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EFFICACY OF ANTI-BACTERIAL ACTION ON SEVEN MEDICINAL PLANTS EXTRACT AGAINST NEONATAL SEPSIS CAUSING BACTERIA-AN IN VITRO STUDY

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Abstract

The use of plant parts and their yield as therapeutic purpose was found from the starting evolution of human being. Medicinal plants, according to World Health Organization, are the finest resource to get a wide range of newer herbal drugs. During the last few decades, the demand of plant originated remedy has been growing rapid all around the humankind. The study was done to screen antibacterial properties of aqueous extract of seven Nepalese medicinal plants against eight isolated blood pathogens which be able to further used to develop new herbal medicines with having antimicrobial properties higher. *Azadiracta indica* (Leaf) (Dohroo, A. *et al.,* 2016), *Tinospora cordifolia* (Leaf), *Punica granatum* (Rind), *Syzygium cumini* (leaf), *Moringa oleifera* (Leaf), *Nyctanthes arbortristis* (Leaf), and *Swertia chirata* (Whole plant) were used in this study. Extract of plants were evaluated against eight isolated pathogens for antibacterial activity by agar well diffusion method. The strongest antibacterial activity was found with *Syzygium cumini* leaf extract against *S. aureus* (30mm), Coagulase Negative Staphylococci (20mm) and *Streptococcus* spp. (20mm) whereas *Punica granatum* rind extract showed strongest antibacterial activity for CONS(20mm), *Streptococcus* spp.(20mm), *E. coli*(16mm) and *Enterobacter* spp.(22mm). *T. cordifolia, N. arbortristis, M. oleifera* leaf extract revealed antibacterial activity, strongest against *P. aeruginosa* (20mm) only. No any plant extracts exposed antibacterial activity for *Proteus* spp. *A. indica* and *S. chirata* extract was ineffective against the tested isolates. Significant antibacterial activity was observed with aqueous extract of *P. granatum* and *S. cumini* against both gram positive and negative (Pseudomonas *aeruginosa*) isolate contributing broad-spectrum activity.

***Keywords*:** Medicinal Plants, Antibacterial, Aqueous, Neonatal sepsis

Introduction

The utilization of plant parts and their yield as herbal medicines was found from starting evolution of human being. "Rig-Veda", thought to be the oldest depository of knowledge for human being in medicinal uses of plants which has been written between 4500-1600 B.C. (Rastogi and Mehrotra, 2002). Medicinal plants, according to World Health Organization, are the finest resource to obtain a wide range of newer herbal drugs Chhikara *et al*., 2018) During the last few decades, the demand of plant originated remedy has been growing rapid all around the globe (Sathiyaraj *et al.*, 2010; Kaur *et al.,* 2016; Singh and Quraishi, 2015; Hantsu *et al.,* 2017). Phytochemical compounds contain in medicinal plants give specific physiological activities on the body of human being due to the presence of bioactive compounds (secondary metabolites) like alkaloids, flavonoids, phenols, quinines, tannins, coumarins, terpenoids, steroids (Khurana and Gajbhiye, 2013; Panghal *et al.*, 2019; Edoga *et al.*, 2005; Priya and Singh 2012; Verma *et al.,* 2015). The concentrations of bioactive compounds may vary with different plants and its parts which result in distinctive medicinal properties of particular plant and their parts (Kaur *et al.,* 2014; Richard *et al.*, 2013; Arora *et al.,* 2013; Kaur & Shantanu, 2015). Pandey & Kaur, 2018). Medical uses of different medicinal plant range from the administration of the root, barks, stems, leaves, flower, seeds or whole plant to the utilize of extract and decoction from the plant parts (Ogbulie *et al.*, 2007; Kaur *et al.*, 2016 and Kumar *et al.,* 2017).

Growing antibiotic resistance to microorganism has created interest globally among researchers for evaluation of different medicinal plants for its antibacterial activities to overcome this problem. Drug resistance in pathogenic microorganisms is supposed to be emerged due to unsystematic use of commercially synthesized drugs having antibacterial property. Various properties of medicinal plants make interest worldwide study that has increased during the last few decades rapidly due to properties for antibacterial and antioxidant activities, low toxic effect and the cheaper alternative to expensive commercial drugs (Chew *et al.*, 2012; Priadarshini *et al.*, 2013). Due to the antimicrobial resistance it is challenge for protection and cure of an rapidly coming out of infections caused by viruses, bacteria, parasites and fungi (Farjana *et al.*, 2014). The leading causes of death with infectious disease by emerging multi resistant pathogens worldwide accountable for 68% of all deaths in 2012 (Kher and Chaurasia, 1997; who, 2000). Therefore, it is highly imperative to screen antimicrobial properties of different medicinal plants which can be further used to make new drug with more effective having antimicrobial potential (Jassal and Thambyrajah, 2018).

