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# Ethnic Knowledge to Combat Fungal & Bacterial Endocarditis in Neonates- Exploring Aptness of Some Indigenous Plants from Kandhamal, Odisha

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**ABSTRACT**

Infective endocarditis in neonates after surgery or due to other hospital related intervention is a rare but serious infection of the heart lining or valves usually caused by bacteria or fungus entering the bloodstream and attaching to the heart. Our intention in this research work is to explore anti-microbial potential of some rare plants from tribal belts of Kandhamal, Odisha against these life-threatening micro-organisms which affect immuno-compromised patients. We have selected four plants (Cayratia trifolia, Sesbania grandiflora, Cordia dichotoma, and Teprosia purpurea) mostly used by Kutia Kandhas of Kandhamal region found in the hill areas of eastern ghat region. We have carried out efficacy of various extracts of these plants against gram (+) ve bacteria (St. aureus, St. viridans, St. epidermidis); gram (–ve) bacteria (Sal. Enterica, E. coli, Pr. Vulgaris, P. aeruginosa) and fungi (A. brasiliensis, C. albicans) as per standard protocol. The results were very encouraging as all those solvents extracts collected from specific parts of these plants have shown anti-microbial property among which, medium polar ethyl acetate extracts were considered as most effective against these microbial species responsible for the above disease.

***Keywords:*** *minimum Inhibitory concentration, disc diffusion method, Zone of Inhibition, Cayratia trifolia, Sesbania grandiflora, Cordia dichotoma and Tephrosia purpurea*

## 1. INTRODUCTION

Neonatal endocarditis is a rare but serious health issue in Asian nations, especially because of pre-existing congenital heart abnormalities and central venous catheters, which are key underlying risk factors and frequently have a higher frequency than in Western cultures. Limited access to healthcare, especially in rural regions, might impede early diagnosis and adequate treatment, potentially leading to consequences (Carapetis et al., 2018). Early diagnosis is not always easy because of its clinical manifestations, which resemble sepsis very often; risk factors and presentation may differ little bit depending on the healthcare facility in different parts of Asean sub-continent. (Daher & Berkowitz, 1995).

The various plants have long been used as medicines by the primitive people as well as traditional healers to treat a wide range of human ailments including a majority of microbial infections (Cowan, 1999; Siddique et al., 2021). Plants are the richest sources of various biochemicals exhibiting antimicrobial properties (Singh et al., 2023). The current scenario of increasing awareness in use of traditional medicines derived from plants has been attracting much interest in developing phyto-sources as alternatives to synthetic compounds because of serious side effects occurred by synthetic antibiotics (Anand et al., 2019). Now-a-days, the emergence of drug-resistant bacteria has become a medical catastrophe and so, there is an urgent need to identify and develop newer phyto-drugs for various microbial infections for the control of both the human and animal diseases having manifold advantages like biodegradability, availability, low toxicity and cost effectiveness (Seukep et al., 2023, Urban-Chmiel et al., 2022). When it is the question of fighting with bacteria or fungus related infection and overcoming the development of resistance in these pathogens, phytochemicals have shown promising outcomes (Khameneh et al., 2021).

Our focus was to investigate the role of various solvent extracts of roots of *Cayratia trifolia* (vitaceae), flowers of *Sesbania grandiflora* (Fabaceae), barks of *Cordia dichotoma* (Boraginaceae) and seeds of *Tephrosia purpurea* (Fabaceae) against a wide range of microbes and to evaluate their effective concentrations in inhibition of the selected pathogenic microbes.

## 2. MATERIALS & METHODS

### *Procured Chemicals & micro-organisms:*

Selected **gram (+) bacteria** used in the present study were *Staphylococcus epidermidis*, *Streptococcus viridans*, and *Staphylococcus aureus* whereas *Pseudomonas aeruginosa*, *Salmonella enterica*, *Escherichia coli* and *Prot. Vulgaris* were taken as gram-negetive bacteria. Similarly, we have chosen *Candida albicans* as well as *Aspergillus brasiliensis* as targeted fungi species for our proposed study. All these microbial strains were procured from microbiologics Inc. through Himedia Lab. Ltd. The chemicals, glassware and standard drugs are procured through Thermofischer scientific & Hindusthan chemicals Ltd.

### *Extraction & finalization of suitable plant extracts for formulation*

The finalized four plant parts were collected from Daringibadi and Amapani hill areas of Kandhamal district during rainy season after proper recognition and validation/certification by taxonomist. These dried plant parts were powdered separately, and all the physical evaluations have been completed after which these were successively extracted with different solvents (Pet. ether, Ethyl acetate & methanol) according to polarity. All extracts were encoded as:

(PECT, EECT & MECT) *Cayratia trifolia*

(PESG, EESG & MESG) *Sesbania grandiflora*

(PETP, EETP & METP) *Tephrosia purpurea*

***Where PE: Pet. Ether; EE: Ethyl acetate & ME: Methanol***

All these extracts were separately investigated to establish folklore claim of anti-microbial property by following all *in vitro* study protocol.

