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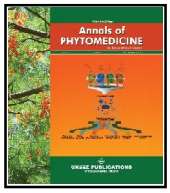

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In vitro antibacterial activity of leaves extract of *Eucalyptus globulus* Labill. and *Tamarindus indica* L. against *Rhodococcus equi*

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Abstract

The present study was carried out at ICAR, NRCE, EPC, Bikaner to evaluate *in vitro* antibacterial activity of ethanolic, chloroformic and SEWE extracts of *Eucalyptus globulus* Labill. and *Tamarindus indica* L. leaves against virulence associated protein A (VapA) gene and virulence associated protein C (VapC) gene positive *Rhodococcus equi*. Ethanolic extracts of these plant leaves showed good *in vitro* antibacterial activity, whereas chloroformic extracts showed no *in vitro* antibacterial activity against *R. equi* using the disc diffusion method. Sequentially extracted water extract (SEWE) of these plant leaves showed good antibacterial activity against *R. equi* using disc diffusion and agar well diffusion methods. Further, sequentially solvent based fractionation of SEWE of these plant leaves in ethanol, methanol and water showed good antibacterial activity against *R equi.* On phytochemical analysis, the most active fraction, water soluble fraction (WSF) of SEWE of *E. globulus* leaves was found positive for catechol tannins, phenolic compounds, saponins and carbohydrates, whereas the most active fraction, ethanol soluble fraction (ESF) of SEWE of *T. indica* leaves was found positive for catechol tannins, phenolic compounds, flavones and carbohydrates. In comparison with currently used antibiotics (azithromycin and rifampicin), the required concentration of the leaves extract of these plants was too high for their possibilities of *in vivo* use. Most effective herbal fractions, WSF of SEWE of *E. globulus* leaves and ESF of SEWE of *T. indica* leaves have shown their minimum inhibitory concentration (MIC) at 3.19 mg/ml and 4.81 mg/ml, respectively. However, the abundant availability of *T. indica* and *E. globulus* plants in India and their antibacterial activity against *R. equi* suggest their potential for use as a disinfectant against *R. equi*.

1. Introduction

Rhodococcus equi is a gram-positive, pleomorphic rod-shaped bacteria, commonly found in soil. It is an important bacterial respiratory pathogen of young foals (Giguère *et al.*, 2011). It is also an important cause of AIDS-associated pneumonia in HIV-infected humans (Kawila *et al.*, 2013). *R. equi* infection causes a subacute or chronic abscessation bronchopneumonia, sometimes with ulcerative typhlocolitis, and may include mesenteric lymphadenitis, osteomyelitis, purulent arthritis, reactive arthritis and ulcerative lymphangitis (Dedar *et al.*, 2017). *R. equi* infection causes tuberculosis-like lesions in the submandibular and other lymph nodes of cattle and pigs whereas, in young goats, granulomatous lesions in the liver are associated with wasting and death (Meijer and Prescott, 2004). *R. equi* is an important cause of foal mortalities and about 17 to 20% of foals are PCR positive on swab sampling from the upper respiratory tract in the studies carried out in Rajasthan and Jammu and Kashmir, respectively (Kumar *et al.*, 2014; Mir *et al.*, 2015). *R. equi* is a facultative intracellular pathogen surviving and replicating in macrophages.

The combination of rifampin and erythromycin is used to treat the disease (Hillidge, 1987; Sweeney *et al.*, 1987). Recently, clarithromycin or azithromycin, newer generation macrolides replaces erythromycin in combination with rifampin (Gigue‘re *et al.*, 2004). Resistant strains of either of these drugs have also been encountered (Asoh *et al.*, 2003; Fines *et al.*, 2001; Gigue‘re *et al.*, 2010; Jacks *et al.*, 2003; Kotze and Eloff, 2002; McNeil and Brown, 1992). It is stated that the increased use of macrolides to control the disease has contributed to the emergence of resistance (Pauw and Eloff, 2014).

The lack of effective alternatives against *R. equi* makes it compulsive to identify novel antimicrobial agents to control and treat *R. equi* infection in foals. Plants have an amazing ability to produce a wide variety of secondary metabolites such as alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinones and coumarins (Bhatt *et al.*, 2019; Bhawana *et al.*, 2021; Das *et al.*, 2010; Desai *et al.*, 2021; Malik *et al.*, 2020; Manju and Pushpa, 2020; Mathew *et al.*, 2019). These biomolecules are the source of plant-derived antimicrobial substances (Bouatrous, 2019; Priyadharshini *et al.*, 2019; Srivastava *et al.*, 2014). Some natural products are highly efficient in the treatment of bacterial infections (Fernebro, 2011).

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Many herb species are now used as traditional medicines for the prevention, treatment and cure of disease and to maintain holistic well-being (Mehrotra, 2021). Many plant-active phytoconstituents are also used for the treatment of multidrug-resistant (MDR) microbial pathogens (Shamna and Poyil, 2021; Seshadri, 2021; Wali *et al.*, 2019).

Bikaner has a large diversity of plant species containing many useful bioactive constituents. In this study, we aimed to investigate the *in vitro* antibacterial activity of leaves extracts of *E. globulus* and *T. indica* against *R. equi*. In the initial screening, extracts showed *in vitro* antibacterial activity against *R. equi*, which could be further exploited for polarity and solubility based fractionation and phytochemical analysis of the most active fractionate.