This study was done to evaluate the antibacterial efficacy in familiar medicinal plants *Azadiracta indica*, *Tinospora cordifolia, Punica granatum*, *Syzygium cumini, Moringa oleifera, Nyctanthes arbortristis,* and *Swertia chirata.*

Materials and Methods

Bacterial cultures

In the current study Bacterial cultures were used of clinical isolates from the cases of neonatal sepsis, collected from National Medical College and Teaching Hospital, Birgunj, Nepal. The bacterial isolate contain of five Gram negative bacterial isolates namely *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Enterobacter* spp., *Proteus* spp. and three Gram positive bacterial isolates namely *Staphylococcus aureus*, Coagulase negative Staphylococci (CONS) and *Streptococcus* spp..

Maintenance of bacterial cultures

The bacterial isolates were sub cultured regularly and maintained in Nutrient agar slant and stored at 4°C., the bacterial cultures were refreshed during all the experiments of this study.

Collection of plant materials

From different geographical regions of Nepal fresh and disease free plant's parts were collected Presented in Table 1. The plants collected were authenticated by Dr. Yogesh Tiwari, Department of Drabya guna, Nepal Ayurved Medical College and Teaching Hospital, Birgunj, Nepal. The collected plant materials were carefully washed under running tap water and shade dried. Then plant materials were subjected to drying at 37°C in a hot air oven for 3-4 hours with intermittent turning the material to avoid burning. The dried plant's parts were crushed by hand then crushed in mixer grinder to coarse powder. The crushed powder were sieved and then stored in airtight plastic bags at room temperature for further extraction process.

Table 1: Medicinal Plants Utilized in this Study

S.No.	Medicinal Plant	Family	Parts collected	Place of Collection	Common Name
1.	*Azadiracta indica*	Meliaceae	Leaves	Bara	Neem
2.	*Moringa oleifera*	Moringaceae	Leaves	Parsa	Shajan
3.	*Nyctanthes arbortristis*	Oleaceae	Leaves	Parsa	Ratorani
4.	*Punica granatum*	Punicaceae	Rind	Parsa	Anar
5.	*Swertia chirata*	Gentianaceae	Leaves, Stem, Root	Nawalprasi	Chiraito
6.	*Syzygium cumini*	Myrtaceae	Leaves	Parsa	Jamun
7.	*Tinospora cordifolia*	Menispermaceae	Leaves	Parsa	Giloy

Laboratory Procedure

Crude extraction

Fifty gram of each coarsely powdered Plant materials was macerated in 300ml of aqueous solvent for a period of 7 days with intermittent shaking. Initially contents of flask were filtered by fold of four layers of muslin cloth and then throughout Whatman filter paper No. 1. In hot air oven at 40°C the filtrate was evaporated. The weight of residues was obtained and stored at 4°C for further use in experiments. The percentage yields of the crude extract were illustrated in figure-1. Residues were dissolved in sterile distilled water and Dimethyl sulfoxide (DMSO) at different concentrations (25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml) for antibacterial effect.

Inoculums preparation by growth method:

5 ml of autoclaved Brain heart infusion (BHI) broth was taken and 3-5 well isolated colonies was inoculated into it, followed by incubated at 37°C for 1 h. Turbidity was adjusted to equivalent of approximately 1–2 × 10^8 colony forming units per ml (CFU/ml). The whole preparation was done according to guidelines of Clinical And Laboratory Standards Institute (CLSI) (CLSI, 2006).

