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Research Article

Antimicrobial Activity of Ethanol Extracts and Isolated Fractions *Shorea robusta* Gaertn f and *Dipterocarpus turbinatus* Gaertn f

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ABSTRACT

Background: *Shorea robusta* and *Dipterocarpus turbinatus* are widely used by the indigenous people of North Eastern state Tripura in their daily life for various purposes. **Objectives:** The present investigation was aimed to evaluate the antimicrobial activity of *S. robusta* and *D. turbinatus* bark extracts and their different fraction by disk diffusion and turbidimetric methods. **Methods:** Plant materials were extracted with different solvents in successive manner and the extracts were screened for antimicrobial activity by disc diffusion. **Results:** As the ethnaollic extracts of both the plants showed good antimicrobial activity, we fractionated the ethanolic extracts with different solvents using a separating funnel to obtain specific solvent soluble fractions. The ethyl acetate soluble fractions and residual ethanolic fractions were effective against several bacterial and fungal pathogens. The ethyl acetate fractions were further fractioned using silica gel column chromatography (60-120) as an adsorbent and the isolated fractions were further tested for their antimicrobial activity by turbimetric methods. **Conclusion:** All isolated fractions had antimicrobial activity, with most of the isolated fractions show activity against gram negative bacteria.

Key words: *Shorea robusta, Dipterocarpus turbinatus*, Isolation, Ethanolic extracts, Antimicrobial, Turbidmetric method.

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INTRODUCTION

Antimicrobial drugs are the greatest contribution of the 20th century to therapeutics. Drugs in this class are designed to inhibit or kill the infecting organism and to have no or minimal effect on the recipient.[1] *Sheorea robusta* (Dipterocarpaceae) is a large deciduous tree found extensively in North East and Central India.[2] The bark is reddish brown-grey in colour, smooth and longitudinally fissured. The plant is traditionally used to treat dysentery and for fumigation, weak digestion, gonorrhea and as an aphrodisiac.[3-5] Scientifically, the plant has been reported for various activities including analgesic,[6,7] antinociceptive, anti-inflammatory,[8,9] oxidative stress,[10] wound healing[11,12] and antiulcer.[13] Bioactive compounds are also isolated from various parts of the plant including the flavonoids 3, 7-dihydroxy-8-methoxy flavones-7-O-α-L rhamnopyranosy (1→4) -α-L rhanmnogluco pyranosyl (1→6) β-glucopyranosyl from the seeds of *S. robusta;*[14] 3, 25-epoxy-1, 2, 3, 11- tetra-hydroxyurs -12-en-28-oic acid and 3, 25-epoxy 1, 2, 3- tri-hydroxyurs -12-en-28 oic from the resin of *S.robusta.*[15]

Dipterocarpus turbinatus (Dipterocarpaceae) is a large woody plant, attaining the height of 30-38 meter and a girth of 2-3 meter. It is found in the tropical forests of North Easter state of India (Tripura, Assam) and Andaman island of India. *D. turbinatus* is used traditionally as anti-diarrheal, astringent and wound healer, as well as to treat ulcers, burns, tuberculoid leprosy.[16-18] In '*Asanadi gana*' an ayurvedic formulation in it is one of the ingredients of 23 plants which were used for the diabetic treatment.[19] The plant is reported for antioxidant,[20] gum tooth ache[21] and cytotoxic activity.[22] Tribal people of Tripura use *D. turbinatus* derived products for various purposes.[23] Five triterpenes, 3-oxo-20-hydroxy-30α- methyl, 17(29)-α-epoxy-28- norlupane (1); 3-oxo-20 hydroxy-30β-methyl-17(29)α-epoxy-28-norlupane (2); 3, 20-dooxo-28, 29-norlupane17α-ol (3); 27-dimethyl-20(s) dammer-23-ene-20-ol-3, 25 dione (4); 3 epi-ceropic acid (5); together with 13 other compound including diterpane, susquiterpenes and triterpenens were isolated from the stem of *D. obturfolius,* characterized and were tested for their cytotoxicity against a small panel of human cancer cell line.[24] Phytochemical investigation of the stem wood of *D. alatus* led to the isolation and characterization of four oligostilbenoids, dipterocarpols A-D and resveratrol oligomers.[25] Diptindonein E was isolated from the acetone extracts of the bark of *D. hasseltii* and cytotoxic activity was examined against murine leukemia P-338.[26] The article focuses on the anti-microbial activity of *S. robusta* and *D. turbinatus* extracts and their various isolated fractions.