2. Materials and Methods

In the present study, the research was carried out at ICAR, NRCE, EPC, Bikaner. The plant specimens were identified by the Botanical Survey of India, Arid Zone Regional Centre, Jodhpur, Rajasthan, India as serial number LK-9 *Tamarindus indica* L. (Family: Caesalpiniaceae) and *Eucalyptus globulus* Labill. (Family: Myrtaceae). Fresh leaves of *T. indica* and *E. globulus* were collected manually from the campus of ICAR, NRCE, EPC, Bikaner, dried in a hot air oven at 50°C and ground in a mixer grinder for preparation of the powder for extract. Chemicals and reagents of analytical grade were used for research work. Disc diffusion method (Nostro *et al.*, 2000; Salie *et al.*, 1996) and agar well diffusion method (Irshad *et al.*, 2012) were used to evaluate *in vitro* antibacterial activity of leaves extracts of *E. globulus* and *T. indica* against VapA and VapC positive *R. equi* using Muller Hinton Broth and Muller Hinton HiVeg Agar.

2.1 Method for the initial screening of *in vitro* antibacterial activity of the ethanolic extract against *R. equi*

In the initial screening of the study, plant leaves were extracted using ethanol (Gberikon *et al.*, 2015). 500 ml absolute ethanol (99.9%) was added in 50 g of powder of each plant leaves, incubated overnight at 37°C in a shaker incubator, sonicated in a sonicator and evaporated the filtrate of sonicated extract in the rotary evaporator machine. The weight of the ethanolic extract was measured against absolute ethanol in a similar volume.

2.2 Polarity based fractionation of the active compound

Further fractionation was done using chloroform and water sequential extraction to separate non-polar and polar compounds by using basic principles (Jeyaseelan *et al.*, 2012).

2.2.1 Preparation of non-polar chloroformic extract

500 ml chloroform (99.9% pure) was added in 50 g plant leaves powder in a clean dry glass bottle, incubated overnight at 37°C in a shaker incubator, then filtered and residual supernatant was washed with chloroform 3-4 times until clean chloroform was observed. Evaporated the filtrate in the rotary evaporator machine. Chloroformic extract was weighed against 99.9% pure chloroform in a similar volume.

2.2.2 Preparation of polar sequentially extracted water extract (SEWE)

Chloroformic washed supernatant was spread on the blotting paper for complete drying. 500 ml distilled water was added in dried supernatant, incubated overnight at 37°C in a shaker incubator, sonicated in a sonicator and evaporated the filtrate of the sonicated extract in the rotary evaporator machine. Sequentially extracted water extract (SEWE) was measured against distilled water in the same volume.

2.3 Solubility based fractionations of polar (SEWE)

Solvent based fractionation of polar SEWE was sequentially done with ethanol, methanol and distilled water. Ethanol soluble fraction (ESF), methanol soluble fraction (MSF) and water soluble fraction (WSF) were collected and tested for their antibacterial activity. Fractions showing the most potent antibacterial activity were further analyzed by phytochemical tests.

2.4 Phytochemical analysis

Fractions showing the most potent antibacterial activity were further tested for phytochemical analysis for flavonoids, flavones, alkaloids, catechol tannins, phlobatannins, saponins, carbohydrates, polyphenols and indoles using a standard procedure (Auwal *et al.*, 2014).

2.5 Evaluation of *in vitro* antibacterial activity

Measured the inhibition zone (IZ) diameter to determine the degree of *in vitro* antibacterial activity of plant leaves extract against the *R. equi* were as followings:

Non-active: when IZ diameter is zero

Mild-active: when IZ is < 10 mm diameter

Moderate-active: when IZ is > 10 mm and < 15 mm diameter

Good-active: when IZ is >15 mm diameter

2.6 Control

Azithromycin and rifampicin 10 mg/l were taken as control.

2.7 Polymerase chain reaction (PCR) technique

Pure colony of *R. equi* was procured from NCVTC, NRCE, Hisar and verified time-to-time for purity by using PCR based on pathogenic VapA and VapC genes having 550 base pair and 700 base pair nucleotide sequences, respectively (Chhabra *et al.*, 2015).

3. Results

3.1 PCR technique

By the PCR technique, we obtained the amplified 550 and 700 bp fragments of the *R. equi* pathogenic VapA and VapC genes, respectively (Figure 2). These pathogenic VapA and VapC genes showed the colony of the *R. equi* was pure (Figure 1).

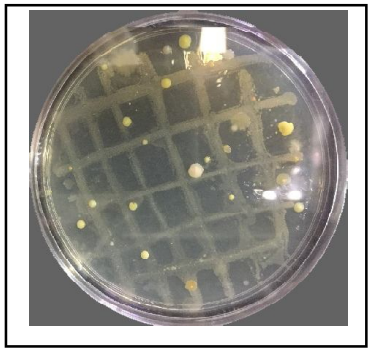


Figure 1: Pure colony of the *R. equi*.