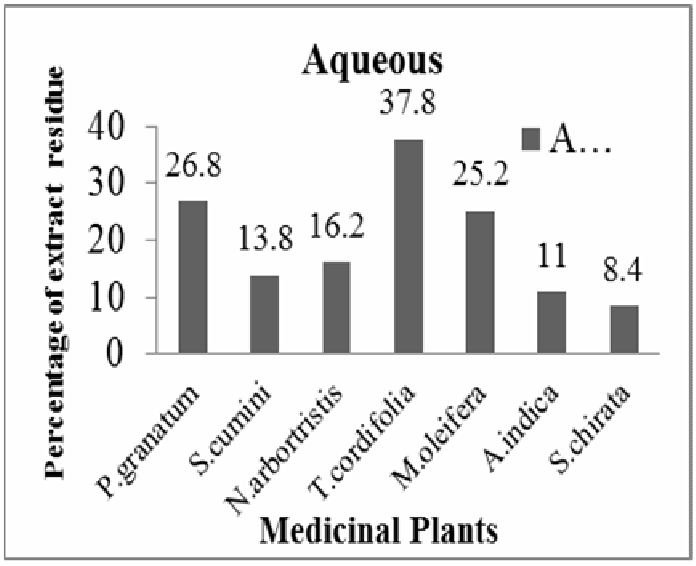

Fig. 1: Percentage yield of Plant Extracts

Antibacterial assay by Agar well diffusion method:

Table 2: Antibacterial efficacy of aqueous extracts of different medicinal plants against bacterial Pathogens (Zone Of Inhibition of Growth in mm including well diameter, average of 4 readings)

Medicinal Plants	Extract Concentration	*S. aureus* [ZOI(mm)]	CONS [ZOI(mm)]	*Streptococcus* spp. [(ZOI)mm]	*K. pneumoniae* [ZOI(mm)]	*E .coli* [ZOI (mm)]	*P. aeruginosa* [ZOI(mm)]	*Enterobacter* spp. [ZOI(mm)]	*Proteus* spp. [ZOI(mm)]
Punica granatum (Rind)	25 mg/ml	12	14	0	0	0	10	13	0
	50mg/ml	14	15	11	0	0	13	16	0
	100mg/ml	15	16	13	0	10	15	18	0
	200mg/ml	17	20	20	0	16	17	22	0
Syzygium cumini	25 mg/ml	13	14	0	0	0	0	0	0
	50mg/ml	25	15	0	0	0	0	0	0
	100mg/ml	27	17	18	0	0	12	0	0
	200mg/ml	30	20	20	0	0	14	0	0
Tinospora cordifolia	25 mg/ml	0	0	0	0	0	0	0	0
	50mg/ml	0	0	0	0	0	16	0	0
	100mg/ml	0	0	0	10	0	18	0	0
	200mg/ml	0	0	0	12	12	20	10	0
Nyctanthes	25 mg/ml	0	10	0	0	0	12	0	0

arbortristis	50mg/ml	0	12	0	0	0	14	10	0
	100mg/ml	12	13	0	0	0	18	12	0
	200mg/ml	14	14	0	0	0	20	14	0
Azadiracta indica	25 mg/ml	0	0	0	0	0	0	0	0
	50mg/ml	0	0	0	0	0	0	0	0
	100mg/ml	0	0	0	0	0	12	0	0
	200mg/ml	0	0	0	0	0	14	0	10
Moringa oleifera	25 mg/ml	0	0	0	0	0	0	0	0
	50mg/ml	0	0	0	0	0	0	0	0
	100mg/ml	0	0	0	0	0	16	0	0
	200mg/ml	0	10	14	10	10	20	9	0
Swertia chirata	25 mg/ml	0	0	0	0	0	0	0	0
	50mg/ml	0	0	0	0	0	0	0	0
	100mg/ml	0	0	0	0	0	0	0	0
	200mg/ml	0	0	0	0	0	0	0	0
Negative control	Distilled water	0	0	0	0	0	0	0	0
Positive control	VA-30mcg	17	17	22	NT	NT	NT	NT	NT
	TGC-15mcg	NT	NT	NT	19	19	25	19	25

[ZOI: Zone of Inhibition, NT: Not Tested, CONS: Coagulase negative Staphylococcus]

For the evaluation of antibacterial activity agar well-diffusion method was used of different concentration relevant well of different concentration. 100 µl of aqueous solvent was poured in well as negative control whereas vancomycin (30mcg/disc) and Tigecycline e seven plants. Mueller-Hinton agar (MHA) plates were prepared, properly labeled and inoculated the aliquot (0.5 McFarland standard bacterial suspension) with sterile swab stick under laminar air flow hood following aseptic conditions. After thirty minutes, five equidistant wells were made in each agar plate with sterilized cork borer of 8 mm diameter, and the agar plugs removed with a sterile forceps. By the use of micropipette, 100 µl of test solution was kept into (15mcg/disc) was used as positive control for gram positive and gram negative isolates respectively.

Each test was duplicated for each bacterial strain. The culture plates were then incubated at 37 °C, and the results were observed after 24hr. The clear zone around each well was measured in mm using ruler in 4 directions and average was calculated. An agar well (8mm) having no clear zone of inhibition (ZOI) was considered as null antibacterial activity. The well having ZOI ≥14mm was regarded as sensitive in case of gram positive bacteria and ZOI ≥15mm was interpretated as sensitive in cases of gram negative bacteria.

