhibition of lipid peroxidation[7,8].

The phosphomolybdenum assay is a quantitative method of evaluating water-soluble and fat-soluble antioxidant capacity (total antioxidant capacity). The ascorbic acid and ethanol extract demonstrated electron-donating capacity, showing its ability to act as chain terminators, transforming relative free radical species into more stable non-reactive products[9].

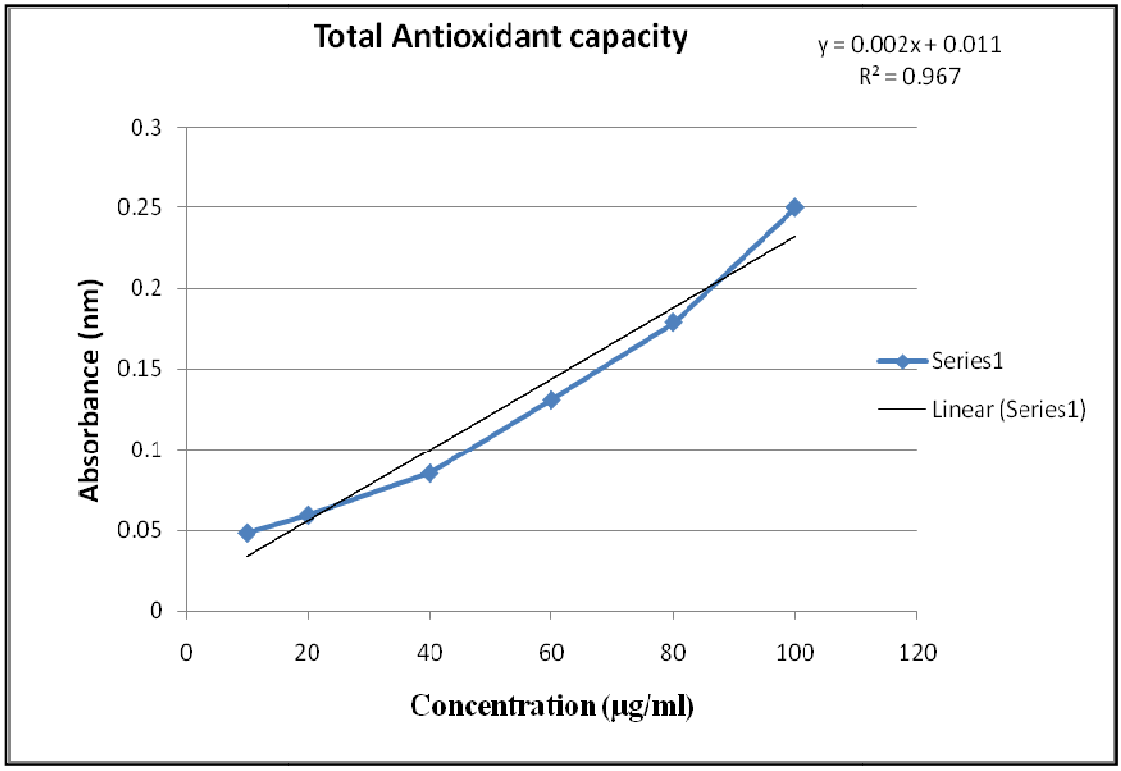


Fig. A-2: Total antioxidant capacity

6.2 ANTI-INFLAMMATORY ACTIVITY:

6.2.1 Acute toxicity test:

Corchorus fascicularis L. leaves extracts did not produce any mortality even at the dose of 3000 mg/kg, p.o. *Corchorus fascicularis* L. was thus found to be non-toxic. On the basis of above results, three doses (100, 200, 400 mg/kg, p.o.) of *Corchorus fascicularis* L. were selected for further pharmacological studies. The results did not show clinical signs and mortality of the animal, therefore an LD50 > 3000 mg/kg body weight may be assumed.

6.2.2 ANTI-INFLAMMATORY ACTIVITY OF *n*-HEXANE AND CHLOROFORM EXTRACTS LEAVES EXTRACT OF *Corchorus fascicularis* Lam.:

6.2.2.1 Carrageenan-induced rat paw edema:

The *n*-hexane and chloroform extracts at the doses of 100, 200 and 400 mg/kg p.o. showed very good results and caused a significant inhibition in the percent rise of carrageenan induced rat paw edema. The 400 mg/kg dose of *n*-hexane extract showed percent rise inhibition of 85.7% and that of chloroform extract showed 67.5%, at 3rd hour as highly significant results summarized in table19. The maximal inhibition in the percent rise of edema volume was achieved at a dose 400 mg/kg ($P<0.01$) of *n*-hexane and chloroform extracts, when compared to standard drug diclofenac sodium (10 mg/kg).