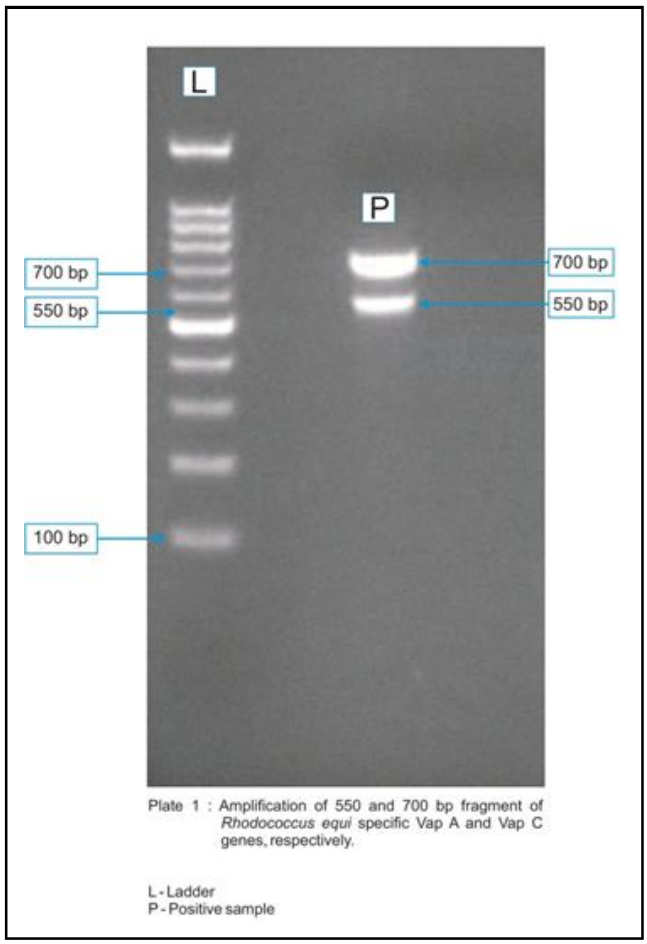


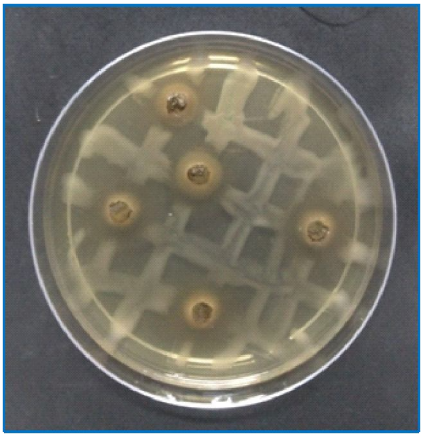
Figure 2: Amplification of 550 and 700 bp fragment of *R. equi* specific VapA and VapC genes, respectively.

3.2 *Eucalyptus globulus* Labill. (Safeda)

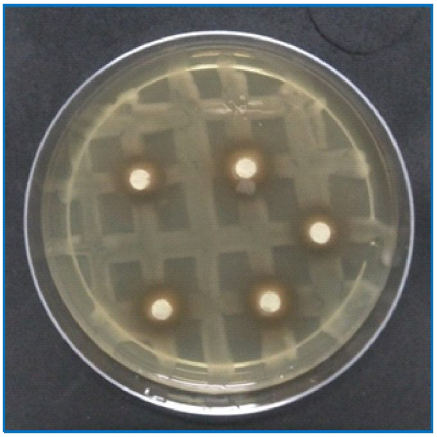
Initial screening of ethanolic extract of leaves of *E. globulus*, further polarity and solubility based fractionation shows the degree of *in vitro* antibacterial activity against *R. equi* in Table 1 and Figure 3 (a to f) and its most active fraction WSF was put on phytochemical analysis and was found positive for having catechol tannins, phenolic compounds, saponins and carbohydrates as shown in Table 2.

Table 1: *In vitro* antibacterial activity of leaves extract and fractions of *E. globulus* against *R. equi*

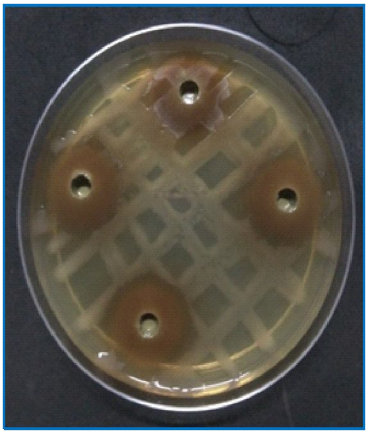
Leaves extract and fraction	Concentration (mg/ml)	Method	Inhibition zone diameter	Degree of *in vitro* antibacterial activity
Ethanolic extract	287.0 mg/ml	Disc diffusion method	16.0 mm	Good
Chloroformic extract	38.4 mg/ml	Disc diffusion method	Zero	None
SEWE	208.8 mg/ml	Disc diffusion method	15.0 mm	Good
SEWE	208.8 mg/ml	Agar well diffusion method	25.0 mm	Good
ESF of SEWE	7.6 mg/ml	Agar well diffusion method	22.0 mm	Good
ESF of SEWE	0.76 mg/ml	Agar well diffusion method	Zero	None
ESF of SEWE	0.38 mg/ml	Agar well diffusion method	Zero	None
MSF of SEWE	26.1 mg/ml	Agar well diffusion method	25.0 mm	Good
MSF of SEWE	2.61 mg/ml	Agar well diffusion method	15.0 mm	Good
MSF of SEWE	1.30 mg/ml	Agar well diffusion method	10.0 mm	Moderate
WSF of SEWE	63.9 mg/ml	Agar well diffusion method	27.0 mm	Good
WSF of SEWE	6.39 mg/ml	Agar well diffusion method	18.0 mm	Good
WSF of SEWE	3.19 mg/ml	Agar well diffusion method	14.0 mm	Moderate



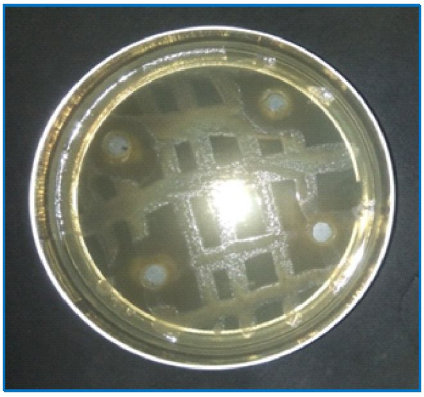
(a) Ethanolic extract showing IZ: 16.0 mm at concentration: 287.0 mg/ml (disc diffusion method)



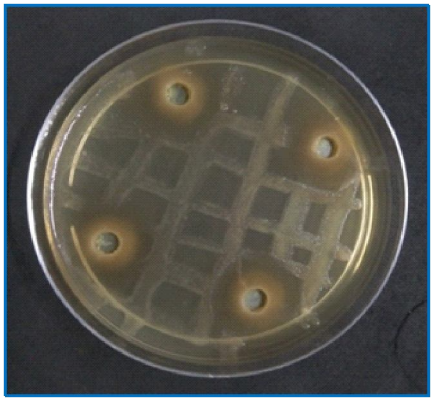
(b) SEWE of leaves showing IZ: 15.0 mm at concentration: 208.8 mg/ml (disc diffusion method)