MATERIALS AND METHODS

Plant materials

Plant materials were procured from the forest of the Gomuti district of North Eastern state Tripura. Plant materials were identified and authenticated by Prof. P. Jayaraman, M.Sc., Ph.D; Director Plant anatomy research centre, Tambaram, Chennai. Voucher specimens (PARC/2012/1276 and PARC/2012/1277) were deposited in our laboratory for further reference. Plant materials were dried under shade and pulverized to a coarse powder in our college laboratory.

Chemicals and reagents

All other reagents for the study were procured from SD Fine lab, Mumbai, India., Pre-coated TLC plates (Merck 20X20 cm) were used for TLC. All media for the study were procured from Hi-Media Mumbai, India. Double beam UV- Visible spectrophotometer (Lab India) was used for physiochemical analysis.

Preparation of extracts and phytochemical study

Coarse powder of plant materials (*S. robusta and D. turbinatus*) were extracted in a successive manner with petroleum ether 60-80, (white colour, 2.965 % w/w and 4.231% w/w) and chloroform (green, 4.11%W/W and 3.65% W/W) by hot-percolation using Soxhlet apparatus. The marc was collected and extracted by cold maceration using 70% ethanol (dark brown, 22% W/W and 18 % W/W) respectively. Solvents were removed by vacuum evaporator, dried and kept in desiccators. All extracts were

stored bellow 10°C. Various phytoconstituents present in the various extracts were detected by standard chemical tests.[27]

Fractionation of the ethanol extracts by solvent fractionation

Ethanol extracts of *S. robusta* and *D. turbinatus* were suspended in water. Then suspended materials were extracted with the organic solvents petroleum ether, chloroform and ethyl acetate to obtain petroleum ether (1.25 and 1.23 % w/w), chloroform, (1.31 and 1.57% w/w), ethylacetate (6 and 3.20 %) and residual ethanol fractions (35 and 32%) of ethanol extracts respectively. All fractions were filtered through Whatman filter paper (40) to remove particles. The particle free fraction was evaporated using a rotary evaporator (Eucator) under reduced pressure at 55°C to obtain dry extracts.[28-30]

Fractionation of the ethyl acetate fraction of ethanol extracts by column chromatography

Fractionation of ethyl acetate fractions of the ethanolic extracts of *S. robusta* and *D. turbinatus* were started by eluting with various solvent mixtures in different ratios according to their polarity. Column flow rate was maintained 30-35 drops/min; 40 mL of fraction was collected and analyzed by TLC.

Thin layer chromatography (TLC) for individual fraction

TLC was carried out on a precoated TLC plate (Merck aluminum sheet 20 X 20cm silica gel $60F_{254}$). Various solvent systems were tested by trial and error to determine the optimal solvent system. After development of the chromatogram, spots were detected under UV light (254 nm and 365 nm), in an iodine chamber, using alcoholic ferric chloride and finally by spraying with 10% alcoholic sulphuric acid.[31,32]

Screening of anti-microbial activity of extracts and isolated fractions

Antimicrobial study for the various extracts and the isolated fraction was carried out by disc diffusion method[33,34] and turbidimetric method.[35,36] *C. albicans* (ATCC -10231) and *A. niger* (ATCC -1015) two fungal pathogens *S. aureus* (ATCC-29213), *Pseudomonas aeruginosa* (ATCC-27853), *E. fecalis* (ATCC-29212) and *E. coli* (ATCC-25022) as bacterial pathogens were used for the study.

Culture media and standard drugs used for antimicrobial agents

Nutrient agar (NA) was used in disc diffusion method and brain heart infusion broth (BHI) was used for turbidimetric assay for both bacterial and fungal pathogens. All media were procured from Hi Media Lab. Ciprofloxacin and fluconazole (Micro Lab, India) was used as reference standard.