The present study indicates that *Corchorus fascicularis* has the pharmacological potential as an anti-inflammatory agent when tested on various animal models. Although the present study did not aim at isolation and identification of bioactive compounds, the phytochemical screening of *n*-hexane and ethanol extracts demonstrated the presence of triterpenoids, steroids, which were suggested to act synergistically to exert the observed pharmacological activity[10], the presence of triterpenoids and steroids in *n*-hexane and chloroform extract could possibly lead to the observed activities. The anti-inflammatory activity of *n*-hexane and chloroform

extracts could also be linked to the ability of the extract to inhibit prostaglandin synthesis[11]. The anti-inflammatory effect is a common property of many triterpenoids[12]. This fact is supported by claims that the carrageenan-induced inflammation is a COX-dependent response and is more effectively controlled with arachidonate cyclo-oxygenase[13], but not arachidonate lipo-oxygenase inhibitors[14] Interestingly, compounds like steroids and triterpenes[15], in part, have been shown to possess anti-inflammatory activity, and the claim made by Attaway and Zaborsky[16] that compounds with anti-inflammatory activity also possess antinociceptive activity seems to support our findings on the *n*-hexane and chloroform extracts as pharmacological activity. Carragenan induced paw edema test is a significant tool for the assessment of anti-inflammatory profile of natural products[17,18,19].

The present results support the ethno-medical application of *Corchorus fascicularis* leaves in the treatment of inflammation diseases. Further experimentation is needed in order to understand the precise mechanism of action in anti-inflammatory activities by the extracts.

Table 11: The anti-inflammatory activity of *n*-hexane and chloroform extracts of Corchorus *fascicularis* L. using the carrageenan-induced paw edema test

Treatment	Dose mg/kg	Percentage increase in paw edema					
		1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
Control		50.3±3.96	68.2±4.40	91.7±1.97	81.9±4.75	83.1±3.78	84.5±4.22
n-Hexane extract	**100**	33.5±5.71*	45.2±7.4	61.1±7.06	57.5±5.23	72.7±4.93	65.7±5.16
	200	35.3±3.75*	46.5±2.71	79.4±4.91	49.3±4.5*	58.1±3.87**	58.4±1.35**
	400	24.7±2.15**	37.5±4.17**	85.7±7.55*	47.3±5.9	63.7±5.57	59.7±5.76*
Chloroform extract	**100**	28.1±1.93	52.5±2.10	63.1±3.67	52.4±2.93**	53.7±2.75	41.5±4.43**
	200	29.9±4.15**	41.8±5.25**	59.7±6.17**	40.5±6.15**	41.5±5.25**	23.8±6.05**
	400	30.1±4.17**	42.8±5.89**	67.5±6.26**	41.5±6.75**	43.5±5.47**	25.8±7.05**
Diclofenac Sodium	**10**	18±0.97**	23.1±0.49**	43.3±1.85**	35.7±3.63**	33.5±4.85**	27.9±3.98**

Values represent mean ± SEM, n=6
One way ANOVA followed by Dunnett's multiple comparison test.
*$p < 0.05$, **$p < 0.01$ compare with control group

6.3 ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF *n*-HEXANE, CHLOROFORM AND ETHANOL LEAVES EXTRACT OF *Corchorus fascicularis* Lam.:

Biological evaluation

The extracts and the isolated compounds from *n*-hexane, chloroform and ethanol extract were preserved in labeled sterile screw capped bottles at –20 ^{0}C. Antibacterial and antifungal activity assays were performed by the modified disc diffusion method[20,21,22,23]. The bacterial and fungal strains used were obtained from National Collection of Industrial Microorganism (NCIM), Pune, India. Petri dishes (5 cm diameter) were filled up to a depth of 3-4 mm with sterile nutrient agar (Hi-Media) for bacteria and Meat extract, Glucose yeast extract, Peptone medium i.e. yeast for fungi. A sterile Whatman filter paper disc of 6 mm diameter preloaded with 100 mcg of target compound in DMSO was placed in the centre of the nutrient agar plates of bacteria and MGYP plates of fungi. Four plugs of bacterial inoculums and fungal inoculums were placed upside down at the quarter circle points 20 mm radius around the drug loaded disc in the Petri dishes. Blank control discs were treated with DMSO, Chloramphenicol for bacteria and Nystatin for fungi were used as standards. The stringent aseptic conditions were maintained during microorganism inoculation and the plates were labeled. The Petri plates were incubated at 37±1^{0}C for 24 hours for antibacterial screening and at 25^0 C for 2-7 days for antifungal screening. The diameter of zone of inhibition of each disc was recorded.

Table 12: Type and source of Micro-organism

Microorganism	Strain Name	Strain reference	Abbreviation used
Gram positive bacteria	*Bacillus subtilis*	NCIM 2250	BS
Gram positive bacteria	*Staphylococcus aureus*	NCIM 2079	SA
Gram negative bacteria	*Escherichia coli*	NCIM 2109	EC
Gram negative bacteria	*Pseudomonas aruginosa*	NCIM 2036	PA
Fungi (Mould)	*Aspergillus niger*	NCIM 545	AN
Fungi (Yeast)	*Candida albicans*	NCIM 3471	CA
NCIM : National Collection of Industrial Microorganisms, National Chemical Laboratory (NCL), Pune -411008 [India]			