Results and Discussion

Presence of bioactive compounds or secondary metabolites in plant, make it having the activity of antibacterial. The secondary metabolites are for the self defense to the plants themselves against bacterial, fungal and viral infections. The results obtained for the antibacterial test performed on different concentration of medicinal plants are presented in Table (2). The results of present study are encouraging because two out of the seven plants showed highly significant antibacterial potential against all gram positive isolates (Figure 2) and three gram negative (Figure 3) isolates (except *Klebsiella pneumoniae* and *Proteus spp.*) showing broad spectrum action .

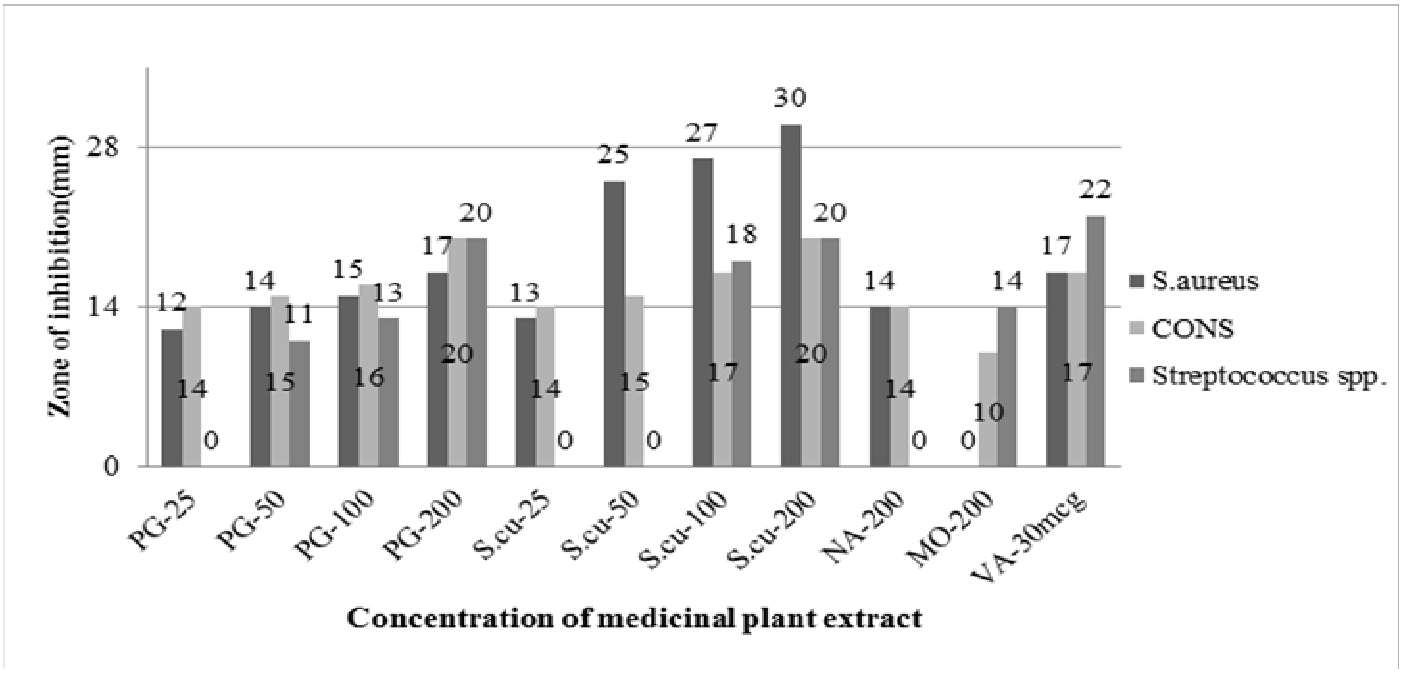

Fig. 2: Plant Extract having antibacterial activity (ZOI≥14) against Gram positive bacteria. [PG-*Punica granatum*: S.cu-*Syzygium cumini*: NA-*Nyctanthes arbortristis*: VA-Vancomycin: ZOI-Zone of Inhibition]