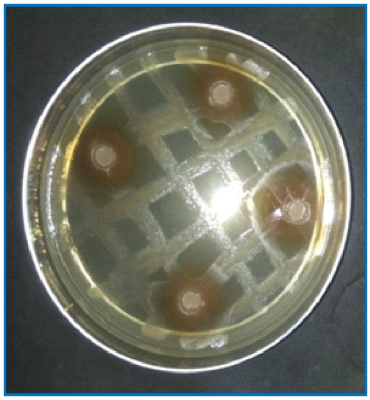
(c) SEWE of leaves showing IZ: 25.0 mm at concentration: 208.8 mg/ml (agar well diffusion method)



(d) ESF of SEWE showing IZ: 22.0 mm at concentration: 7.6 mg/ml (agar well diffusion method)



(e) MSF of SEWE showing IZ: 25.0 mm at concentration: 26.1 mg/ml (agar well diffusion method)



(f) WSF of SEWE showing IZ: 27.0 mm at concentration: 63.9 mg/ml (agar well diffusion method)

Figure 3: ***In vitro*** **antibacterial activity of leaves extract and the fractions of** ***E. globulus*** **against** ***R. equi*** **showing in (a) to (f).**

Table 2: Phytochemical analysis of WSF of SEWE of *E. globulus* leaves

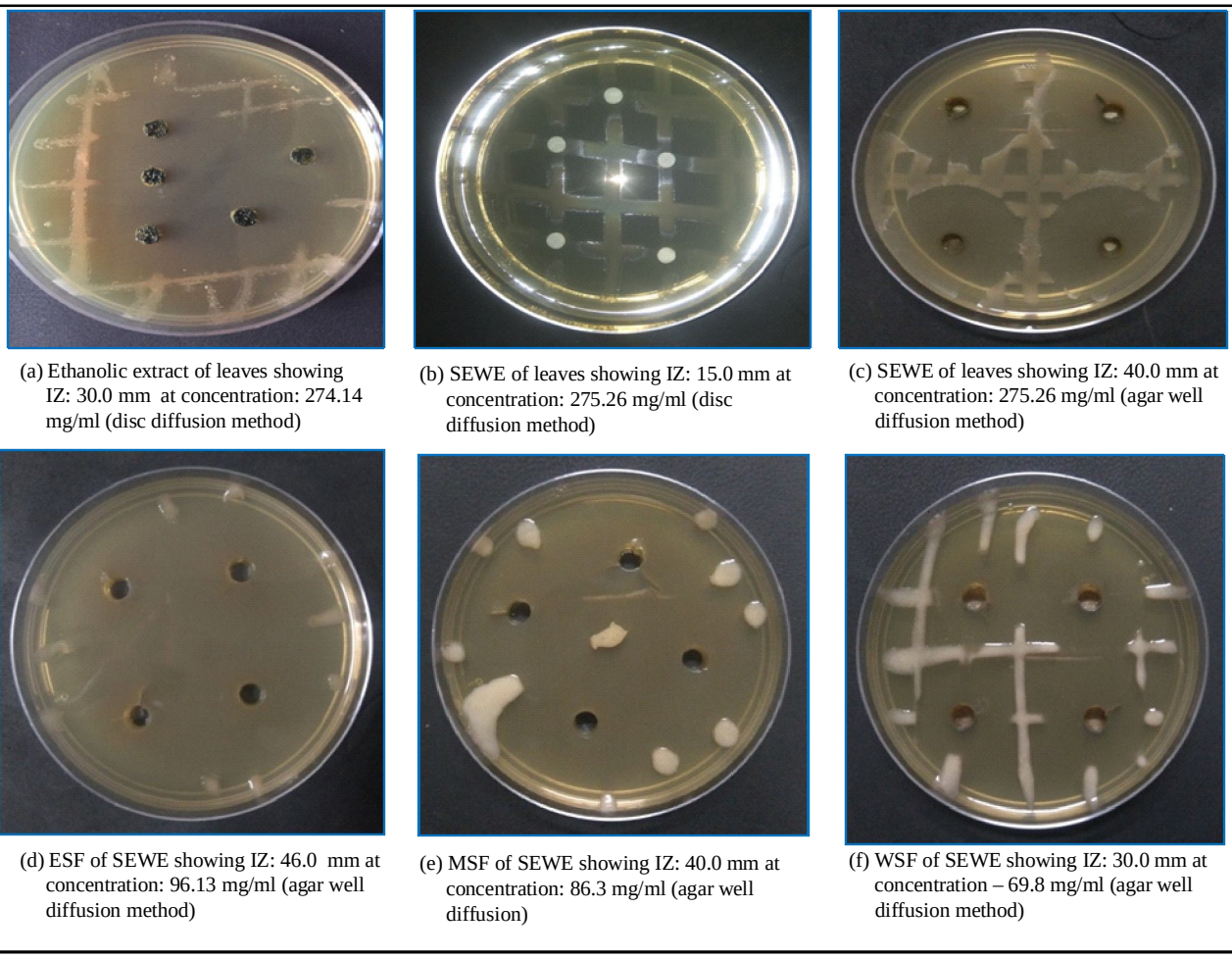
Name of tests	Phytochemicals	Results
Ferric chloride test	Catechol tannins	Positive
Frothing test	Saponins	Positive
Dragendroff test	Alkaloids	Negative
Molisch test	Carbohydrates	Positive
HCL test	Phlobatannins	Negative
Shibita reaction test	Flavonoids	Negative
Folins and ciocalteus reagent	Phenolic compounds	Positive
Ehrlich reagent	Indole	Negative

3.3 *Tamarindus indica* L. (Imli/tamarind)

Initial screening of ethanolic extract of leaves of *T. indica*, further polarity and solubility based fractionation shows the degree of *in vitro* antibacterial activity against *R. equi* in Table 3 and Figure 4 (a to f) and phytochemical analysis was done on most active fraction ESF and was found positive for having phenolic compounds, flavones, catechol tannins and carbohydrates as shown in Table 4.