6.3.1 Antibacterial and antifungal activity of *n*-hexane, chloroform and ethanol extract:

The concentrated extracts were filtered using Whatman filter paper No.1. The filtrate was evaporated under reduced pressure and dried using rotary evaporator at about 55^0C. The dried extracts were preserved in labeled sterile screw capped bottles at –20 ^{0}C. The test solution of extracts and standard solution were prepared. The concentration of extract was set to 0.1 mg/ml in dimethylsulphoxide. The drug used in standard preparation was chloramphenicol of IP grade. The antibacterial activity was performed using 24 hours cultures of *E. coli, P. aeruginosa, B. subtilis,* and *S. aureus*, developed in Muller Hinton agar medium. The bacterial strains were used and obtained from NCIM, Pune. The aliquot of 1ml quantity of test and standard solution was transferred in 6 mm well. The stringent aseptic conditions were maintained during microorganism inoculation and the plates were labeled. The test and standard solution were allowed to diffuse in wells for 2 hours at room temperature. The Petri plates were incubated at 37 ± 1^0C for 24 hours. The diameter of zone of inhibition of each well was recorded. The results of antibacterial activity were tabulated in Table-13 and 14.

Table 13: Antibacterial activity of extracts of *C. fascicularis* L. against bacterial test organism

	Zone Of Inhibition in mm Concentration in 100 µg/ml			
Microorganism	**Ethanol Extract**	**n-Hexane Extract**	**Chloroform Extract**	**Standard**
E. coli	17.76	13.61	15.00	20.52
S. aureus	16.30	15.41	14.18	30.94

Note: Values are inhibition zone (mm), and an average of triplicate.

Each extract has concentration - 100 µg/ml

Standard Drugs: Chloramphenicol (10 µg/disc) for antibacterial stud

Incubation conditions for bacteria—1 day at 37^0 C.

Table 14: Antifungal activity of extracts of *C. fascicularis* L. against bacterial test Organism.

	Zone Of Inhibition in mm Concentration in 100 µg/ml			
Microorganism	**Ethanol Extract**	**n-Hexane Extract**	**Chloroform Extract**	**Standard**
C. albicans	9.80	8.15	9.73	9.53

Note: Values are inhibition zone (mm), and an average of triplicate.

Each extract has concentration of 100 µg/ml

Standard Drugs: Nystatin (50 µg/disc) for antifungal study

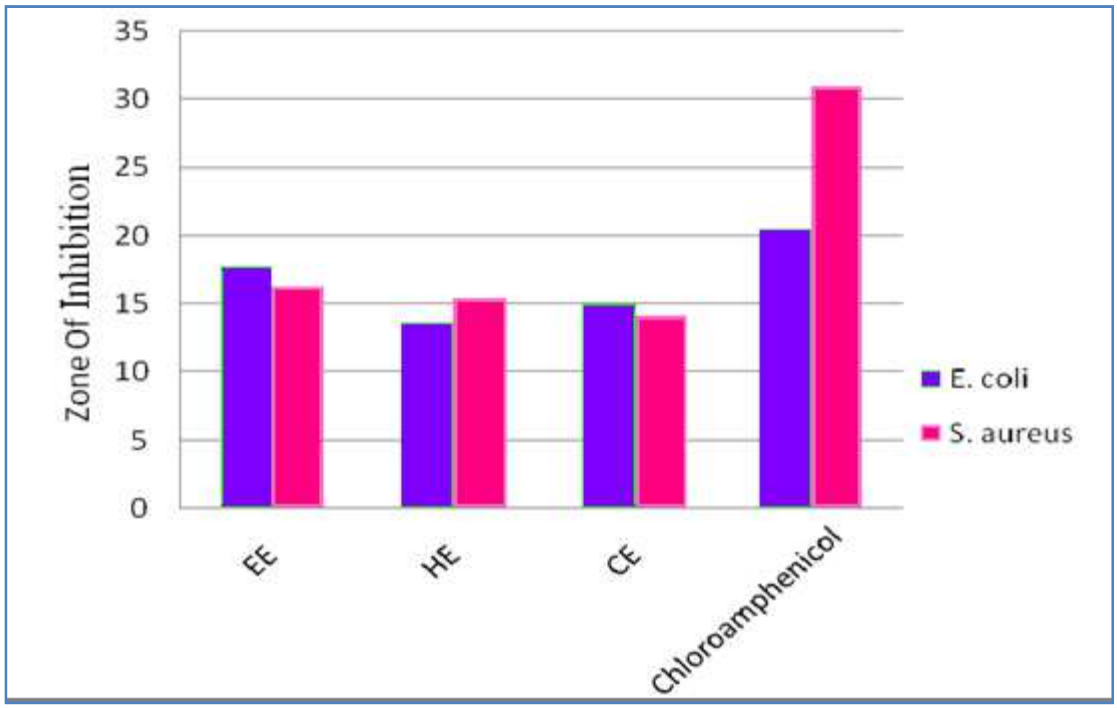
Incubation conditions for fungi—2 days at 27^0 C.

The anti microbial activity of all the extracts of *C. fascicularis L.* were studied with concentration 100 µg/ml against two pathogenic bacterial strains and one fungal strain. Antibacterial and antifungal potential of extracts assessed in terms of zone of

inhibition of bacterial growth. The results of antimicrobial activities are presented in Table 13, 14. The growth of inhibition zone measured ranged from 15-18 mm for sensitive bacteria and ranged from 08-10 mm for fungal strains. The graphical results are presented in Figure AM-1 and AM-2.

