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Original Article

Antibrucellosis Activity of Medicinal Plants from Western Ghats and Characterization of Bioactive Metabolites

Sri Raghava, Sharanaiah Umesha*

ABSTRACT

Background: Brucellosis is one of the most prevalent bacterial zoonosis which is transmitted to humans from animals. As an alternative to conventional antibiotics, medicinal plants are valuable resources for new agents against antibiotic-resistant strains. **Objective:** To evaluate the antibrucellosis activity of different medicinal plants collected from the Western Ghats against *Brucella abortus, Brucella melitensis, Brucella suis*. Identification and characterization of the bioactive metabolites of the potent antibrucellosis agent by Thin Layer Chromatography and Gas chromatography mass spectroscopy. **Methods:** Antibacterial assay was carried for the ethanolic extract of different medicinal plants, the potential and effective medicinal plants extract was subjected for purification by TLC and the bioactive metabolites were characterized by the GC MS analysis. **Results:** *Acacia nelotica, Terminalia arjuna, Eugenia jambolana* and *Callistemon citrinus* showed the antibrucellosis activity comparatively *Callistemon citrinus* had the strong antibrucellosis activity. Further the crude sample was purified by TLC profiling, compounds with different retention factor were screened for antibrucellosis activity, and the bioactive metabolites were identified by GC-MS analysis. **Conclusion:** For the first time the different medicinal plants from Western Ghats were screened for the antibrucellosis activity. The crude and TLC purified C*allistemon citrinus* ethanolic extract exhibited strong antibrucellosis activity. The bioactive compounds identified were reported for the first time and the bioactive metabolites identified exhibited as potential antibacterial agents against brucellosis and other Human pathogens.

Key words: Antibrucellosis, Bioactive compounds, GC-MS, TLC, Medicinal plant.

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INTRODUCTION

Brucellosis, a bacterial zoonosis and major public health concern due to its high morbidity rate. The prevalence of infection in humans is directly associated with occurrence in animals, particularly in domestic ruminants.[1] Among *Brucella* species *B. melitensis*, *B. abortus* and *B. suis* are pathogenic for humans. While brucellosis occurs worldwide, it is endemic in the Mediterranean basin, the Middle East, Western Asia, Africa and Latin America.[2]

Infection of brucellosis causes significant economic losses by comparatively low milk production in livestock, abortion, weak off-springs, public health and international trade implications.[3] The real rate is estimated to be 10 to 25 times more than annual reports.[4] *Brucella* is non-motile, small, gram negative, non-spore forming, and strictly aerobic coccobacilli. It is mostly positive for catalase and oxidase tests and shows various results in urease tests.[5] Brucellae genus shows little variation genetically, presently eleven *Brucella* species have been recognized, they are genetically very similar although each have different host preferences.[6] Brucellae are highly potent pathogen in animals, humans and also effective biological agents for use in biological weapons even at very low concentration of 10 bacteria. Brucellae are easily transmitted to humans via aerosols and these make bacteria most attractive for defence researchers.[7]

Infectious diseases pose a severe health concern worldwide. The development of drug resistant pathogens due to haphazard use of antibiotics has increased the need for new source of antimicrobial agents. This has encouraged screening of new plant species for potential medicinal and antioxidant properties.[8,9] In general, the Gram-negative bacteria show less sensitivity to plant extracts possibly as a result of their extra lipopolysaccharide and protein cell wall that provides a permeability barrier to the antibacterial agent.[10] Furthermore, the Gram-positive bacteria are more sensitive to the plant extracts because of the single layer of their cell wall, while the double membrane of Gram- negative bacteria should make them less sensitive.[11]

Medicinal plants have been recognized as a part of the evolution of human healthcare for thousands of years. Medicinal components from plants play an important role in traditional as well as in modern medicine. Antimicrobial resistance is progressively becoming a serious threat to global public

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health. According to World Health Organization (WHO) report on antimicrobial resistance in 2014, overcoming the antibiotic resistance is the major challenge for the next millennium.[12] Screening of plants for antimicrobial agents has gained importance, because WHO is encouraging and promoting the development and utilization of medicinal plant resources in the traditional system of medicine.