Preparation of inocula

For the preparation of the inoculate 24hr the culture was emulsified in 3 mL sterile saline following the McFarland turbidity to obtain a concentration of 10^8 cells/mL. The suspension was standardized by adjusting the optical density to 0.1 at 600 nm. One hundred microlitres (100 µl) of cell suspension with approximately 10^6 -10^8 bacteria per milliliter was placed in petri dishes and dispersed over agar.[37]

Determination of zone of inhibition by disc diffusion method

Antimicrobial activities of the different solvent extracts and different solvent soluble fraction of ethanolic extracts of *Shorea robusta* and *Dipterocarpus turbinatus* were screened by the disc diffusion method. Different concentrations were prepared (20 mg/mL, 30 mg/mL and 50 mg/mL) for the ethanol extracts of *S. robusta* and *D. turbinatus*. Different solvents soluble fractions of *S.robusta* and *D. turbinatus* were made in the concentration of 10mg/mL, 20mg/mL and 30 mg/mL using dimethyl sulphoxide (DMSO) as solvent. The nutrient agar (Hi-Media) was prepared and sterilized. Wells were made in the plates with the help of a sterile stainless steel-borer (6 mm diameter). Five holes per plates were made into the set agar containing the bacterial culture, 100 microliter of the tested extracts was added to the well in different concentrations. For each bacterial strain controls were maintained where pure solvents, instead of extract as negative control. After inoculation, the plates were allowed to diffuse and then incubated at 37°C for 24hr. Fungal suspensions were adjusted to 10^7 cells/mL. The zone of inhibition was determined after incubation for 48hr at 27°C. Specified test drug extracts (20, 30 and 50 mg/mL) and fractions (10, 20, 30 mg/mL) were used respectively. Ciprofloxacin (CPF) 100µg/ml and fluconazole (FLZ 100 µg/ml), were used as reference standard. All tests were performed in triplicates and repeated twice.[38] Results were recorded by measuring the zone of inhibition in mm surrounding the wells. Each assay was performed in triplicates and repeated twice.

Determination of MIC (minimum inhibitory concentration) for isolated fractions by serial dilution method[35,36]

Anti-microbial assays for isolated fractions were quantified by serial dilution method using 9 dilutions of each tested extracts. BHI (brain heart infusion broth) was used for the study. In the initial tube, 20µL of test compound was added into the 380 µL of BHI broth. For dilutions, 200 µL of BHI broths was added into the next 9 tubes separately. Then from the initial tube 200 µL was transferred to the first tube containing 200 µL of BHI broth. This was considered as 10^{-1} dilution. From 10^{-1} diluted tube 200 µL was transferred to second tube to make 10^{-2} dilution. The serial dilutions were repeated up to 10^{-9} dilution or each drug. From the maintained stock cultures of required organisms, 5 microliter was taken and added into 2ml of BHI (brain heart infusion) broth. In each serially diluted tube 200 µL of above culture suspension was added. The tubes were incubated and observed for turbidity. For strict anaerobes, tubes were incubated in anaerobic jars for 48-72 hr and calculate the MIC.[39]

RESULTS

Fractionation of the ethanol extracts by separating funnel

Ethanolic extracts of both the plant were fractioned by different solvent using separating funnel the results for the different solvent soluble fractions for ethanolic extracts is mentioned in the Table 1.

Table 1: Fractionation of the ethanol extracts by separating funnel.

S. No	Solvents	S. robusta		D. turbinatus	
		% of Yield (W/W)	Colour	% of Yield (W/W)	Colour of fraction
01	Petroleum Ether	1.25	White	1.23	White
02	Chloroform	1.31	Green sticky	1.57	Green and sticky
03	Ethyl acetate	6.00	Yellowish brown	3.20	Light brown
04	Ethanol	35.00	Dark brown	32.00	Dark brown

Fractionation of ethyl acetate fraction by column chromatography

Four different fractions were collected from the ethyl acetate fraction of ethanolic extracts of *Shorea robusta* and named, SR C, SR D, SR E and SRF. We collected 5 different fractions from ethyl acetate fraction of ethanolic extract of *Dipterocarpus turbinatus* and named them DT B, DT C, DT D, DT E and DT F. Table 2 and Table 3 summaries the results for the isolation of the different fraction for *Shorea robusta* and *Dipterocarpus turbinatus*.