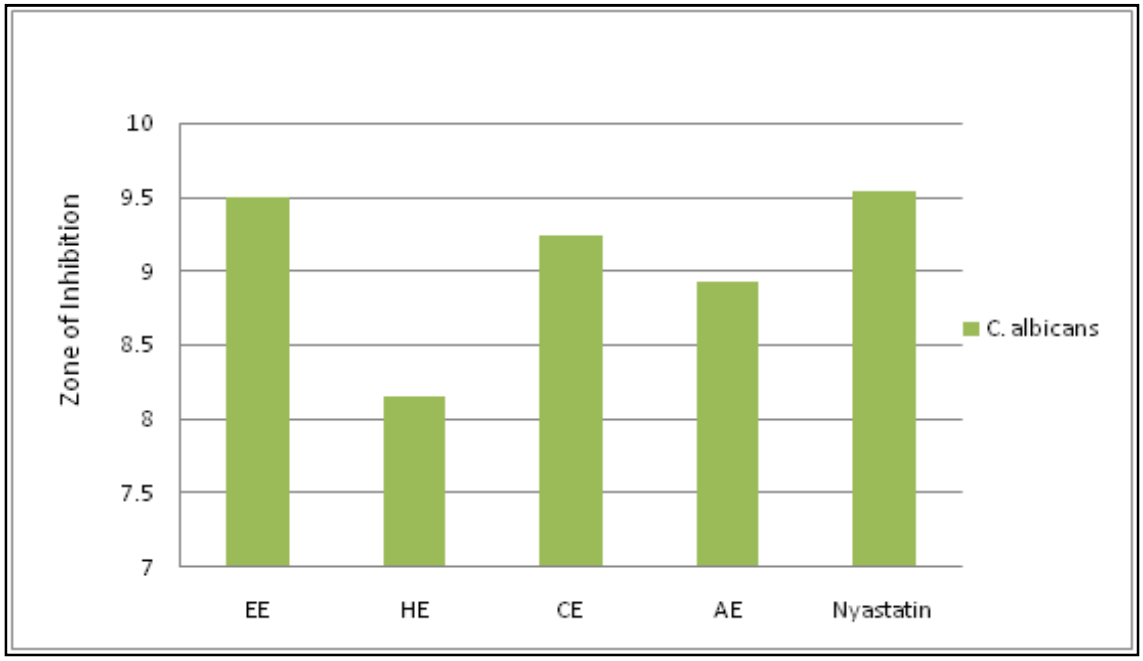
The inhibitory effect of *C. fascicularis* L. leaves ethanol, n-Hexane, chloroform and aqueous extracts showed at 17.76, 13.61, 15.00, 15.01 mm for *E. coli*, 16.30, 15.41, 14.18, 15.09 mm for *S. aureus* and 9.80, 8.15, 9.73, 8.92 for *C. albicans* respectively, The results showed that *C. fascicularis* L. leaves extracts were found to be effective against all the microbes tested.

Figure AM- 1: Antibacterial Activity Against *E. coli* and *S. Aureus*



(#EE- Ethanol extract, HE- n-Hexane extract, CE- Chloroform extract, AE- Aqueous extract)

Figure AM-2: Antifungal Activity Against *C. albicans.*



(#EE- Ethanol extract, HE- n-Hexane extract, CE- Chloroform extract, AE- Aqueous extract)

6.3.2 MIC, MBC and MFC of Isolated compounds of Leaves extracts of *Corchorus fascicularis* L.

Minimum Inhibitory Concentration (MIC), minimum bactericidal Concentration (MBC) and minimum fungicidal concentration values of each isolated compounds of unsaponifiable matter of n-hexane extract, chloroform extract and ethanol extract were determined. The results are summarized in table 15 to 20.

Table 15: Results of MIC and MBC (µg/ml) of isolated compounds of unsaponifiable matter of *n*-hexane extracts by tube dilution method:

Sr. No.	Microorganisms	MIC		MBC		Control DMSO	Standard Drug
		HEC-1	HEC-2	HEC-1	HEC-2		
1.	*Escherichia coli (NCIM 2110)*	> 256	> 256	256	256	-	0.25
2.	*Staphylococcus aureus (NCIM 2079)*	> 32	> 8	32	8	-	0.25
3.	*Bacillus subtilis (NCIM 2250)*	>64	> 8	64	8	-	0.50
4.	*Pseudommonas aeruginosa (NCIM 2036)*	>32	> 16	32	16	-	1.0

Note: Values are Minimal Inhibitory Concentration (µg/ml) and Minimal Bactericidal Concentration (µg/ml).

Standard Drugs: Amoxycillin for antibacterial, Ketoconazole for antifungal study.