Table 3: ***In vitro*** **antibacterial activity of leaves extract and the fractions of** ***T. indica*** **against** ***R. equi***

Leaves extract and fraction	Concentration (mg/ml)	Method	Inhibition zone diameter	Degree of *in vitro* antibacterial activity
Ethanolic extract	274.14 mg/ml	Disc diffusion method	30.0 mm	Good
Chloroformic extract	156.5 mg/ml	Disc diffusion method	Zero	None
SEWE	275.26 mg/ml	Disc diffusion method	15.0 mm	Good
SEWE	275.26 mg/ml	Agar well diffusion method	40.0 mm	Good
SEWE	27.53 mg/ml	Agar well diffusion method	36.0 mm	Good
ESF of SEWE	96.13 mg/ml	Agar well diffusion method	46.0 mm	Good
ESF of SEWE	9.61 mg/ml	Agar well diffusion method	20.0 mm	Good
ESF of SEWE	4.81 mg/ml	Agar well diffusion method	15.0 mm	Good
MSF of SEWE	86.3 mg/ml	Agar well diffusion method	40.0 mm	Good
MSF of SEWE	8.63 mg/ml	Agar well diffusion method	15.0 mm	Good
MSF of SEWE	4.31 mg/ml	Agar well diffusion method	11.0 mm	Moderate
WSF of SEWE	69.8 mg/ml	Agar well diffusion method	30.0 mm	Good
WSF of SEWE	6.98 mg/ml	Agar well diffusion method	Zero	None
WSF of SEWE	3.49 mg/ml	Agar well diffusion method	Zero	None



(a) Ethanolic extract of leaves showing IZ: 30.0 mm at concentration: 274.14 mg/ml (disc diffusion method)

(b) SEWE of leaves showing IZ: 15.0 mm at concentration: 275.26 mg/ml (disc diffusion method)

(c) SEWE of leaves showing IZ: 40.0 mm at concentration: 275.26 mg/ml (agar well diffusion method)

(d) ESF of SEWE showing IZ: 46.0 mm at concentration: 96.13 mg/ml (agar well diffusion method)

(e) MSF of SEWE showing IZ: 40.0 mm at concentration: 86.3 mg/ml (agar well diffusion)

(f) WSF of SEWE showing IZ: 30.0 mm at concentration – 69.8 mg/ml (agar well diffusion method)

Figure 4: ***In vitro*** **antibacterial activity of leaves extract and the fractions of** ***T. indica*** **against** ***R. equi*** **showing in (a) to (f).**

Table 4: Phytochemical analysis of ESF of SEWE of *T. indica* leaves

Name of tests	Phytochemicals	Results
Ferric chloride test	Catechol tannins	Positive
Frothing test	Saponins	Negative
Dragendroff test	Alkaloids	Negative
Molisch test	Carbohydrates	Positive
HCL test	Phlobatannins	Negative
Shibita reaction test	Flavones	Positive
Folins and Ciocalteus reagent	Phenolic compounds	Positive
Ehrlich reagent	Indole	Negative

3.4 Control: Azithromycin and Rifampicin

Azithromycin and rifampicin were taken as control showing inhibition zone diameters of 25.0 mm and 20.0 mm, respectively, at the concentration of 10.0 mg/l against *R. equi* (Figure 5 and Figure 6).

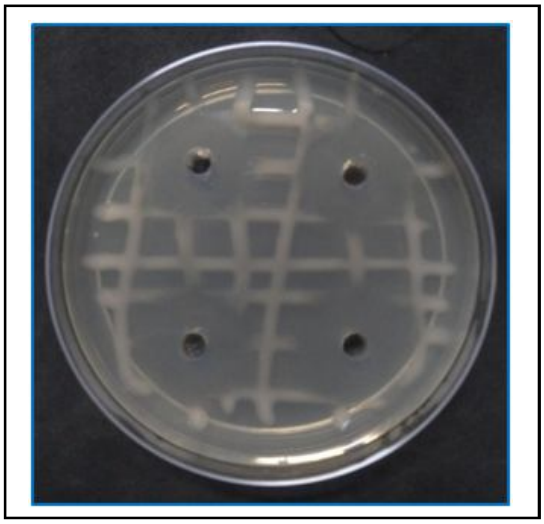


Figure 5: Control azithromycin IZ: 25.0 mm concentration: 10.0 mg/l (agar well diffusion method).

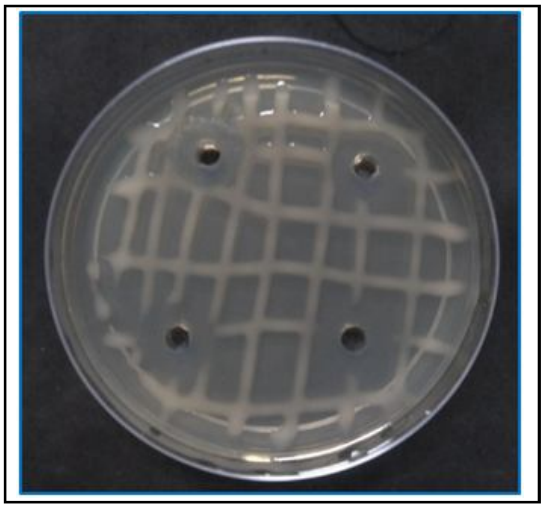


Figure 6: Control rifampicin IZ: 20.0 mm concentration: 10.0 mg/l (agar well diffusion method).