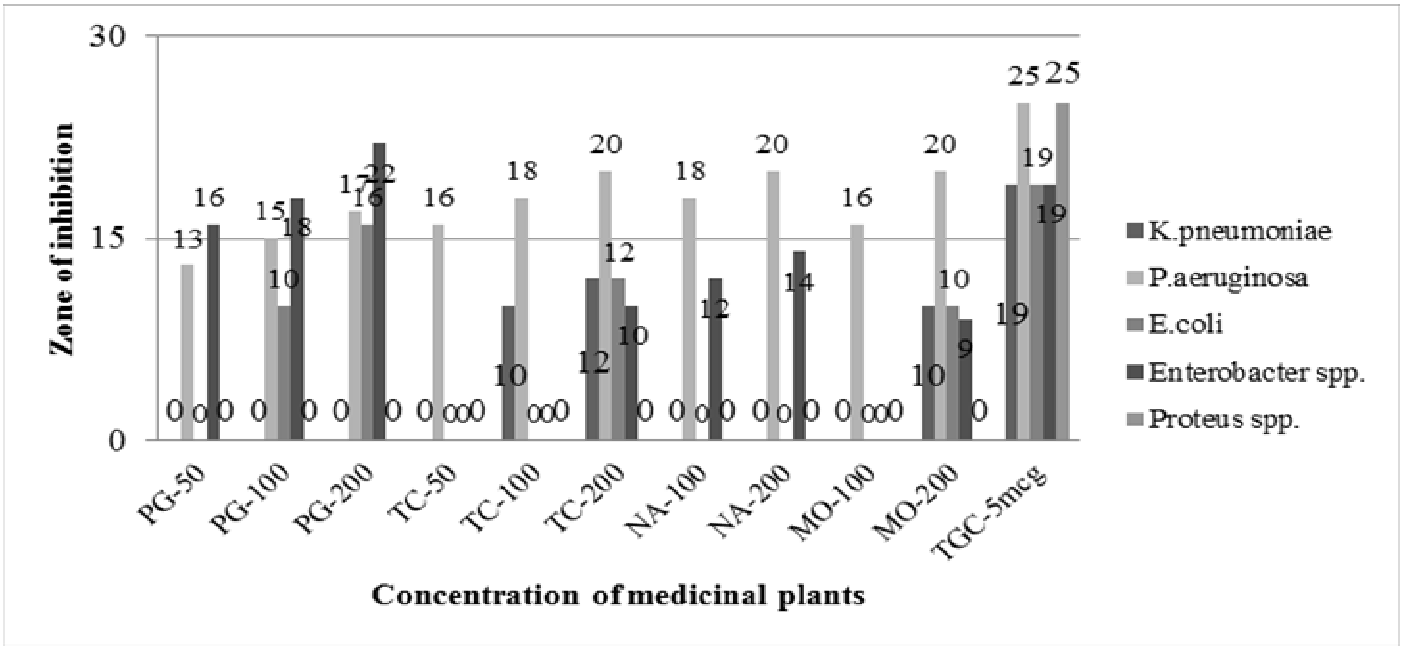

Fig. 3 : Plant Extract shown antibacterial activity (ZOI≥15) against Gram negative bacteria.[PG: *Punica granatum*, TC: *Tinospora cordifolia*, NA: *Nyctanthes arbortristis*, MO: *Moringa oleifera*, TGC: Tigecycline, ZOI: Zone of Inhibition]

The aqueous extract showed maximum inhibitory effect only on *Staphylococcus aureus* (30mm), followed by *CONS* (20mm), *Streptococcus spp.*(20mm), *Pseudomonas aeruginosa* (20mm) *and Enterobacter spp.*(22mm) and moderate antibacterial effect against *Escherichia coli (16mm)* whereas resistance towards *Klebsiella pneumoniae*(12mm) and no inhibitory effect on *Proteus* spp.. The strongest antibacterial activity was found with *Syzygium cumini* leaf extract against *S. aureus* (30mm), CONS (20mm) and *Streptococcus* spp. (20mm)whereas, *Punica granatum* rind extract showed strongest antibacterial activity for CONS(20mm), *Streptococcus* spp.(20mm), *E. coli*(16mm) and *Enterobacter* spp.(22mm) Figure-4. *T. cordifolia, N. arbortristis, M. oleifera* leaf extract revealed antibacterial activity, strongest against *P. aeruginosa* (20mm) only. No any plant extracts showed antibacterial activity for *Proteus* spp., *A. indica* and *S. chirata* extract was unable to show antibacterial activity against the tested isolates.