### *Stock Solution Preparation*

The items like Sterile graduated pipettes (1 mL to 10 mL sizes), Pasteur pipettes, Sterile capped tubes, distilled microbe-free water, medium for nutrient-broth, standard drug (Chloramphenicol and ketoconazole) in powder form, solvent for making standard drug solution were arranged properly before our study. The test and control organisms' overnight broth culture were used to make the stock solution.

As desired, stock dilutions of concentration 100 & 1000 microgram/litre reference drugs were made from the original stock solution (10 gram/litre). The test tube rack was arranged with 2 rows of 12 sterile-capped tubes containing standard medication solution (in first tube of each row). This was prepared from the prepared stock solution. The content of the universal bottle was carefully mixed well from which 2 mL content was transferred to first tube of above arranged rows. After completion of this initial process, 4 mL broth was poured and mixed to 4 mL remaining content of universal bottle. 2 mL from this mixture was transferred to the adjacent tube in each row. The process of dilution was carried out in the same manner. However, in the final tube of both rows, 2 mL content was present without antibiotics. The test organism content (single drop) of our previously prepared culture was diluted in desired broth (1:1000) which was added further to one row. The control organism of known sensitivity was added to the second row (also diluted). Given that the test mixture comprised $10^6$ organisms/mL, the size of the inoculums had a substantial impact on the test's outcome. After the above steps, all the tubes were passed through the process of incubation for eighteen hours at a standard temperature of 37 degree Celsius. As a benchmark for determining total inhibition, a tube containing two milliliters of broth containing the organism was infected and refrigerated at 4 degrees Celsius for the whole night (Panda et al., 2012; Barry et al, 2004).

***Zone of Inhibition study*** (Arullappan et al., 2009)

We have followed disc diffusion method which is widely used for assessing antimicrobial activity of all the selected plant extracts. The microbial strains were cultured for 24 hours in petri discs with 20 milliliters of agar media. Sterilized filter paper discs (Whatman no.1) were placed on the inoculated agar after being individually impregnated with plant extracts (50-250 mg per mL) and standard drugs (5 to 200 µg/mL) concentrations. A final inoculum of 108 CFU/mL was obtained by adjusting the inoculum size. Both the bacteria and the fungus were incubated for 24 and 72 hours at 37º C, respectively. For assessing anti-microbial potential, the diameter of the inhibitory zone which formed around the disc was measured. The antibacterial and antifungal activity were compared using standard drugs in different concentrations (5 to 200 µg/mL) as positive controls. For every concentration of plant extracts, every test was conducted in triplicate, and the outcomes are shown in tables 1 to 4.

***Minimum inhibitory concentration***

After decanting 75 µl of sterile nutritional broth medium into each well of a sterile 96-well microplate, the dilution susceptibility testing method was employed to calculate the MIC. The highest concentration of plant extract, i.e. 75 µl, was put into the first well. After 75 µl of the mixture was transferred to the second well, the dilution technique went on for the subsequent wells, resulting in a sequence of dilutions of 1/2, 1/4, 1/8, 1/32, 1/64, 1/128, 1/256, 1/512, and 1/1024, respectively. 1.5 µl of the inoculum solution were added to each well. Following a 24-hour incubation period at 37º C, the tubes were inspected for turbidity, which was interpreted as growth, and non-turbidity, which being defined as no growth. The sample's highest dilution (lowest concentration), which displayed clear fluid without the emergence of turbidity, was determined by interpreting the MIC values. Positive controls and solvent blanks were also used. Every test was conducted in triplicate. Table 5 shows the MIC values of all the relevant plant material extracts for all of the microorganisms used (Kowalska-Krochmal & Dudek-Wicher, 2021).

**Table – 1: Data representation of *Cayratia trifolia* root extracts' Zone of Inhibition against specific gram positive, gram negetive bacteria and fungus**