Incubation conditions for bacteria—1 day at 37^0 C, for fungi—2 days at 27^0 C

The compound **HEC-1(Oleanolic acid)** and **HEC-2 (Ursolic acid)** showed significant MIC and MBC values. The compound **HEC-1 (Oleanolic acid)** showed minimum inhibition concentration (MIC), minimum bactericidal concentration (MBC) for, *S. aureus* and *B. subtilis*. The compound **HEC-2 (Ursolic acid)** showed minimum inhibition concentration (MIC), minimum bactericidal concentration (MBC) for, *S. aureus* and *B. subtilis*. The compound **HEC-2 (Ursolic acid)** showed more potent for minimum inhibition concentration (MIC), minimum bactericidal concentration (MBC) as compared to **HEC-1 (Oleanolic acid).**

Table 16: Results MIC and MFC (µg/ml) of isolated compounds of unsaponifiable matter of *n*-hexane extracts by tube dilution method:

Sr. No.	Microorganisms	MIC		MFC		Control DMSO	Standard Drug
		HEC-1	HEC-2	HEC-1	HEC-2		
1.	*Candida albicans (NCIM 3471)*	>32	>16	32	16	-	0.50
2.	*Aspergillus niger (NCIM 545)*	>32	>16	32	16	-	0.25

Note: Values are Minimal Inhibitory Concentration (µg/ml) and Minimal Bactericidal Concentration (µg/ml).
Standard Drugs: Amoxycillin for antibacterial and Ketoconazole for antifungal study.
Incubation conditions for bacteria—1 day at 37^0 C, for fungi—2 days at 27^0 C

The compound **HEC-1(Oleanolic acid)** and **HEC-2 (Ursolic acid)** showed significant MIC and MFC values. The compound **HEC-1 (Oleanolic acid)** showed minimum inhibition concentration (MIC), minimum fungicidal concentration (MFC) for, *C. albicans* and *A. niger.*

The compound **HEC-2 (Ursolic acid)** and **HEC-2 (Ursolic acid)** have shown potent minimum inhibition concentration (MIC), minimum fungicidal concentration (MFC) for, *C. albicans* and *A. niger.*

Table 17: Results of MIC and MBC (µg/ml) of isolated compounds of chloroform extracts by tube dilution method:

Sr. No.	Microorganisms	MIC		MBC		Control DMSO	Standard Drug
		CEC-1	CEC-2	CEC-1	CEC-2		
1.	*Escherichia coli (NCIM 2110)*	> 512	> 512	512	512	-	0.25
2.	*Staphylococcus aureus (NCIM 2079)*	> 32	> 16	32	16	-	0.25
3.	*Bacillus subtilis (NCIM 2250)*	>128	> 32	128	32	-	0.50
4.	*Pseudommonas aeruginosa (NCIM 2036)*	>32	> 32	32	32	-	1.0

Note: Values are Minimal Inhibitory Concentration (µg/ml) and Minimal Bactericidal Concentration (µg/ml).

Standard Drugs: Amoxycillin for antibacterial and Ketoconazole for antifungal study.

Incubation conditions for bacteria—1 day at 37^0 C, for fungi—2 days at 27^0 C

The compound **CEC-1(Stigmasterol)** and **CEC-2 (β-sitosterol)** showed significant MIC and MBC values. The compound **CEC-1 (Stigmasterol)** showed minimum inhibition concentration (MIC), minimum bactericidal concentration (MBC) for, *S. aureus* and *P. aeruginosa*.

The compound **CEC-2 (β-sitosterol)** showed minimum inhibition concentration (MIC), minimum bactericidal concentration (MBC) for, *S. aureus*. The compound **CEC-2 (β-sitosterol)** was found more potent for minimum inhibition concentration (MIC), minimum bactericidal concentration (MBC) as compared to **CEC-1(Stigmasterol)**.

Table 18: Results MIC and MFC (µg/ml) of isolated compounds of chloroform extracts by tube dilution method:

Sr. No.	Microorganisms	MIC		MFC		Control DMSO	Standard Drug
		CEC-1	CEC-2	CEC-1	CEC-2		
1.	*Candida albicans (NCIM 3471)*	> 8	>16	8	16	-	0.50
2.	*Aspergillus niger (NCIM 545)*	>32	>32	32	32	-	0.25

Note: Values are Minimal Inhibitory Concentration (µg/ml) and Minimal Bactericidal Concentration

Standard Drugs: Amoxycillin for antibacterial and Ketoconazole for antifungal study

Incubation conditions for bacteria—1 day at 37^0 C. for fungi—2 days at 27^0 C.

The compound **CEC-1(Stigmasterol)** and **CEC-2 (β-sitosterol)** showed significant MIC and MFC values. The compound **CEC-1(Stigmasterol)** showed minimum inhibition concentration (MIC), minimum fungicidal concentration (MFC) more potent for, *C. albicans.*

The compound **CEC-1(Stigmasterol)** showed more potent minimum inhibition concentration (MIC), minimum fungicidal concentration (MFC) for, *C. albicans* and *A. niger* as compared to **(β-sitosterol)**.

Table 18: Results of MIC and MBC (µg/ml) of isolated compounds of ethanol extracts by tube dilution method

Sr.	Microorganisms	MIC	MBC	Control	Standard

No.		EEC-1	EEC-2	EEC-1	EEC-2	DMSO	Drug
1.	*Escherichia coli (NCIM 2110)*	> 512	>1024	512	1024	-	0.25
2.	*Staphylococcus aureus (NCIM 2079)*	> 256	> 128	256	128	-	0.25
3.	*Bacillus subtilis (NCIM 2250)*	> 256	> 512	256	512	-	0.50
4.	*Pseudommonas aeruginosa (NCIM 2036)*	>1024	>1024	1024	1024	-	1.0

Note: Values are Minimal Inhibitory Concentration (µg/ml) and Minimal Bactericidal Concentration (µg/ml).