Antimicrobial activity of the different solvent soluble fraction of ethanolic extracts of Shorea robusta and Dipterocarpus turbinatus

Antimicrobial activity for the different extracts from *Shorea robusta and Dipterocarpus turbinatus* and different solvent soluble fractions (petroleum ether, chloroform, ethyl-acetate soluble fraction) form ethanolic extract of *S. robusta* and *D. turbinatus* was conducted by well diffusion using concentrations of 20 mg/mL, 30 mg/mL and 50mg/mL of plant extracts. The results of the antimicrobial activity study for the different extract is given in the Table 4.

Antimicrobial activity for the different solvent soluble fraction was carried out in 3 different concentrations *viz* 10mg/mL, 20mg/mL and 30mg/mL. Results for the antimicrobial study of different solvent soluble fraction of ethanolic extract of *S. robusta* and *D. turbinatus* is denoted in the Table 5.

Determination of MIC values for the isolated fraction form ethyl acetate fraction of ethanolic extracts of S. robusta and *D. turbinatus*

MIC values for the different fraction obtained by column chromatography from the ethyl acetated fraction of ethanolic extract of *S. robusta* and *D. turbinatus* were determined by turbidimetric methods. The results for the antimicrobial activity are represented in the Table 6.

DISCUSSION

Traditionally the plants are used for antimicrobial agent in India.[16] Plant compounds are of interest as a source of safer and more effective substitute than synthetically produced antimicrobial substance. Pathogenic organisms are getting resistance to the existing drug. (new). In this study our aim was to isolate the chemical entities having antimicrobial activity in both the plant *i.e* in *Shorea robusta* and *Dipterocarpus turbinatus*. A single plant may contain many constituents. Individual component can be separated by fractions with different solvents to isolate individual entities. There is various technique of fractionation to isolates new chemical entities.[40] Fractionation of the ethanol extract was carried out by using solvent non polar to polar solvents and collect ethanolic fraction for further studies of separation.[41]

Column chromatography is one of the convenient techniques to technique to isolate phyto constituents. Using column chromatography we have isolated five different fractions from *Shorea robusta* and five different fractions from *Dipterocaropus turbinatus*. Antimicrobial activity was

Table 2: Isolated fraction from ethylacetate fraction of ethanolic extract of Shorea robusta by column chromatography.

S. No	Eluted solvents	Name	Qty and Colour	R_f value and solvent system
01	$CHCl_3$:EtOAc (75:25)	SR C	80mg and green	0.54(Ethyl acetate: methanol: water; 100:13.5:10)
02	$CHCl_3$:EtOAc (50:50)	SR D	100mg and greenish yellow	0.41 (Ethyl acetate: methanol: water; 100:13.5:10)
03	$CHCl_3$:EtOAc (25:75)	SR E	60 mg and brownish yellow	0.51 (Ethyl acetate: methanol: water; 100:13.5:10) 0.46 (toluene : ethylformate : formic acid 50:40:10)
04	EtOAc	SR F	125mg and brown	0.65 (Ethyl acetate: methanol: water; 100:13.5:10) 0.71 (toluene : ethylformate : formic acid 50:40:10)

Table 3: Isolated fraction from ethyl acetate fraction of ethanolic extract of *Dipterocarpus turbinatus* by column chromatography.

S. No	Eluted solvents	Name	Qty and Colour	R_f value and solvent system
01	$CHCl_3$	DT B	60mg and green	0.8 (Ethyl acetate: methanol: water; 100:13.5:10)
02	$CHCl_3$:EtOAc (75:25)	DT C	75mg and green	0.4 (Ethyl acetate: methanol: water; 100:13.5:10)
03	$CHCl_3$:EtOAc (50:50)	DT D	90mg and greenish yellow	0.41 (Ethyl acetate: methanol: water; 100:13.5:10)
04	$CHCl_3$:EtOAc (25:75)	DT E	80 mg and brownish yellow	0.61 (Ethyl acetate: methanol: water; 100:13.5:10) 0.66 (toluene : ethylformate : formic acid 50:40:10)
05	EtOAc	DT F	135mg and brown	0.65 (Ethyl acetate: methanol: water; 100:13.5:10) 0.72 (toluene : ethylformate : formic acid 50:40:10)

Table 4: Determination of Antimicrobial effects (measuring zone of inhibition in mm) of the different extracts of *Shorea robusta* and *Dipterocarpus turbinatus*.