| Conc$^{n}$ | | Gram negetive bacteria | | | Gram positive bacteria | | | | *Fungus* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | ***Staphyloco. epidermidis*** | ***Staphylococcus aureus*** | ***Streptococcus viridans*** | ***Salmonella enterica*** | ***Escherichia coli*** | ***Proteus vulgaris*** | ***Pseudomonas aeruginosa*** | ***Aspergillus brasiliensis*** | ***Candida albicans*** |
| Petroleum ether Extract(PECT) | | | | | | | | | | |
| 50 | mg/mL | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| 100 | | ∞ | 0.7±0.11 | ∞ | 0.4±0.02 | 0.3±0.02 | ∞ | 0.7±0.05 | 0.3±0.02 | 0.2±0.02 |
| 150 | | 0.3±0.12 | 1.3±0.17 | 0.2±0.04 | 1.1±0.05 | 1.5±0.12 | ∞ | 1.8±0.20 | 0.8±0.05 | 0.8±0.14 |
| 200 | | 0.6±0.05 | 3.6±0.18 | 0.7±0.06 | 1.7±0.21 | 2.8±0.22 | 0.6±0.02 | 2.4±0.42 | 1.5±0.16 | 1.6±0.23 |
| 250 | | 1.0±0.08 | 7.7±0.32 | 1.4±0.15 | 2.3±0.26 | 6.1±0.35 | 1.5±0.13 | 4.4±0.15 | 2.8±0.25 | 2.8±0.21 |
| Ethyl acetate Extract (EECT) | | | | | | | | | | |
| 50 | mg/mL | 0.5±0.04 | ∞ | ∞ | ∞ | 2.1±0.13 | ∞ | 0.6±0.04 | 1.9±0.25 | 0.6±0.02 |
| 100 | | 1.6±0.12 | 1.2±0.28 | 0.5±0.06 | 0.8±0.21 | 5.9±0.35 | 0.9±0.04 | 2.8±0.35 | 2.6±0.29 | 3.7±0.23 |
| 150 | | 4.4±0.22 | 3.5±0.12 | 1.3±0.21 | 4.7±0.33 | 8.9±0.68 | 3.2±0.12 | 6.1±0.32 | 5.7±0.41 | 11.9±0.72 |
| 200 | | 11.8±1.12 | 9.0±0.44 | 4.4±0.22 | 8.9±0.52 | 12.3±1.14 | 7.3±0.63 | 9.4±0.64 | 12.3±0.74 | 14.3±1.31 |
| 250 | | **19.5±1.51** | 12.1±1.18 | 7.8±0.15 | 12.5±1.14 | **17.8±1.05** | 13.6±0.9 | 12.5±1.06 | **22.8±2.14** | **27.1±2.35** |
| Methanol Extract (MECT) | | | | | | | | | | |
| 50 | mg/mL | ∞ | ∞ | ∞ | ∞ | 0.4±0.03 | ∞ | ∞ | 0.4±0.03 | 0.2±0.01 |
| 100 | | 0.3±0.05 | ∞ | ∞ | 0.2±0.02 | 1.1±0.14 | ∞ | 1.2±0.12 | 0.7±0.04 | 1.2±0.05 |
| 150 | | 0.8±0.12 | 0.4±0.15 | 0.4±0.06 | 0.9±0.03 | 2.5±0.24 | 0.3±0.2 | 2.7±0.26 | 2.1±0.15 | 3.8±0.21 |
| 200 | | 3.4±0.16 | 2.6±0.25 | 1.6±0.17 | 2.7±0.15 | 4.9±0.32 | 2.4±0.22 | 4.2±0.26 | 2.9±0.19 | 6.9±0.28 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 250 | | 7.2±0.26 | 6.6±0.32 | 3.5±0.12 | 4.3±0.22 | 7.2±0.73 | 4.9±0.32 | 7.3±0.24 | 5.4±0.27 | 9.4±0.73 |
| | | Chloramphenicol | | | | | | Ketoconazole | | |
| 5 | µg/mL | 4.5±0.12 | 15.8±1.22 | 9.5±0.22 | 20.7±1.78 | 19.7±2.24 | ∞ | 10.7±1.42 | 6.8±0.05 | 17.9±1.26 |
| 25 | | 11.7±0.26 | 23.6±1.36 | 15.2±1.29 | 25.6±1.75 | 26.9±2.65 | 0.2±0.02 | 15.8±1.29 | 13.9±1.32 | 18.7±1.24 |
| 50 | | 16.3±1.32 | 26.8±2.12 | 23.4±1.54 | 31.9±2.97 | 33.5±2.89 | 5.3±0.34 | 23.9±1.62 | 21.5±1.45 | 29.8±1.66 |
| 100 | | 20.3±1.55 | 31.5±2.48 | 28.6±2.37 | 35.4±2.68 | 38.9±3.04 | 8.4±0.79 | 27.8±2.17 | 26.4±1.87 | 36.1±1.89 |
| 200 | | 27.7±2.62 | 37.7±2.33 | 35.5±2.27 | 42.8±3.54 | 42.7±2.69 | 14.9±1.0 | 30.6±2.43 | 32.2±2.06 | 42.2±2.26 |