Standard Drugs: Amoxycillin for antibacterial and Ketoconazole for antifungal study.

Incubation conditions for bacteria—1 day at 37^0 C, for fungi—2 days at 27^0 C

The compound **EEC-1 (Quercetin)** and **EEC-2 (Catechin)** showed significant MIC and MBC values. The compound **EEC-1 (Quercetin)** showed minimum inhibition concentration (MIC), minimum bactericidal concentration (MBC) for, *S. aureus* and *B. subtilis* .

The compound **EEC-2 (Catechin)** showed minimum inhibition concentration (MIC), minimum bactericidal concentration (MBC) for, *S. aureus*. The compound **EEC-2 (Catechin)** was found more potent for minimum inhibition concentration (MIC), minimum bactericidal concentration (MBC) as compared to **EEC-1 (Quercetin).**

Table 20: Results MIC and MFC (µg/ml) of isolated compounds of ethanol extracts by tube dilution method:

Sr. No.	Microorganisms	MIC		MFC		Control DMSO	Standard Drug
		EEC-1	EEC-2	EEC-1	EEC-2		
1.	*Candida albicans (NCIM 3471)*	>128	>512	128	512	-	0.50
2.	*Aspergillus niger (NCIM 545)*	>512	>256	512	256	-	0.25

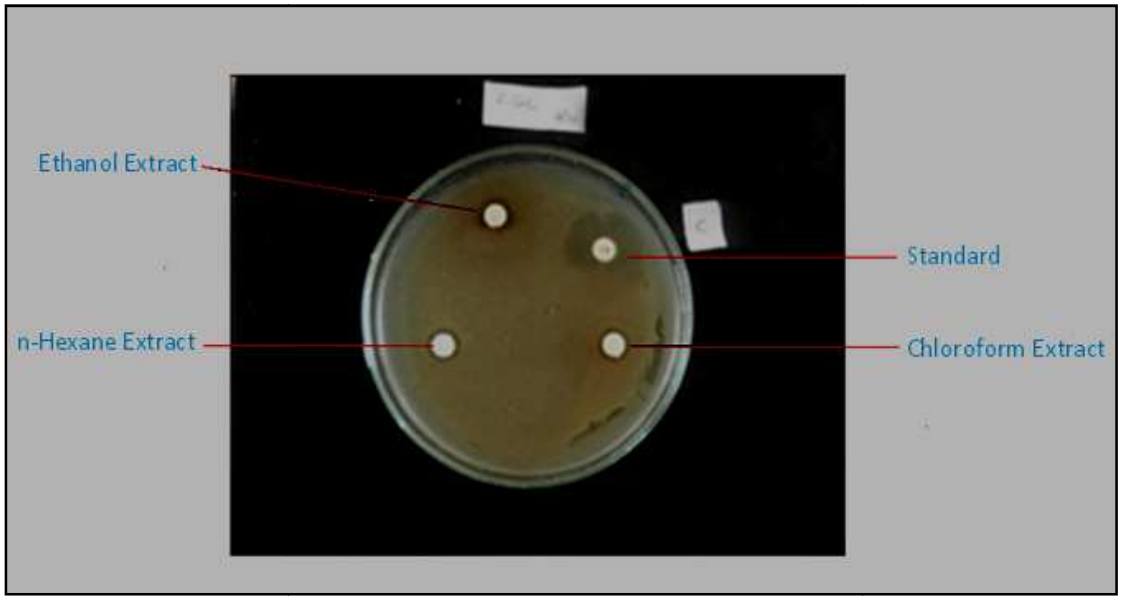
Note: Values are Minimal Inhibitory Concentration in (µg/ml) and Minimal Bactericidal Concentration in (µg/ml).
Standard Drugs: Amoxycillin for antibacterial and Ketoconazole for antifungal study.
Incubation conditions for bacteria—1 day at 37^0 C, for fungi—2 days at 27^0 C.

The compound **EEC-1 (Quercetin)** and **EEC-2 (Catechin)** showed significant MIC and MFC values. The compound **CEC-1(Stigmasterol)** showed minimum inhibition concentration (MIC), minimum fungicidal concentration (MFC) more potent for, *C. albicans*. The compound **EEC-1 (Quercetin)** showed more potent minimum inhibition concentration (MIC), minimum fungicidal concentration (MFC) for, *C. albicans*.