4. Discussion

In the present study, discs were dipped in solvents (ethyl alcohol 99.9% and chloroform 99.9%) and discs dried until the solvents were completely evaporated. So, the concentration of these chemical solvents in the dry discs was zero. Ethanol is well known to dissolve both polar and non-polar compounds because of its polar nature due to presence of hydroxyl group (OH^-) and non-polar nature due to presence of the ethyl (C_2H_5) group. Chloroform dissolves non-polar compounds and distilled water dissolves polar compounds.

In the present study, *E. globulus* was the second most active plant against *R. equi*. Out of all fractions, WSF has shown the highest antibacterial potential with 27.0 mm IZ diameter at 63.9 mg/ml concentration and minimum inhibitory concentration (MIC) at 3.19 mg/ml showing 14.0 mm IZ diameter. Its most active fraction WSF was put on phytochemical analysis and was found positive for having catechol tannins, phenolic compounds, saponins and carbohydrates.

Phloretin is a known polar phenolic compound in *Eucalyptus* species and reported in *E. globulus* (Burgos *et al.*, 2018). Phloretin has antibacterial, antitumor, antioxidant and immunomodulatory efficacy. The activity of phloretin against gram-positive and gram-negative bacteria in *in vitro* studies has been reported earlier (Barreca *et al.*, 2014).

Phenyl propanoic pathway intermediates, including p-coumaric acid, caffeic acid, ferulic acid and sinapic acids have been demonstrated with *in vitro* antimicrobial activities and as phenolic compounds with less complex catechol and coumarin, have also been shown to exhibit bactericidal and fungicidal activities in stump wood and stump bark extract of *E. globulus* (Luís *et al.*, 2014). So, it seems predominately phloretin with other phenolic substances might have shown antibacterial activity against *R. equi*. So, there is a need for further study of the possibility of *in vivo* use of phloretin against *R. equi* infection in foals.

In the present study, *T. indica* was the most active plant against *R. equi* and the inhibition zone was largest than *E. globulus*. Out of all fractions, ESF has shown the highest antibacterial potential with 46.0 mm IZ diameter at 96.13 mg/ml concentration and minimum inhibitory concentration (MIC) at 4.81 mg/ml shown 15.0 mm IZ diameter. Its most active fraction ESF was put on phytochemical analysis and was found positive for having phenolic compounds, flavones, catechol tannins and carbohydrates.

Vitexin is an apigenin flavone reported in *T. indica* leaves (Soemardji, 2007; Lim, 2012; Amado *et al*., 2016) and Orientin is a flavone compound and 8-C glucoside of luteolin also reported in *T. indica* leaves and have antibacterial activity (Lin *et al*., 2004; Gumgumjee *et al*., 2012; Amir *et al*., 2016). So, there is a need for further study of the possibility of *in vivo* use of vitexin and orientin against *R. equi* infection in foals.

Macrolides (erythromycin/azithromycin) and rifampicin combination is the most effective and prevalent treatment against *R. equi* in foals, but resistant strains of *R. equi* are also being observed (Cisek *et al*., 2014). In the present experiment, commercially available azithromycin and rifampicin were used at 10 mg/l and both antibiotics have shown a good zone of inhibition. While most effective herbal fractions of *T. indica* and *E. globulus* have shown their MIC at 4.81 mg/ml (ESF) and 3.19 mg/ml (WSF), respectively. It shows that quantitatively currently used antibiotics have about 200 to 500 times more antimicrobial efficacy than the most active fractions of *T. indica* and *E. globulus*. It depicts that even, if the extracts are considered nontoxic and not interfered with by digestive and metabolic processes then there will be the requirement of extracts at 200 to 500 g for a 100 kg foal. So, it suggests that there is a need to find a more purified compound of these extracts to see the possibilities of *in vivo* use. However, there are possibilities for direct use of *T. indica* and *E. globulus* leaves and their water extract against *R. equi* as farm disinfectant.

5. Conclusion

On phytochemical analysis, the most active fraction, WSF of SEWE of *E. globulus* leaves was found positive for catechol tannins, phenolic compounds, saponins and carbohydrates whereas, the most active fraction, ESF of SEWE of *T. indica* leaves was found positive for catechol tannins, phenolic compounds, flavones and carbohydrates. In comparison with currently used antibiotics (azithromycin and rifampicin), the required concentration of the leaves extract of these plants was too high for their possibilities of *in vivo* use. Most effective herbal fractions, WSF of SEWE of *E. globulus* leaves and ESF of SEWE of *T. indica* leaves have shown their minimum inhibitory concentration (MIC) at 3.19 mg/ml and 4.81 mg/ml, respectively. However, the abundant availability of *T. indica* and *E. globulus* plants in India and their antibacterial activity against *R. equi* suggest their potential for use as a disinfectant against *R. equi*.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Amado, J.R.R.; Prada, A.L.; Arranz, J.C.E.; Rosés, R.P.; Quevedo, H.M.; Keita, H.; Zapata, E.P.; Fernandes, C.P. and Carvalho, J.C.T. (2016). Antioxidant and hepatoprotective activity of a new tablets formulation from *T. indica*. Evidence Based Complementary Altern. Med., pp:1-7.

Amir, M.; Khan, M.A.; Ahmad, S.; Akhtar, M.; Mujeeb, M. Amir, M.; Khan, M.A.; Ahmad, S.; Akhtar, M.; Mujeeb, M.; Ahmad, A.; Khan, S.A. and Al-Abbasi, F.A. (2016). Ameliorating effects of *Tamarindus indica* fruit extract on anti-tubercular drugs induced liver toxicity in rats. Nat. prod. Res., **30**(6):715-719.

Asoh, N.; Watanabe, H.; Guyon, M.F.; Watanabe, K.; Oishi, K.; KosIt'sakulchai, W.; Sanchai, T.; Kunsuikmengrai, K.; Kahintapong, S.; Khantawa, B.; Tharavichitkul, P.; Sirisanthana, T. and Nagatake, T. (2003). Emergence of rifampin resistant *Rhodococcus equi* with several types of mutations in the *rpoB* gene among AIDS patients in Northern Thailand. J. Clin. Microbiol., **41**(6):2337-2340.