Name of the extracts	Concmg/mL	Name of Microorganism					
		Gram positive		Gram negative		Fungal	
		P. aeruginosa	*S. aureus*	*E. coli*	*E. faecalis*	*C. albicans*	*A. niger*
PEESR	20	-	-	-	-	-	-
	30	7.2	-	-	-	7.2 ± **0.21**	-_
	50	8.8 ±0.17	7.2± 0.33	8.1 ± 0.44	8.5 ± 0.72	8.5 ± 0.87	7.5 ± 0.27
PEEDT	20	-	-	-	-	-	-
	30	7.4±0.23	7.2 ± 0.12	-	-	-	-
	50	8.9 ±0.28	7.3 ±.567	7.5 ± 0.7	7.8 ± 1.66	7.8 ± 1.667	7.3 ± 0.02
CEESR	20	9.1 ± 0.73	9.3 ± **0.53**	10.5 ±0.35	11.3 ± 1.32	9.5±.022	9.3 ± 1.37
	30	10.5 ± 0.38	9.7 ±0.4	11.3 ±1.00	12.5± 1.45	10.2 ± **0.02**	9.8 **± 0.22**
	50	10.6 ±0.22	10.2± 0.33	13.1 ±0.10	13.5 ± 0.211	10.5 ± 0.08	9.5 ± 0.27
CEEDT	20	8.5 ±0.23	8.4 ± **0.53**	10.1 ± 0.357	11.5 ± 1.32	8.45 ± 0.34	9.0 ± 0.53
	30	10.8 ± 0.12	9.7 ± 0.21	10.9 ±0.21	13.1± 2.45	10.2 ± **0.02**	10.1**± 0.22**
	50	10.8 ± 0.081	10.2 ± 0.5	13.3 ± 0.44	14.1 ± 0.11	13.5 ± 0.75	10.5 ± 0.35
EESR	20	18.3± 0.71	19.1 ± 0.89	19.7 ± 0.88	18.8 ± 0.91	18.7 ± 0.85	16.9 ± 0.88
	30	19.5± 0.91	21.7 ± 0.41	19.1 ± 0.21	22.32 ± 0.1	22.1 ± 0.41	20.3 ±0.12
	50	22.4 ± 0.48	21.3±0.37	22.9 ±0.45	23.6±0.72	21.0±0.23	23.3±0.18
EEDT	20	15.3 ±2.44	18.1± 1.10	19.0 ± 0.74	12.6 ±2.404	18.3±2.404	17.2 ±2.40
	30	22.8 ±1.48	21.3±1.28	22.3±1.4	24.0±1.8	21.83±0.4	21.1 ±.01
	50	23 ± 0.0577	24 ± 0.774	25.3±2.404	25.2 ± 0.8	23.4± 0.02	22.1 ± 1.5

Where, PEESR- Petroleum ether extract of *Shorea robusta*; PEEDT- Petroleum ether extracts of *Dipterocarpus turbinatus*; CHESR- Chloroform extracts of *Shorea robusta*; CHEDT- Chloroform extracts of *Dipterocarpus turbinatus*; EAESR- Ethyl acetate extracts of *Shorea robusta*, EESR Ethanol extracts of *Shorea robusta*, EEDT- Ethanol extracts of *Dipterocarpus turbinatus*.

Table 5: Antimicrobial activity of the different fractions of *Shorea robusta* and *Dipterocarpus turbinatus*.