The usage of herbal plants as traditional health remedies is the most preferable by 80% of the world population in Asia, Latin America and Africa and has been reported to have minimal side effects.[13] For treatment of brucellosis, a combination of antibiotics that penetrates the macrophage should be used. The choice treatment for human brucellosis caused by *B. melitensis* field strains is a combination of long-acting tetracyclines and streptomycin. Additionally, studies have shown that for treatment of patients with *B. melitensis* vaccine strains Rev1, a gentamicin/doxycycline combination may be the first choice.[1] In general, tetracycline/aminoglycoside combinations are the most common antibiotics used for brucellosis treatment. However, because of high rates of treatment failure or relapses due to emerging resistance, the treatment of brucellosis is still problematic. Thus, new antibacterial compounds are becoming necessary for brucellosis treatment. Medicinal plants have always been sources for new drug discovery. Plants readily synthesize substances for their defence against insects, herbivores, and micro organisms.[14] Moreover, they might produce secondary antimicrobial metabolites as a part of their normal growth and development or in response to stress.[15]

Hence the objective of the study is to screen for antibrucellosis activity of ethonolic extracts of *Acacia nilotica, Withania somnifera, Eugenia jambolana, Callistemon citrinus, Clerodendrum inerme, Terminalia arjuna, Thevetia peruviana, Leucas aspera, Hemidesmus indicus, Gloriosa superba, Cymbopogon citrates, Acorus calamus, Cinnamon, Thuja occidentalis* and *Santhalum album* against antibrucellosis activity *in vitro*. Purification of crude ethanolic plant extract by TLC profiling and identification of the bioactive metabolites by GC-MS.

MATERIALS AND METHODS

Bacterial reference strains

Brucella strains -*Brucella abortus, Brucella melitensis, Brucella suis* were procured from Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, Uttar Pradesh, India. They were tested for the purity, biochemical and molecular characteristics before use. Type III Biosafety containment was used to culture the bacteria. *Escherichia coli* (1610), *Salmonella typhimurium* (98) *Bacillus cereus* (430), *Shigella flexneri* (1457), *Vibrio cholera* (3904), *Pseudomonas aeruginosa* (1688), *Vibrio paraheamolyticus* (451), *Bacillus subtilis* (6939) and *Enterobacter aerogenes* (13048) bacterial strains were obtained from Microbial Typing Culture Collection (MTCC), Chandigarh, India and American Tissue Culture Collection (ATCC) and were cultured as per the protocol prescribed by MTCC and ATCC respectively.

Plant collection and identification

Different medicinal plants were collected from Western Ghats of Karnataka, India. The taxonomic identification of these plants was done by Prof. G. R. Shivamurthy, former professor, Department of Botany, University of Mysore, Karnataka, India.

Plant materials collection and processing

The plant leaves were thoroughly washed with tap water to remove dusts and other unwanted materials accumulated on the leaves from their natural environment. The dust free leaves were allowed to dry under shade in the laboratory for 20 days. The dried leaves were powdered by using electric blender. Finally, fine powder was collected from the powdered leaves by sieving through the muslin cloth and used for extraction.

Extraction procedure

Twenty gram of powdered plant material was put in a 200 mL conical flask and 100 mL of ethanol solvent was added. Conical flask was covered with aluminium foil and kept in a reciprocating shaker for 24 h for continuous agitation at 130 rev/min for thorough mixing and also complete extraction of active materials to dissolve in the solvent. Then, extract was filtered by using muslin cloth followed by Whatman No 1 filter paper and finally the solvent from the extract was removed by using rotary vacuum evaporator at water bath temperature of 50°C. Finally, the residues were collected and used for the experiment.