*Mean ± SEM, Study conducted in triplicate. '∞': Inhibition not observed*

**Table – 2: Data representation of *Sesbania grandiflora* flower extracts' Zone of Inhibition against specific gram positive, gram negetive bacteria and fungus**

| Concⁿ | | Gram( +) ve bacteria | | | Gram( -) ve bacteria | | | | *Fungus* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | ***Staphyloco. epidermidis*** | ***Staphylococcus aureus*** | ***Streptococcus viridans*** | ***Salmonella enterica*** | ***Escherichia coli*** | ***Proteus vulgaris*** | ***Pseudomonas aeruginosa*** | ***Aspergillus brasiliensis*** | ***Candida albicans*** |
| Petroleum ether Extract(PESG) | | | | | | | | | | |
| 50 | mg/mL | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| 100 | | 0.4±0.02 | 0.2±0.02 | 0.4±0.03 | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| 150 | | 1.1±0.11 | 0.8±0.06 | 1.2±0.08 | 0.4±0.03 | 0.7±0.13 | 0.2±0.01 | 0.5±0.12 | 0.7±0.02 | 0.2±0.01 |
| 200 | | 2.5±0.24 | 3.4±0.17 | 2.6±0.26 | 2.4±0.16 | 2.3±0.18 | 0.9±0.04 | 2.4±0.26 | 2.1±0.14 | 0.8±0.13 |
| 250 | | 3.6±0.45 | 8.2±0.72 | 4.1±0.49 | 5.3±0.24 | 5.7±0.29 | 2.7±0.31 | 6.9±0.45 | 3.2±0.32 | 1.3±0.15 |
| Ethyl acetate Extract (EESG) | | | | | | | | | | |
| 50 | mg/mL | 0.2±0.05 | ∞ | ∞ | ∞ | 0.9±0.14 | ∞ | ∞ | 1.6±0.21 | ∞ |
| 100 | | 2.4±0.14 | 0.6±0.03 | ∞ | 0.3±0.02 | 4.2±0.17 | 0.3±0.06 | 1.2±0.08 | 2.3±0.29 | 3.2±0.24 |
| 150 | | 3.8±0.31 | 2.4±0.12 | 0.5±0.11 | 3.8±0.23 | 7.4±0.25 | 2.6±0.17 | 3.5±0.15 | 4.9±0.38 | 12.2±0.63 |
| 200 | | 8.9±0.75 | 7.6±0.24 | 3.9±0.26 | 7.8±0.41 | 11.9±0.44 | 5.9±0.38 | 7.9±0.28 | 11.7±0.87 | 16.2±0.82 |
| 250 | | **16.6±1.12** | 10.3±0.83 | 8.2±0.52 | 11.3±0.93 | **17.6±0.08** | 12.4±0.8 | 10.3±0.68 | **23.4±2.65** | **27.2±1.94** |
| Methanol Extract (MESG) | | | | | | | | | | |
| 50 | | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| 100 | | ∞ | ∞ | ∞ | ∞ | 0.6±0.0 | 0.5±0.0 | 0.8±0.15 | 0.4±0.01 | 0.4±0.02 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | mg/mL | | | | | 2 | 3 | | | |
| 150 | | 0.7±0.08 | ∞ | ∞ | 0.6±0.04 | 1.7±0.14 | 1.3±0.11 | 2.4±0.17 | 1.6±0.12 | 2.9±0.23 |
| 200 | | 2.4±0.23 | 1.2±0.13 | 0.7±0.21 | 3.9±0.31 | 2.7±0.26 | 3.4±0.26 | 3.8±0.32 | 2.2±0.17 | 5.3±0.29 |
| 250 | | 6.9±0.37 | 5.4±0.29 | 2.8±0.37 | 5.8±0.42 | 5.4±0.28 | 6.2±0.28 | 7.4±0.36 | 6.8±0.24 | 11.8±0.72 |
| | | | | Chloramphenicol | | | | Ketoconazole | | |
| 5 | µg/mL | 4.9±0.52 | 15.9±1.23 | 9.4±0.96 | 20.7±1.78 | 19.7±2.24 | ∞ | 10.7±1.42 | 6.8±0.05 | 17.9±1.26 |
| 25 | | 11.8±0.97 | 23.7±1.85 | 15.1±1.26 | 25.6±1.75 | 26.9±2.65 | 0.2±0.02 | 15.8±1.29 | 13.9±1.32 | 18.7±1.24 |
| 50 | | 16.4±1.47 | 26.4±2.02 | 23.7±1.82 | 31.9±2.97 | 33.5±2.89 | 5.3±0.34 | 23.9±1.62 | 21.5±1.45 | 29.8±1.66 |
| 100 | | 20.4±1.64 | 31.2±2.57 | 28.9±2.32 | 35.4±2.68 | 38.9±3.04 | 8.4±0.79 | 27.8±2.17 | 26.4±1.87 | 36.1±1.89 |
| 200 | | 27.9±2.68 | 37.3±2.41 | 35.5±2.94 | 42.8±3.54 | 42.7±2.69 | 14.9±1.0 | 30.6±2.43 | 32.2±2.06 | 42.2±2.26 |