Auwal, M.S.; Saka, S.; Mairiga, I.A.; Sanda, K.A.; Shuaibu, A. and Ibrahim, A. (2014). Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of *Acacia nilotica* (Thorn mimosa). Vet. Res. Forum., **5**(2):95-100.

Barreca, D.; Bellocco, E.; Laganà, G.; Ginestra, G. and Bisignano, C. (2014). Biochemical and antimicrobial activity of phloretin and It's glycosilated derivatives present in apple and kumquat. Food Chem., **160**:292-297.

Bhatt, P.R.; Pandya, K.B.; Patel, U.D.; Modi, C.M.; Patel, H.B. and Javia, B.B. (2019). Antidiabetic, antioxidant and anti-inflammatory activity of medicinal plants collected from nearby area of Junagadh, Gujarat. Ann. Phytomed., **8**(2):75-84.

Bhawana, S.; Sharma, S.C. and Alam, A. (2021). Phytochemical screening and GC-MS analysis of *T. indica* (Angiosperms: Fabaceae). Ann. Phytomed., **10**(1):215-221.

Bouatrous, Y. (2019). Antibacterial activity of an essential oil and various extracts of the medicinal plant *Thymus hirtus* sp. algeriensis Boiss. and Reut. Ann. Phytomed., **8**(2):108-114.

Burgos, E.G.; Liaudanskas, M.; Viškelis, J.; Žvikas, V.; Janulis, V. and Serranillos, M.P.G. (2018). Antioxidant activity, neuroprotective properties and bioactive constituents analysis of varying polarity extracts from *Eucalyptus globulus* leaves. J. Food Drug Anal., **26**(4):1293-1302.

Chhabra, S.; Khurana, S.K.; Kapoor, P.K.; Singha, H.; Singh, Y. and Khirbat, R. (2015). Characterization of *Rhodococcus equi* isolates from foals with respiratory problems using a multiplex PCR for the Vap genes. Adv. Anim. Vet. Sci., **3**(1):28-32.

Cisek, A.A.; Rzewuska, M.; Witkowski, L. and Binek, M. (2014). Antimicrobial resistance in *Rhodococcus equi*. Acta Biochim. Pol., **61**(4):633-638.

Das, K.; Tiwari, R.K.S. and Shrivastava, D.K. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agents: Current methods and future trends. J. Med. Plant Res., **4**(2):104-111.

Dedar, R.K.; Vaid, R.K.; Anand, T.; Singh, J.; Virmani, N.; Khurana, S.K. and Kumar, S. (2017). *Rhodococcus equi* diarrhoea and suppurative pneumonia in marwari filly: A case report. Vet. Pract., **18**(2):245-248.

Desai, S.D.; Pai, S.R. and Desai, N.S. (2021). A Review on *Terminalia arjuna* (Roxb.) Wight and Arn.: The wonder medicinal plant with prodigious potential in therapeutics. Ann. Phytomed., **10**(1):62-73.

Fernebro, J. (2011). Fighting bacterial infections: Future treatment options. Drug Resist. Updates, **14**(2):125-139.

Fines, M.; Pronost, S.; Maillard, K.; Taouji, S. and Leclercq, R. (2001). Characterization of mutations in the rpoB gene associated with rifampin resistance in *Rhodococcus equi* isolated from foals. J. Clin. Microbiol., **39**(8):2784-2787.

Gberikon, G.M.; Adeoti, I.I. and Aondoackaa, A.D. (2015). Effect of ethanol and aqueous solutions as extraction solvents on phytochemical screening and antibacterial activity of fruit and stem bark extracts of *Tetrapleura tetrapteraon, Streptococcus salivarus* and *Streptococcus mutans.* Int. J. Curr. Microbiol. App. Sci. **4**(5):404-410.

Gigue're, S.; Jacks, S.; Roberts, G.D.; Hernandez, J.; Long, M.T. and Ellis, C. (2004). Retrospective comparison of azithromycin, clarithromycin and erythromycin for the treatment of foals with *Rhodococcus equi* pneumonia. J. Vet. Intern. Med., **18**(4):568-573.

Gigue're, S.; Lee, E.; William, E.; Cohen, N.D.Y. and Chaffin, M.K. (2010). Determination of the prevalence of antimicrobial resistance to macrolide antimicrobials or rifampin in *Rhodococcus equi* isolates and treatment outcome in foals infected with antimicrobial resistant isolates of *R. equi.* J. Am. Vet. Med. Assoc., **237**(1):74-81.

Giguère, S.; Cohen, N.D.; Chaffin, M.K.; Hines, S.A.; Hondalus, M.K.; Prescott, J.F. and Slovis, N.M. (2011). *Rhodococcus equi*: Clinical manifestations, virulence and immunity. J. vet. Intern. Med., **25**(6):1221-1230.

Gumgumjee, N.M.; Khedr, A. and Hajar, A.S. (2012). Antimicrobial activities and chemical properties of *T. indica* leaves extract. Afr. J. Microbiol. Res., **6**(32):6172-6181.

Hillidge, C.J. (1987). Use of erythromycin-rifampin combination in treatment of *Rhodococcus equi* pneumonia. Vet. Microbiol., **14**(3):337-342.

Irshad, S.; Mahmood, M. and Perveen, F. (2012). *In vitro* antibacterial activities of three medicinal plants using agar well diffusion method. Res. J. Biol., **2**(1):1-8.

Jacks, S.S.; Gigue're, S. and Nguyen, A. (2003). *In vitro* susceptibilities of *Rhodococcus equi* and other common equine pathogens to azithromycin, clarithromycin and 20 other antimicrobials. Antimicrob. Agents Chemother., **47**(5):1742-1745.