Fraction	Con. mg/mL	Name of Microorganism					
		Gram positive		Gram negative		Fungal	
		P. aeruginosa	*S. aureus*	*E. coli*	*E. faecalis*	*C. albicans*	*A.niger*
PEFEESR	10	-	-	-	-	-	-
	20	7.1 ±0.0	7.2 ±0.21	7.2 ±0.11	7.5 ±0.04	7.7 ±0.05	7.3 ±0.02
	30	7.6 ±0.13	7.70 ± 0.321	7.5 ± 0.52	7.5 ± 0.55	7.8 ±0.02	7.7 ±0.128
PEFEEDT	10	-	-	-	-	-	-
	20	7.1 ±.0.4	7.0 ±.00	7.2 ±0.33	7.5 ±0.223	7.2 ± **0.02**	7.0± 0.05
	30	7.3 ± 0.39	7.2± 0.33	7.6 ± 0.44	7.5 ± 0.721	7.8 ± 0.0087	7.5 ± 0.27
CFEESR	10	8.3± 0.23	8.2±0.56	9.5±0.8	8.8±0.64	8.0 ±0.32	8.1±0.22
	20	9.2 ± 0.84.	9.2 ± 0.02	9.5 ± 0.112	9.3 ±. 0.54	9.2 ± **0.73**	9.5 ± 0.09
	30	10.3 ± 0.54	10.5 ± 0.33	10.1 ± 0.44	10.5 ± 0.21	10.5 ± 0.087	9.5 ± 0.32
CFEEDT	10	8.0 ±0.19	7.5±0.54	8.5 ±0.64	8.0 ± 0.35	8.0 ± 0.54	8.1 ±0.8
	20	9.4 ± 0.1	9.2 ±0.33	9.3 ± 0.33	9.5 ± 0.07	9.5 ± **0.021**	8.8 ±0.55
	30	10.3 ± 0.89	10.2± 0.07	10.0 ± 0.11	10.5 ± 0.721	10.0 ± 0.0	9.5 ± 0.23
EAFEESR	10	15.1 ±.0198	14.3 ± 0.665	10.9 ± 0.21	12.8 ± 0.54	11.5 ± 0.11	12.9 ± 0. 25
	20	16.1 ±0.441	14.5 ±0.112	15.1 ± 1.24	14.5 ±0.02	16.2 ± **0.02**	13.5 ± **0.39**
	30	19.3 ±0.112	18.5 ± 0.33	18.0 ± 0.55	17.5 ± 0.721	18.0 ± 0.0087	15.5 ± 0.2732
EAFEEDT	10	13.5 ± 0.54	14.1 ± 0.34	17.3±.57	16.2 ± 0.57	13.5 ± 0.54	15.4 ± 0.123
	20	15.2 ± 0.54	16.2 ±0.234	18.7 ±.0.4	18.9±.0.44	18.4 ± **0.021**	17.5 ±0.23
	30	17.2 ± 0.45	18.6 ± 0.33	21.3 ± 0.45	21.9 ± 0.45	18.5 ± 0.00	17.5± 0.27

Table : 5 cont'

EFEESR	10	16.2 ± 0.01	14.2 ±0.55	12.0 ±0.35	12.5 ±0.978	11.0 ± 0.77	9.00 ±0.023
	20	19.1 ± .041.	18.9 ± 0.79	14.5 ±0.34	13.0 ±1.2	10.2 **± 0.021**	10.00 ±0.47
	30	20.3 ± 0.88	20.0± 0.33	21.7 ± 0.44	18.5 ± 0.72	18.5 ± 0.87	17.5 ± 0.27
EFEEDT	10	17.2 ± 0.12	14.2 ±0.55	18.0 ±0.35	18.5 ±0.978	17.0 ± 0.77	15.0 ±0.023
	20	18.1 ± .44	18.9 ± 0.79	20.5 ±0.34	21.0 ±1.2	18.2 **± 0.021**	18.0 ±0.47
	30	21.3 ± 0.88	21.0± 0.33	22.1 ± 0.10	21.5 ± 0.72	24.5 ± 0.87	21.5 ± 0.32

Where,

PEFEESR-Petroleum ether fraction of ethanolic extracts of *Shorea robusta*. PEFEEDT- Petroleum ether fraction of ethanolic extracts of *Dipterocarpus turbinatus* CFEESR- Chloroform fraction of ethanolic extracts of *Shorea robusta*. CFEEDT- Chloroform fraction of ethanolic extracts of *Dipterocarpus turbinatus EAFEESR*- Ethylacetate fraction of ethanolic extracts of *Shorea robusta; EAFEEDT*- Ethylacetate fraction of ethanolic extracts of *Dipterocarpus turbinatus, EFEESR*-Ethanolic fraction of ethanolic extracts of *Shorea robusta*, *EFEEDT*- Ethanolic fraction of ethanolic extract of *Dipterocarpus turbinatus*.