*Mean ± SEM, Study conducted in triplicate. '∞': Inhibition not observed*

**Table – 3: Data representation of *Cordia dichotoma* bark extracts' Zone of Inhibition against specific gram positive, gram negetive bacteria and fungus**

| Conc$^{n}$ | | Gram( +) ve bacteria | | | Gram( -) ve bacteria | | | | *Fungus* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | ***Staphyloco. epidermidis*** | ***Staphylococcus aureus*** | ***Streptococcus viridans*** | ***Salmonella enterica*** | ***Escherichia coli*** | ***Proteus vulgaris*** | ***Pseudomonas aeruginosa*** | ***Aspergillus brasiliensis*** | ***Candida albicans*** |
| Petroleum ether Extract(PECD) | | | | | | | | | | |
| 50 | mg/mL | ∞ | - | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| 100 | | ∞ | 0.3±0.02 | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| 150 | | ∞ | 0.8±0.01 | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | 0.2±0.03 |
| 200 | | ∞ | 1.4±0.23 | ∞ | 0.2±002 | 0.3±0.04 | ∞ | 0.2±0.04 | 1.3±0.14 | 0.6±0.13 |
| 250 | | 0.4±0.02 | 1.7±0.28 | 0.2±0.01 | 0.4±0.03 | 0.4±0.07 | 0.4±0.05 | 0.6±0.13 | 2.8±0.31 | 1.4±0.23 |
| Ethyl acetate Extract (EECD) | | | | | | | | | | |
| 50 | mg/mL | 0.5±0.03 | 4.2±0.13 | ∞ | ∞ | 1.2±0.14 | ∞ | 0.7±0.06 | 0.7±0.04 | ∞ |
| 100 | | 2.3±0.13 | 8.3±0.62 | ∞ | 0.8±0.16 | 3.9±0.27 | 0.7±0.13 | 2.9±0.25 | 2.9±0.27 | ∞ |
| 150 | | 5.8±0.26 | 14.7±0.63 | ∞ | 2.8±0.25 | 7.2±0.35 | 2.5±0.22 | 6.4±0.38 | 6.7±0.35 | 0.5±0.04 |
| 200 | | 11.2±0.49 | 18.6±1.06 | 0.3±0.05 | 5.7±0.34 | 10.5±0.39 | 8.9±0.32 | 9.8±0.36 | 14.3±0.75 | 3.2±0.17 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 250 | | **16.4±0.95** | **21.6±1.68** | 2.4±0.13 | 10.8±0.35 | **18.2±0.22** | **14.9±1.02** | **15.1±0.35** | **20.8±1.93** | 5.4±0.23 |
| Methanol Extract (MECD) | | | | | | | | | | |
| 50 | mg/mL | ∞ | 1.2±0.12 | ∞ | ∞ | 0.4±0.07 | ∞ | 0.3±0.04 | ∞ | ∞ |
| 100 | | ∞ | 2.7±0.15 | ∞ | ∞ | 0.9±0.15 | 0.2±0.02 | 1.6±0.18 | ∞ | ∞ |
| 150 | | 0.3±0.02 | 7.4±0.26 | ∞ | 0.7±0.05 | 1.8±0.28 | 0.7±0.15 | 2.4±0.22 | 1.5±0.24 | ∞ |
| 200 | | 1.1±0.12 | 9.2±0.53 | ∞ | 2.8±0.26 | 2.3±0.27 | 3.4±0.27 | 4.1±0.27 | 3.7±0.46 | 0.1±0.02 |
| 250 | | 1.9±0.23 | 12.6±0.83 | 0.8±0.14 | 4.3±0.42 | 5.8±0.34 | 5.9±0.32 | 5.6±0.32 | 6.1±0.62 | 0.7±0.03 |
| | | Chloramphenicol | | | | | | Ketoconazole | | |
| 5 | µg/mL | 4.9±0.52 | 15.9±1.23 | 9.4±0.96 | 17.3±1.26 | 20.7±1.78 | 19.7±2.24 | - | 6.8±0.05 | 17.9±1.26 |
| 25 | | 11.8±0.97 | 23.7±1.85 | 15.1±1.26 | 22.8±1.93 | 25.6±1.75 | 26.9±2.65 | 0.2±0.02 | 13.9±1.32 | 18.7±1.24 |
| 50 | | 16.4±1.47 | 26.4±2.02 | 23.7±1.82 | 27.5±2.36 | 31.9±2.97 | 33.5±2.89 | 5.3±0.34 | 21.5±1.45 | 29.8±1.66 |
| 100 | | 20.4±1.64 | 31.2±2.57 | 28.9±2.32 | 30.8±2.15 | 35.4±2.68 | 38.9±3.04 | 8.4±0.79 | 26.4±1.87 | 36.1±1.89 |
| 200 | | 4.9±0.52 | 15.9±1.23 | 9.4±0.96 | 38.4±2.59 | 42.8±3.54 | 42.7±2.69 | 14.9±1.06 | 32.2±2.06 | 42.2±2.26 |