Jeyaseelan, E.C.; Tharmila, S.; Sathiyaseelan, V. and Niranjan, K. (2012). Antibacterial activity of various solvent extracts of some selected medicinal plants present in Jaffna Peninsula. Int. J. Pharm. Biol. Arch., **3**(4):792-796.

Kawila, R.; Chaiwarith, R. and Supparatpinyo, K. (2013). Clinical and laboratory characteristics of penicilliosismarneffei among patients with and without HIV infection in Northern Thailand: A retrospective study. BMC Infect. Dis., **13**(1):464.

Kotze, M. and Eloff, J.N. (2002). Extraction of antibacterial compounds from *Combretum microphyllum* (Combretaceae). S. Afr. J. Bot., **68**(1):62-67.

Kumar, K.; Maherchandani, S.; Kashyap, S.K.; Bohara, D.L. and Vaid, R.K. (2014). Prevalence of *Rhodococcus equi* in equine environments in arid zone. Indian J. Comp. Microbiol. Immunol. Infect. Dis., **35**(1):34-38.

Lim, T.K. (2012). *Tamarindus indica.* In Edible medicinal and non-medicinal plants. Springer Dordrecht, pp: 879-905.

Lin, Q.; Feng, S.; Cen, Y.; Yang, Y. and Wang, L. (2004). Study on the antibacterial and antiviral activity compositions of *Trollium chinensis* Bunge. J. Zhejiang Univ. Sci. Ed., **31**(4):412-415.

Luís, Â.; Neiva, D.; Pereira, H.; Gominho, J.; Domingues, F. and Duarte, A.P. (2014). Stumps of *Eucalyptus globulus* as a source of antioxidant and antimicrobial polyphenols. Mol., **19**(10):16428-16446.

Malik, T.; Madan, V.K. and Prakash, R. (2020). Herbs that heal: Floristic boon to the natural healthcare system. Ann. Phytomed., **9**(2):6-14.

Manju, P. and Pushpa, A. (2020). Phytochemical analysis and *in vitro* free radical scavenging activity of rhizome of *Zingiber officinale* Rosc. Ann. Phytomed., **9**(2):257-262.

Mathew, S.E.; Ramavarma, S.K.; Babu, T.D.; Kuzhivelil, B.T. and Raghavamenon, A.C. (2019). Preliminary assessment on phytochemical composition, cytotoxic and antitumor efficacy of *Simarouba glauca* DC. leaf methanolic extract. Ann. Phytomed., **8**(2):121-126.

McNeil, M.M. and Brown, J.M. (1992). Distribution and antimicrobial susceptibility of *Rhodococcus equi* from clinical specimens. Eur. J. Epidemiol., **8**(3):437-443.

Mehrotra, N. (2021). Herbs that heal: Nature's pharmacy endowed remedies for better health. Ann. Phytomed., **10**(1):6-22.

Meijer, W.G. and Prescott, J.F. (2004). *Rhodococcus equi.* Vet. Res., **35**(4):383-396.

Mir, I.A.; Kumar, B.; Taku, A.; Bhardwaj, R.K.; Bhat, M.A. and Badroo, G.A. (2015). Prevalence and antibiogram study of *Rhodococcus equi* in equines of Jammu and Kashmir, India. J. Equine Sci. **26**(1):21-24.

Nostro, A.; Germano, M.P.; D'Angelo, V.; Marino, A. and Cannattelli, M.A. (2000). Extraction methods and bioautography for evaluation of medicinal plants for antimicrobial activity. Lett. Appl. Microbiol., **30**(5):379-384.

Pauw, E. and Eloff, J.N. (2014). Which tree orders in Southern Africa have the highest antimicrobial activity and selectivity against bacterial and fungal pathogens of animals? BMC Complementary Altern. Med., **14**(317):1-12.

Priyadharshini, P.; Raj, A. and Warrier, R.R. (2019). Phytochemical and antimicrobial efficacy of *in vivo* and *in vitro* tissues of *Aegle marmelos* (L.) Corrêa. Ann. Phytomed., **8**(1):140-147.

Salie, F.; Eagles, P.F.K. and Leng, H.M.J. (1996). Preliminary antimicrobial screening of four South African asteraceae species. J. Ethnopharmacol., **52**(1):27-33.

Seshadri, V.D. (2021). Identification of antibacterial phytochemicals in *Terminalia arjuna* (Roxb.) Wight and Arn. and *Andrographis paniculata* (Burm.f.) Nees for the treatment of multidrug resistant (MDR) bacterial pathogens: An *in silico* analysis. Ann. Phytomed., **10**(1):141-159.

Shamna, K.P. and Poyil, M.M. (2021). Isolation of multidrug resistant bacterial pathogens and screening against *Coleus amboinicus* L. extracts. Ann. Phytomed., **10**(2):409-415.

Soemardji, A. A. (2007). *T. indica* or "Asam Jawa": The sour but sweet and useful. Visit. Profr. Inst. Nat. Med. Univ. Toyama Japan., pp: 1-20.

Srivastava, J.; Chandra, H; Nautiyal, A.R. and Kalra, S.J.S. (2014). Antimicrobial resistance (AMR) and plant derived antimicrobials (PDAms) as an alternative drug line to control infections. 3 Biotech., **4**(5):451-460.

Sweeney, C.R.; Sweeney, R.W. and Divers, T.J. (1987). *Rhodococcus equi* pneumonia in 48 foals: Response to antimicrobial therapy. Vet. Microbiol., **14**(3):329-336.

Wali, A.F.; Hamad, E.A.; Khazandar, A.A.; Al-Azzawi, A.M.; Sarheed, O.A.; Menezes, G.A. and Alam, A. (2019). Antimicrobial and *in vitro* antioxidant activity of *Salvia officinalis* L. against various re-emergent multidrug resistance microbial pathogens. Ann. Phytomed., **8**(2):115-120.

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