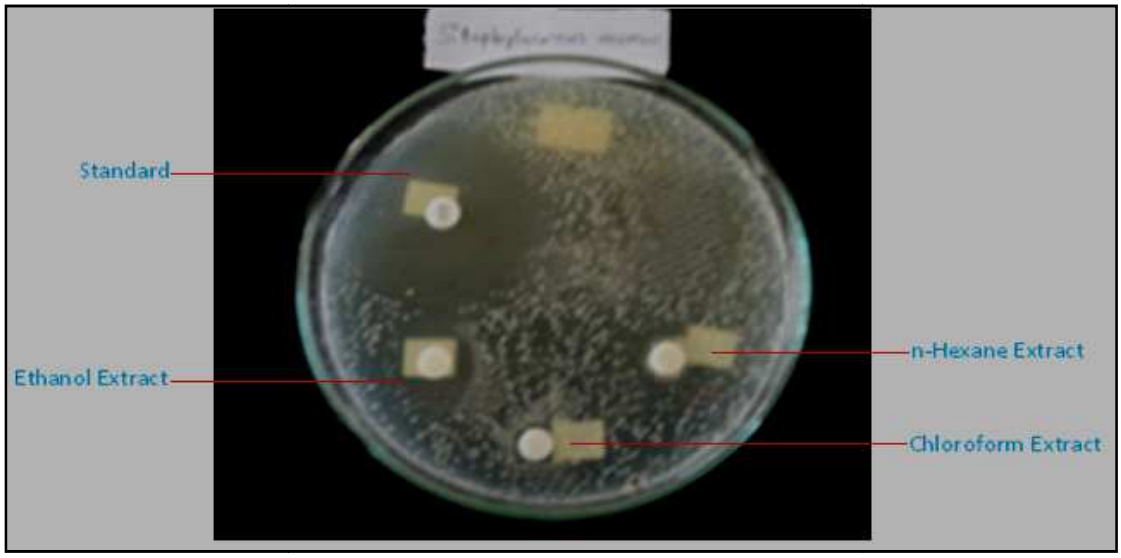
The compound **EEC-2 (Catechin)** showed more potent minimum inhibition concentration (MIC), minimum fungicidal concentration (MFC) for, *A. niger*. The compound **EEC-1 (Quercetin)** showed more potent minimum inhibition concentration (MIC), minimum fungicidal concentration (MFC) as compared to **EEC-2 (Catechin)**.

From the above results, it can be concluded that the *Corchorus fascicularis* L. leaves extracts can be used as broad-spectrum antimicrobial agent after extensive investigation. These results may provide a basis for the isolation of compounds of biological interest from *Corchorus fascicularis* L. for potent activity.

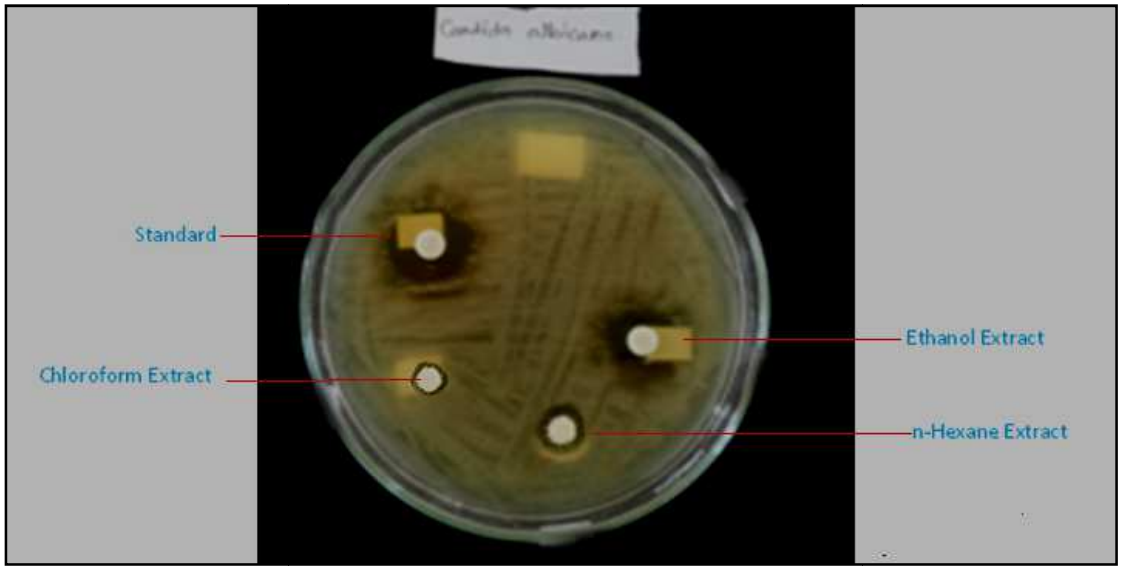
Photographs of Antibacterial and Antifungal Activity



Photograph of *E. Coli*



Photograph *S. aureus*



Photograph *C. albicans*

6.4 CONCLUSION:

6.4.1 PRELIMINARY PHYTOCHEMICAL STUDY.

Triterpenes and steroids were found in *n*-hexane and chloroform extracts of leaves of *Corchorus fascicularis* L. respectively, while flavonoids were observed in ethanol extract of leaves of *Corchorus fascicularis* L. Highly polar chemicals such as flavonoids were found in ethanol, which is also supported by ethanol extracts percentage yield which was quite higher than both of the other percentage yield of extracts.