Table 6: Determination of MIC for the isolated fractions (turbidimetric method) from ethyl acetate fraction of ethanolic extract of *Shorea robusta* and *Dipterocarpus turbinatus*.

Name of the fractions	Name of the organism					
	P. aeruginosa	*S. aureus*	*E. coli*	*E. faecalis*	*C. albicans*	*A. niger*
	(µg/mL)	(µg/mL)	µg/mL)	µg/mL)	(µg/mL)	(µg/mL)
SR C	50	50	25	12.5	12.5	25
SR D	50	50	12.5	12.5	25	25
SR E	25	50	25	25	25	12.5
SR F	100	100	50	50	50	50
DT B	50	50	50	25	6.25	50
DT C	50	100	50	25	12.5	50
DT D	100	100	50	50	6.25	100
DT E	100	50	100	50	0.4	50
DT F	100	100	50	50	50	50
Ciprofloxacin(10µg)	4	2	2	2	_____	_____
Fluconazole (30µg:	_____	_____	_____	_____	16	8

Where MIC, minimum inhibitory concentration.

carried out for the extracts and isolated fractions. Entire isolated fraction shows significant activity against bacterial and fungal pathogens, but all of our isolates show more effective against gram negative bacteria and against *C.albicans*

CONCLUSION

Our study shows the both extract and isolated fraction of *Shorea robusta* and *Diptercarpus turbinatus* shows promising antimicrobial activity against bacterial and fungal pathogen. Further study is require in molecular level to confirm the mechanism and find out the structure of the isolated compound.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

$CHCl_3$: Chloroform; EtOAc- Ethyl acetate; **Mg:** Milligram; **mL:** Milliliter; **W/W:** Weight by weight; **%:** Percentage; **MIC:** Minimum inhibitory concentration.

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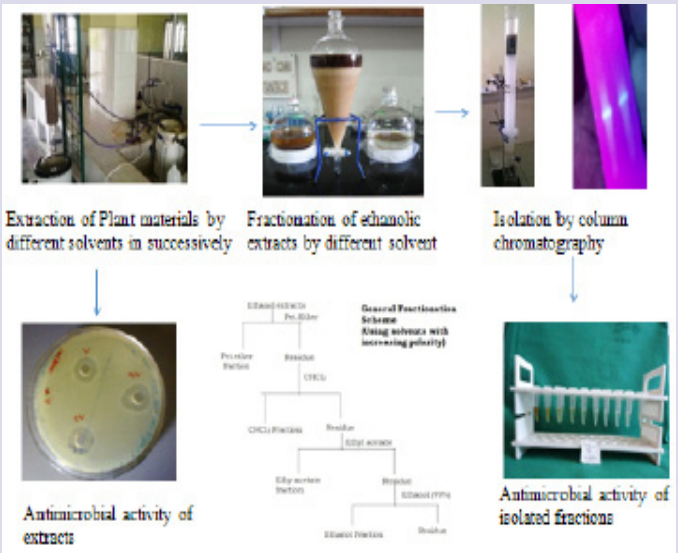
PICTORIAL ABSTRACT



SUMMARY

- Extraction of the plant materials (*Shorea robusta and Dipterocarpus turbinatus*) by using different solvent in successive manner.
- Antimicrobial activity for the different extract was done and ethanolic extract was found to be more active.
- Fractionation of the ethonlic extract was done using separating funnel with different solvent and performs antimicrobial activity for the different fractions.
- Isolation of the different fraction was done form the ethylacetate fraction of ethnolic extract of *Shorea robusta* and *Dipterocarpus turbinatus* and studied the antimicrobial activity by turbidimetric method.