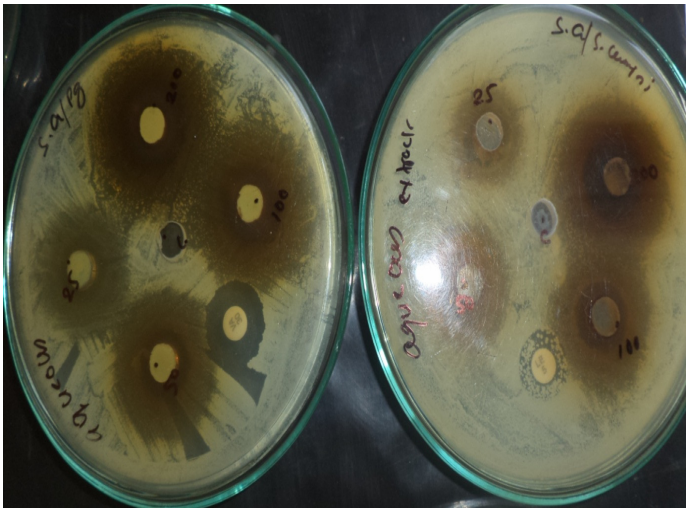

Fig. 4: Antibacterial effect (ZOI) of *Punica granatum* (pg) and *S. cumini* against *S. aureus* (s.a)

In the current investigation, the *Punica granatum* rind extract exhibited high degree of inhibitory activity against most of the eight tested organism (except *K. pneumoniae* and *Proteus* spp.) followed by *S. cumini* leaf extract. *Punica granatum* rind extract exerted significant antibacterial activity against tested pathogens at concentration of 50,100 and 200mg/ml. However it is ineffective against *K. pneumoniae* and *Proteus spp*. under above concentration. But it has shown strongest antibacterial activity for *Enterobacter* spp.(22mm). Likewise *S. cumini* leaf also shown significant antibacterial activity against tested pathogens at highest concentration of 200mg/ml. but it is ineffective against *K. pneumoniae*, *Proteus* spp., *E. coli* and *Enterobacter* spp. under above concentration. Significantly, it has shown strongest antibacterial activity against *S. aureus* (30mm) than other extract (Singh *et al.,* 2018).

T. cordifolia, *N. arbortristis*, *M. oleifera* leaf extract showed strongest antibacterial activity against *P. aeruginosa* (20mm) at its highest concentration of 200mg/ml. *N. arbortristis* and *M. oleifera* leaf extract showed intermediate antibacterial effect against *S. aureus*, CONS, *Enterobacter* spp. and *Streptococcus* spp. respectively. Other isolates were ineffective for this extract. Whereas *T. cordifolia* leaf extract showed insignificant antimicrobial activity against all isolate except *P. aeruginosa*.

Prevalence of antibiotic resistance against pathogenic bacteria has been increasing since last few decades. Eventually it has been increasing occurrence of infectious diseases in developed as well as developing countries and has raised the curiosity for the researchers to search for new anti-bacterial component to cure from the various diseases caused by multidrug resistance pathogenic bacteria. There are various reasons for development of multidrug resistance like chromosomal mutations, plasmids, transposons, and integrons. Multidrug-resistant organisms once established persist and spread globally, leads to failures in the management of different type of infections. To cure from the infections caused by multidrug-resistant organisms natural antimicrobial agents are one of the choices in front of us.

According to previous reports, aqueous extracts of *Syzygium cumini*, *T. cordifolia*, *N. arbortristis*, *M. oleifera*, *A. indica leaves*, *S. chirata* whole plant and *Punica granatum* rind demonstrated antimicrobial activity against different microorganisms.

Earlier Studies established antibacterial effect of aqueous leaf extract of *Syzygium cumini* against gram positive bacteria *S. aureus* (Chanudom *et al.*, 2014; Tahir *et al.*, 2012) and *Streptococcus mutans* (Tahir *et al.*, 2012). Similar findings have been noticed in this study as it was found effective against *S. aureus* and *Streptococcus* spp.. Despite this, aqueous leaf extract of *Syzygium cumini* was found effective against CONS.

A study (Tahir *et al.*, 2012) showed antibacterial effect of aqueous leaf extract of *Syzygium cumini* against gram negative bacteria *E. coli* and *P. aeruginosa.* In contrast to this finding, this study demonstrated leaf extract of *Syzygium cumini* ineffective against the gram negative bacterial (*Klebsiella pneumoniae, E. coli, P. aeruginosa, Enterobacter* spp., *Proteus* spp.) isolates from neonates (Digvijay & Bhardwaj 2017).