*Mean ± SEM, Study conducted in triplicate. '∞': Inhibition not observed*

**Table –4: Data representation of *Tephrosia purpurea* seeds extracts' Zone of Inhibition against specific gram positive, gram negetive bacteria and fungus**

| Conc$^{n}$ | | Gram( +) ve bacteria | | | Gram( -) ve bacteria | | | | *Fungus* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | ***Staphyloco. epidermidis*** | ***Staphylococcus aureus*** | ***Streptococcus viridans*** | ***Salmonella enterica*** | ***Escherichia coli*** | ***Proteus vulgaris*** | ***Pseudomonas aeruginosa*** | ***Aspergillus brasiliensis*** | ***Candida albicans*** |
| Petroleum ether Extract(PECT) | | | | | | | | | | |
| 50 | mg/mL | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| 100 | | ∞ | 0.4±0.67 | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| 150 | | ∞ | 0.9±0.12 | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | 0.2±0.16 |
| 200 | | 0.2±0.22 | 1.5±0.21 | ∞ | 0.1±08 | 0.3±0.76 | ∞ | 0.2±0.04 | 1.3±0.11 | 0.6±0.10 |
| 250 | | 1.1±0.72 | 1.9±0.22 | 0.3±0.16 | 0.5±0.01 | 0.4±0.03 | 0.4±0.08 | 0.6±0.13 | 2.8±0.20 | 1.4±0.22 |
| Ethyl acetate Extract (EECT) | | | | | | | | | | |
| 50 | mg/ | 0.9±0.18 | 4.3±0.17 | ∞ | ∞ | 1.2±0.12 | ∞ | 0.7±0.06 | 0.7±0.09 | ∞ |
| 100 | | 2.5±0.16 | 8.5±0.41 | ∞ | 0.7±0.1 | 3.9±0.2 | 0.7±0.1 | 2.9±0.25 | 2.9±0.12 | ∞ |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | mL | | | | 2 | 7 | 0 | | | |
| 150 | | 6.6±0.22 | 14.2±0.28 | ∞ | 2.9±0.22 | 7.2±0.38 | 2.5±0.65 | 6.4±0.38 | 6.7±0.28 | 0.5±0.08 |
| 200 | | 10.7±0.42 | 18.1±1.05 | 0.5±0.27 | 6.3±0.67 | 10.5±0.31 | 8.9±0.33 | 9.8±0.36 | 14.3±0.15 | 3.2±0.11 |
| 250 | | **15.8±0.95** | **21.6±1.68** | 2.7±0.16 | 10.4±0.02 | **18.1±0.22** | **14.1±1.13** | **15.0±0.05** | **21.9±1.22** | 5.4±0.32 |
| Methanol Extract (MECT) | | | | | | | | | | |
| 50 | mg/mL | ∞ | 1.5±0.18 | ∞ | ∞ | 0.4±0.02 | ∞ | 0.3±0.04 | ∞ | ∞ |
| 100 | | ∞ | 2.6±0.33 | ∞ | ∞ | 0.9±0.14 | 0.2±0.14 | 1.6±0.18 | ∞ | ∞ |
| 150 | | 0.7±0.07 | 7.7±0.28 | ∞ | 0.9±0.052 | 1.8±0.25 | 0.7±0.17 | 2.4±0.22 | 1.5±0.16 | ∞ |
| 200 | | 1.4±0.18 | 9.5±0.17 | 0.2±0.32 | 3.8±0.17 | 2.3±0.21 | 3.4±0.09 | 4.1±0.27 | 3.7±0.25 | 0.1±0.21 |
| 250 | | 2.1±0.48 | 13.6±0.25 | 0.9±0.54 | 5.3±0.26 | 5.8±0.30 | 5.9±0.1 | 5.6±0.32 | 6.1±0.44 | 0.7±0.05 |
| | | Chloramphenicol | | | | | Ketoconazole | | | |
| 5 | µg/mL | 5.1±0.42 | 14.9±1.33 | 8.9±0.26 | 21.7±1.21 | 19.7±2.22 | ∞ | 10.7±1.42 | 6.8±0.08 | 17.9±1.18 |
| 25 | | 10.3±0.22 | 24.7±1.61 | 15.5±1.03 | 26.6±1.27 | 26.9±2.34 | 0.2±0.17 | 15.8±1.29 | 13.9±1.19 | 18.7±1.08 |
| 50 | | 16.5±1.40 | 29.4±2.09 | 22.9±1.72 | 32.9±2.07 | 33.5±2.17 | 5.3±0.25 | 23.9±1.62 | 21.5±1.37 | 29.8±1.16 |
| 100 | | 20.4±1.64 | 34.2±1.27 | 27.9±2.68 | 34.4±2.33 | 38.9±3.23 | 8.4±0.28 | 27.8±2.17 | 26.4±1.65 | 36.1±1.24 |
| 200 | | 27.9±2.68 | 38.3±1.08 | 34.5±2.11 | 41.8±3.45 | 42.7±2.72 | 14.9±1.06 | 30.6±2.43 | 32.2±2.12 | 42.2±2.12 |