6.4.2 PHYTOCHEMICAL INVESTIGATION.

Chromatographic separation of phytoconstituents from *n*-hexane extract revealed two compounds from extract of leaves of *Corchorus fascicularis* L. Chemical identification, spectral analysis and interpretation of data of these isolated compounds using UV, IR, ^{1}H-NMR, ^{13}C-NMR and LC-MS, suggested that compounds HEC-1 and HEC-2 are triterpenoids, oleanolic acid and ursolic acid, having molecular formulae $C_{30}H_{48}O_3$ and $C_{30}H_{50}O_3$ respectively.

Oleanolic acid and Ursolic Acid were not reported from the leaves of *Corchorus fascicularis* L., their presence is being reported for the first time.

Chromatographic separation of phytoconstituents from chloroform extract revealed two compounds from the extract of leaves of *Corchorus fascicularis* L. Chemical identification, spectral analysis and interpretation of data of these isolated compounds using UV, IR, ^{1}H-NMR, ^{13}C-NMR and LC-MS, suggested that compound HEC-1 and HEC-2 are sterols, stigmasterol and β-sitosterol, having molecular formulae $C_{29}H_{48}O$ and $C_{29}H_{50}O$ respectively.

Stigmasterol and β-sitosterol were not reported from the leaves of *Corchorus fascicularis* L., their presence is being reported for the first time.

Chromatographic separation of phytoconstituents from ethanol extract revealed two compounds from the extract of leaves of *Corchorus fascicularis* L. Chemical identification, spectral analysis and interpretation of data of these isolated compounds

using UV, IR, ^{1}H-NMR, ^{13}C-NMR and LC-MS, suggested that compound EEC-1 and EEC-2 are flavonoids, quercetin and catechin, having molecular formulae $C_{15}H_{10}O_7$ and $C_{15}H_{14}O_6$ respectively.

Quercetin and Catechin were not reported from the leaves of *Corchorus fascicularis* L., their presence is being reported for the first time.

6.4.3 BIOLOGICAL STUDIES.

6.4.3 Antioxidant Activity:

The free radical scavenging activity of the *n*-hexane, chloroform and ethanol extracts of leaves of *Corchorus fascicularis* L. were evaluated by the DPPH assay. The DPPH radical scavenging activity of the ethanol extract revealed higher antioxidant activity, considering the fact that quenching properties were obtained only from the crude extract and were comparable with ascorbic acid. Scavenging of the DPPH radical is related to the inhibition of lipid per-oxidation.

The Ethanol extract had comparable radical scavenging potential to reference standard due to presence of phenolic compounds.

The total antioxidant capacity of the *n*-hexane, chloroform and ethanol extracts of leaves of *Corchorus fascicularis* L. were evaluated by the phosphomolybdenum assay. The phosphomolybdenum assay is a quantitative method of evaluating water-soluble and fat-soluble antioxidant capacity (total antioxidant capacity). The ascorbic acid and ethanol extract demonstrated electron-donating capacity, showing its ability to act as chain terminators, transforming relative free radical species into more stable non-reactive products.

The total antioxidant capacity of the ethanol extract (equivalent to ascorbic acid) was found to be the highest of all the extracts and was concentration-dependent.

The present results support the ethno-medical applications of the leaves of *Corchorus fascicularis* L. in the antioxidant effect.

6.4.3 Anti-inflammatory activity:

The *n*-hexane and chloroform leaves extracts of *Corchorus fascicularis* L. at the doses of 100, 200 and 400 mg/kg p. o. showed prominent results and caused a significant inhibition in the percent rise of carrageenan induced rat paw edema. The 400 mg/kg dose of *n*-hexane extract showed percent rise inhibition of 85.7% and of chloroform extract showed 67.5%, at 3rd hour as highly significant results against

carrageenan induced rat paw edema. The maximal inhibition in the percent rise of edema volume was achieved at a dose 400 mg/kg ($P<0.01$) of *n*-hexane and chloroform of leaves extracts, when compared to standard drug diclofenac sodium.

The presence of triterpenoids and steroids in *n*-hexane and chloroform extract could possibly lead to the observed activities. The anti-inflammatory activity of *n*-hexane and chloroform extract could also be linked to the ability of the extract to influence the peripheral and central COX activity or prostaglandin synthesis.

The present results support the ethno-medical applications of the leaves of *Corchorus fascicularis* L. in the treatment of inflammation diseases.

6.4.3 Antimicrobial Activity:

Antibacterial and antifungal activity assays of leaves of *Corchorus fascicularis* L. were performed by the modified disc diffusion method.

The results revealed that the ethanol and n-Hexane extracts showed significant antibacterial activity as compared to chloroform extract. The n-Hexane and ethanol extracts exhibited prominent activity against bacteria like *Escherichia coli, Staphylococcus aureus.* Ethanol extract was found more potent as compared to standard drug for fungi *Candida albicans.*

Minimum Inhibitory Concentration (MIC), minimum bactericidal Concentration (MBC) and minimum fungicidal Concentration (MFC) values of each isolated compounds of *Corchorus fascicularis* L. were determined. The results of each isolated compounds for MIC, MBC and MFC values were potent against bacteria and fungi.

From the results, it can be concluded that the leaves of *Corchorus fascicularis* L. extracts can be used as broad-spectrum antimicrobial agents. The isolated compounds from leaves of *Corchorus fascicularis* L. showed strong MIC, MBC and MFC therefore, leaves are useful for treating various microbial diseases.

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