In previous studies, aqueous extract of *Punica granatum* rind showed antibacterial effect against gram positive bacteria *S. aureus* (Hagir *et al.*, 2016; Khan *et al.*, 2014; Malviya *et al.*, 2014; Ali *et al.*, 2011; Chebaibi *et al.*, 2013; Mahajan *et al.*, 2014; Banu *et al.,* 2017) along with gram negative bacteria *P. aeruginosa* (Hagir *et al.*, 2016; Ali *et al.*, 2011; Chebaibi *et al.*, 2013; Mahajan *et al.*, 2014), *K. pneumoniae* (Khan *et al.*, 2014; Malviya *et al.*, 2014; Mahajan *et al.*, 2014), *Enterobacter* spp. (Malviya *et al.*, 2014), *E. coli* (Hagir *et al.*, 2016; Khan *et al.*, 2014; Mahajan *et al.*, 2014) and *Proteus vulgaris* (Hagir *et al.*, 2016). The findings were comparable to this study as *P. granatum* rind extract showed antibacterial effect for *S. aureus* and gram negative bacteria *E. coli, P. aeruginosa* and *Enterobacter* spp.. The antibacterial effect of *P. granatum* rind extract in this study was found insensitive against some gram negative bacteria (*K. pneumoniae* and *Proteus* spp.). This finding was similar to a previous study (Chebaibi *et al.*, 2013). In addition, *P. granatum* rind extract was found sensitive for CONS and *Streptococcus* spp. in this study.

Aqueous leaf extract of *T. cordifolia* showed sensitive only for *P. aeruginosa*. Similar finding was traced in previous studies (Patil *et al.*, 2017; Santhi and Nelson, 2013; Farooq and Koul, 2019). A study (Mohana *et al.*, 2008) revealed least antibacterial activity of aqueous leaf extract of *T. cordifolia* against *Klebsiella pneumoniae* and *E. coli.* Comparable findings were noticed in this study. Some studies showed aqueous leaf extract of *T. cordifolia* resistance towards gram negative bacteria *P. vulgaris* (Santhi and Nelson, 2013) *E.* coli (Patil *et al.*, 2017; Santhi and Nelson, 2013) and *E. aerogens* (Patil *et al.*, 2017). This study also demonstrated aqueous leaf extract of *T. cordifolia* resistance against *Proteus* spp. In addition, resistance was also noticed against *S. aureus*, *Streptococcus* spp., CONS; and least antibacterial activity against *Enterobacter* spp. in this study.

Aqueous extract of *Nyctanthes arbortristis* leaf showed antibacterial activity against gram positive bacteria *S. aureus* (Jain and Singh, 2013; Geetha *et al.*, 2014), *K. pneumoniae* Geetha *et al.*, 2014, *E. coli* (Jain and Singh, 2013), *P. aeruginosa* (Jain and Singh, 2013), *P. vulgaris* (Jain and Singh, 2013) and *P. mirabilis* (Geetha *et al.*, 2014) in earlier research works. A previous research established lesser antibacterial activity against *E. coli*, *K. pneumoniae*, and *P. aeruginosa* (Senthilnath *et al.*, 2013). This study showed antibacterial effect of aqueous extract of *Nyctanthes arbortristis* leaf against gram positive bacteria (*S. aureus*, and CONS) and gram negative bacteria(*P. aeruginosa*) while *Enterobacter* spp. was least sensitive and Other isolates (*Streptococcus* spp., *Klebsiella pneumoniae*, *E. coli*, *Proteus* spp.) were resistant.