*Mean ± SEM, Study conducted in triplicate. '∞': Inhibition not observed*

**Table – 5: Minimum inhibitory concentration data compilations of various solvent extracts against selected micro-organisms**

| Micro-organisms | *Cayratia trifolia* (Root) | | | *Sesbania grandiflora* (Flower) | | | *Cordia dichotoma* (Bark) | | | *Tephrosia purpurea* (Seed) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **PECT** | **EECT** | **MECT** | **PESG** | **EESG** | **MESG** | **PECD** | **EECD** | **MECD** | **PETP** | **EETP** | **METP** |
| Gm. +ve Bacteria | | | | | | | | | | | | |
| *Staphylo. epidermidis* | 18.75 | 12.5 | 25 | **12.5** | 12.5 | **18.75** | 31.25 | 0.78 | 18.75 | **12.8** | 13.0 | **17.95** |
| *Staphylo. aureus* | 12.5 | 12.5 | 18.75 | **12.5** | 12.5 | 50 | **6.25** | **0.39** | **3.12** | **13.5** | 13.5 | 48 |
| *Strepto. viridans* | 37.5 | 25 | 18.75 | 25 | 37.5 | 50 | 62.5 | 6.25 | 31.25 | 25 | 37.5 | 49 |
| Gm. -ve Bacteria | | | | | | | | | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Salmonella enterica* | 25 | 12.5 | 25 | 37.5 | 12.5 | 18.75 | **12.5** | 1.56 | 9.37 | 37.5 | 12.5 | 17.75 |
| *Escherichia coli* | 25 | **6.25** | 12.5 | 18.75 | **3.12** | **12.5** | **12.5** | **0.78** | **3.12** | 18.75 | **3.12** | **13.15** |
| *Proteus vulgaris* | 25 | **6.25** | 18.75 | 37.5 | 6.25 | **12.5** | 62.5 | 1.56 | 12.5 | 37.5 | 6.25 | **12.76** |
| *Pseudo. aeruginosa* | **12.5** | 12.5 | **6.25** | 18.75 | 6.25 | **12.5** | **12.5** | 3.12 | **3.12** | 18.75 | 6.25 | **12.52** |
| *Fungi* | | | | | | | | | | | | |
| *Aspergillus brasiliensis* | **12.5** | **0.78** | 6.25 | 18.75 | **0.78** | 6.25 | **6.25** | **0.78** | **9.37** | 18.75 | **0.78** | 6.80 |
| *Candida albicans* | **12.5** | 6.25 | 12.5 | 18.75 | **0.78** | **3.12** | 18.75 | 18.75 | 12.5 | 18.75 | **0.78** | **3.45** |

## 3. RESULT & DISCUSSION

### *Zone of inhibition study outcome*

For ethyl acetate extracts of specific plant components, the zone of inhibitions (ZOI) was shown to be at its greatest. The following was the highest ZOI found for the ethyl acetate plant extracts of all four plant species against the aforementioned microorganisms:

Regarding inhibition of gram-positive microbes, The EA extract of *C. trifolia* demonstrated a maximum inhibition of 19.5 mm against *Staphylococcus epidermidis* at 250 mg/mL conc$^{n}$, which was comparable to the activity of standard chloramphenicol in its prescribed dose.

Likewise, in regard to gram-negative bacteria, it showed maximum inhibition of 17.8 mm in a concentration of 250mg/mL against *Escherichia coli* which was comparable to the inhibition incurred in the concentration of 5µg/mL of Chloramphenicol. All the two used fungi, namely, *Aspergillus brasiliensis* and *Candida albicans* incurred maximum inhibition of 22.8mm and 27.1mm respectively by the EA extract of *C. trifolia* at 250mg concentration. This result was quite similar significantly as typical inhibitions caused by ketoconazole at 50 µg/mL.

Regarding the inhibition of gram-positive microbes, ethyl acetate extract of *S. grandiflora* demonstrated a maximum inhibition of 16.6 mm against *Staphylococcus epidermidis* at 250 mg/mL dose, which is equivalent to the inhibition observed at 50 µg/mL conc$^{n}$ of the common medication, chloramphenicol,

Similarly, in case of gram-negative microbes, it showed maximum inhibition of 17.6 mm in a concentration of 250 mg/mL against Escherichia *coli* which was more or less comparable to the inhibition incurred by chloramphenicol at its lowest dose concentration. In case of fungi, this extract showed maximum inhibition of 23.4mm against *Aspergillus brasiliensis* and 27.2 mm against *Candida albicans* which were comparable to the inhibitions incurred by 50 µg/mL .ketoconazole.

250 mg/mL dose of ethyl acetate extract of ***Cordia dichotoma*** displayed proper ZOI i.e. 21.6 & 16.6 mm. against *St. aureus* and *Staphylococcus epidermidis* respectively which were comparable with the inhibitions incurred by the standard drug, Chloramphenicol at its lowest dose i.e. 25/50 µg/mL.