The past studies showed antibacterial effect of aqueous extract of A. indica leaf against gram positive bacteria *S. aureus* (Farjana *et al.*, 2014; Patil *et al.*, 2017; Gupta *et al.*, 2013), *Streptococcus mutans* (Nayak *et al.*, 2011; Bhuva and Dixit, 2015), and gram negative bacteria *E. coli* (Gupta *et al.*, 2013), *P. aeruginosa* (Patil *et al.*, 2017; Bhuva and Dixit, 2015), and *Proteus vulgaris* (Patil *et al.*, 2017). Some of the researchers have found moderate activity with *E. coli* (Farjana *et al.*, 2014) and *Klebsiella* spp (Farjana *et al.*, 2014). The finding of resistance towards *E. coli* (Khan *et al.*, 2014; Patil *et al.*, 2017), *Enterobacter aerogens* (Patil *et al.*, 2017), *K. pneumoniae* (Khan *et al.*, 2014), *S. aureus* (Khan *et al.*, 2014) resembles with this study. The finding from this study also showed least antibacterial activity on *P. aeruginosa* and *Proteus* spp., while resistance towards *Streptococcus* spp. and CONS (Chakarborty *et al.,* 2015). Study carried out on aqueous extract of *M. oleifera* leaf in earlier period exhibited antibacterial effect for both gram positive *S. aureus* (Rajamanickam and Sudha, 2013; Osman *et al.*, 2015; Peixoto *et al.*, 2011, Kumar *et al.*, 2017) and gram negative bacteria *K. pneumoniae* (Osman *et al.*, 2015), *E. coli* (Osman *et al.*, 2015), *P. aeruginosa* (Rajamanickam and Sudha, 2013) indicates broad spectrum activity. Another study demonstrated no effect against *E. coli* (Rajamanickam and Sudha, 2013, Kaur, *et al.*, 2016), *K. pneumoniae* (Rajamanickam and Sudha, 2013), *Proteus* spp. (Rajamanickam and Sudha, 2013) and *P. aeruginosa* (Osman *et al.*, 2015). In this study aqueous extract of *M. oleifera* leaf was highly active on *P. aeruginosa* and least active on CONS, *Streptococcus* spp. *K. pneumoniae*, *E. coli*, *Enterobacter* spp., represents wide range of activity (Singh *et al.*, 2018)

Prior research exhibited antibacterial activity of Aqueous extract of *S. chirata* whole plant on *S. aureus* (Malik *et al.*, 2011; Khalid *et al.*, 2011), *E. coli* (Malik *et al.*, 2011; Ahirwal *et al.*, 2011) whereas least antibacterial effect against *K. pneumoniae* and *S. aureus* in another research (Roy *et al.*, 2015). This study resembles many studies as it did not show antibacterial effect against *K. pneumoniae* (Malik *et al.*, 2011), *P. aeruginosa* (Khalid *et al.*, 2011; Ahirwal *et al.*, 2011), *S. aureus* (Ahirwal *et al.*, 2011), *S. pyogenes* (Ahirwal *et al.*, 2011), *P. mirabilis* (Ahirwal *et al.*, 2011), *E. coli* (Roy *et al.*, 2015).

In the present study, aqueous extract of selected medicinal plants exhibited the antibacterial activity in the order of *P. granatum*> *S. cumini*> *N. arbortristis*> *M. oleifera*> *T. cordifolia* >*A. indica* but *S. chirata* does not showed antibacterial activity to any of the isolated bacteria. This study also showed *P. granatum* and *S. cumini* exhibited antibacterial effect on the Gram-positive bacteria than Gram-negative bacteria may be due to the diversity in morphological symphony between Gram-positive and Gram-negative bacteria.

Gram negative bacteria contain lipopolysaccharide in their cell wall causing the impermeability to chemical substances having antimicrobial property. The Gram-positive bacteria composed peptidoglycan in cell wall, which makes more permeable to substances that have antibacterial potential than lipopolysaccharide layer of cell wall of gram negative bacteria. Gram-negative bacteria have complex cell wall composition than Gram positive bacteria. For this reason, Gram-positive bacteria are more vulnerable to chemical substances with antibacterial potential than Gram negative bacteria (Chanda and Baravalia, 2010, Jamatia *et al.,* 2017).

The solvent used for the extraction determine which compounds are extracted during the extraction procedure. In the traditional medicine solvent used primarily is water, either in boiled, hot or cold form. Water is the most polar solvent because of it extract the polar compounds during extraction which have ability to spread and liquefy in different culture media used in the study. Plants having null antibacterial potential does not denote that the bioactive compounds are not present in the plant Negative antibacterial activity may be presence of insufficient quantities of antimicrobial substances.

Plants with antimicrobial potential have bioactive phytochemical components such as phenolic acids, tannins, coumarins, flavonoids, alkaloids, quinines and terpenoids (Cowan, 1999). The Presence of such bioactive compound in these medicinal plants makes it antibacterial in nature.

Conclusions

The results obtained in the present investigation showed very significant antibacterial activity of aqueous extract of *P. granatum* and *S. cumini* against both gram positive (*Staphylococcus aureus*, *Streptococcus* spp. and CONS) and gram negative(*Pseudomonas aeruginosa*) neonatal sepsis causing bacteria. From this study result it is secure that these extracts can confidently inhibit the growth of isolated pathogens thereby prevent the blood pathogens and provide safe, easy, effective and practical solution to find out bioactive natural compound that may provide as basic source for the development of new antimicrobial product to defeat the problem of neonatal death due to bacterial sepsis as well as to defeat the emerging resistance strain.

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