Almost all the gram-negative bacteria (excluding *Sal. enterica*), were also better inhibited at 250 mg/mL of the EACD extract to 18.2 mm, 14.9 mm, and 15.1 mm, respectively. The inhibition results were comparable to those obtained at 5, 200 and 25 µg/mL concentrations of chloramphenicol.

Maximum zones of inhibition noted in case of EECD bark extract at 250 mg dose against the fungi, *Aspergillus brasiliensis* i.e. 20.8 mm, which were comparable with that of inhibitions resulted in case of ketoconazole at concentration of 50 microgram.

Maximum inhibitions of 22.6 mm and 15.8 mm were demonstrated by ethyl acetate extract of *Tephrosia purpurea* seeds at a concentration of 250 mg/mL against *Staphylococcus aureus* and *Staphylococcus epidermidis*, respectively. These inhibitions were comparable to those caused by the standard medication, chloramphenicol, at concentrations of 25 and 25 µg/mL, respectively.

Similarly, all gram-negative microbes *Pseudo. aeruginosa*, *Proteus vulgaris* and *E. coli*, were better inhibited in 250mg/mL concentration of the EA extract to an extent of 15 mm, 14.1mm and 18.1 mm respectively and the inhibition results were well comparable with the inhibitions incurred by 25, 200 & 5 micrograms of Chloramphenicol. At 250 mg/mL of the ethyl

acetate extract of Cordia dichotoma bark, the maximum zones of inhibition seen against the fungus *Aspergillus brasiliensis* were 21.9 mm. These zones of inhibition were comparable to those obtained with 50 µg/mL concentrations of the common medication ketoconazole.

***Minimum Inhibitory Concentration (MIC)***

In the inhibition study of various extracts against the gram-positive microbes, the lowest MIC were observed in almost all the ethyl acetate extracts with an exception of only pet. ether extract of *C. trifolia*. PECT & EECT had the lowest MIC. In case of *S. grandiflora*, pet. Ether extract (PESG) had the lowest MIC of 12.5 against both *Staphylococcus epidermidis* and *Staphylococcus aureus*. In the case of *C. dichotoma*, the EECD, PECD and MECD had the lowest MIC of 0.39, 6.25 and 3.12 respectively against *Staphylococcus aureus*. Similarly, in the case of *T. purpurea*, petroleum ether extract (PETP) has also shown moderate MIC of 13.5 & 12.8 against *Staphylococcus aureus* and *Staphyloco. epidermidis.*

During observation regarding the lowest MIC of plant extracts towards gram negative microbes, the Pet. Ether extract of *C. trifolia* had 12.5 against *P. aeruginosa*, Ethyl acetate extract (EECT) had 6.25 against both *E. coli* and *P. vulgaris* and MECT had 6.25 against *P. aeruginosa*. The Pet. Ether extract of *S. grandiflora* had 12.5 against *V. cholera*, Ethyl acetate extract (EESG) had 3.12 against *E. coli* and Methanolic extract (MESG) had 12.5 towards all taken gram-negative bacteria except *S. enterica*. The Pet. Ether extract (PECD) of *C. grandiflora* had 12,5 towards all taken gram-negative bacteria except *P. vulgaris* whereas EECD had 0.78 against *E. coli* and MECD had 3.12 against both *P. aeruginosa* as well as *E. Coli*. The Pet. Ether extract (PETP) of *T. purpurea* had 12,8, 13.5 against all the two staphylococcus species.

In the observation of the lowest MIC of plant extracts against fungi, the Pet. Ether extract of *C. trifolia* (PECT) had the lowest MIC of 12.5 both against *A. brasiliensis* and *C. albicans*, the EECT had 0.78 against *A. brasiliensis*; the EESG *had* MIC of 0.78 against *A. brasiliensis* and *C. albicans* and MESG had 3.12 against *C. albicans*. The PECD, EECD and MECD of *Cordia dichotoma* had 6.25, o.78 and 9.37 respectively against fungi, i.e. *A. brasiliensis*. The EETP extract of *Tephrosia purpurea* had MIC of 0.75 against *A. brasiliensis* and *C. albicans* and METP had 3.45 against *C. albicans*.

## 4. CONCLUSION

Neonatal endocarditis is a resilient, non-contagious infection of the heart's valves or lining during stay in hospitals, which is mainly brought on by bacteria, though fungi may also be responsible. Due to seriousness regarding complicacies, treating this infection requires combinations of 2-3 antibiotics along with other supplementary medicines as per situation. The adverse effects of these modern antibiotics are well proven which leads to search for naturally occurring bioactive secondary metabolites. From the above results it can be concluded that our selected plants parts have proved to be potentially effective in controlling microbial infection-related complicacies. However, a proper investigation of the isolated secondary metabolites from these selected plants will be more beneficial in developing promising safe alternatives while dealing with endocarditis.

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