Antibacterial susceptibility assay

The test isolate was grown in Muller-Hinton Broth (Merck, USA) medium at 37 °C for 22 h. Final inoculum bacterial numbers were adjusted to 10^8 CFU/ml. A total of 0.1 ml of bacterial suspension was poured on each plate containing Muller-Hinton Agar (MHA). The lawn culture was prepared by sterile cotton swab and allowed to remain in contact for 1 min. Different concentrations of ethanolic extracts (1, 5, 10, 25, 50, 100, 200, and 300 mg/ml) from each plant were prepared. The sterile filter paper discs (6-mm diameter) were saturated by 50 µl of different concentrations of each extract and then were placed on lawn cultures.[16,17] The Petri dishes were subsequently incubated at 37°C for 24 h and the inhibition zone around each disc was measured in mm. As positive controls, discs (Difco, USA) containing streptomycin 10 µg, gentamicin 10 µg and Ciprafloxin 10 µg were used. Further the TLC profiling was carried out for the extract with strongest antibrucellosis activity.

Thin Layer Chromatography profiling (TLC)

TLC system equipped with a sample applicator was used for application of samples. Five ul of leaf ethanol extracts was separately applied on 5 × 10 cm chromatographic pre-coated silica gel plates (TLC grade, Merck, USA) as the stationary phase. The TLC plates were developed in a twin trough glass chamber containing mixture of chloroform and methanol (99: 1 v/v) as the mobile phase. The plates were removed when the solvent front has moved to the defined level, subsequently allowed to dry. After drying, the spots on the developed plates were visualized under visible (white), short UV (254 nm), and long UV (366 nm) light. Extract was expressed by its retention factor (R_f). Values were calculated for each spot using the following formula:

$$R_f = \frac{\text{distance travelled by the solute from the point of application to the center of spot}}{\text{distance travelled by the solvent front}}$$

Preparative TLC was carried out to isolate the separated compounds based on R_f values was done to obtain substantial quantities for antimicrobial test.

Minimum inhibitory concentration MIC

The test isolate was grown in Muller-Hinton Broth (Merck, USA) medium at 37°C for 22 h. Final inoculum bacterial numbers were adjusted to 10^8 CFU/ml. A total of 0.1 ml of bacterial suspension was poured on each plate containing Muller-Hinton Agar. The lawn culture was prepared by sterile cotton swab and allowed to remain in contact for 1 min. Different concentrations of TLC purified ethanolic extracts (25, 50, 100, 200, 400, 800 and 1200 µg/ml) from each plant were prepared. The sterile filter paper discs (6-mm diameter) were saturated by 50 µl of different concentrations of each extract and then were placed on lawn cultures.[16,17] The Petri dishes were subsequently incubated at 37°C for 48 to 72 h anaerobically and the inhibition zone around each

disc was measured in mm. As positive controls, discs (Difco, USA) containing gentamicin 10 µg.

Gas chromatography-mass spectrometry

A Hewlett-Packard 5890 Series II Chromatograph equipped with a FID detector and HP-2 fused silica columns (25 m × 0.32 mm, 0.25 µm film thicknesses) was used. The samples, dissolved in hexane, were injected in the split less mode into helium carrier gas. Injector and detector temperatures were maintained at 250°C. The column temperature was programmed from 60°C (after 2 min) to 220°C at 4°C/min, and the final temperature was held for 20 min. Peak areas and retention times were measured by electronic integration of by computer. The relative amounts of individual components are based on the peak areas obtained, without FID response factor correction. GC-MS analyses were carried out on a Hewlett-Packard 5970A mass selective detector (MSD), directly coupled to HP 5790A gas chromatograph. A 26 m × 0.22 mm column, coated with 0.13 µm of CP-Sil 5CB was employed, using helium carrier gas. The oven temperature program was 60°C (3 min), then 5°C/min to 250°C (30 min). Other conditions were the same as described under GC. Electron ionization (EI) mass spectra were acquired over a mass range of 10-400 Da at a rate of 2/s.

Identification of the compounds

The identification of the compounds present in the TLC purified ethanolic extracts were based on direct comparison of the retention times and mass spectral data with those for standard compounds, and by computer matching with the Wiley 229, Nist MS Library.

Statistical analysis

All experiment/measurements were made in triplicate, and all the values are expressed as the mean ± SE of three independent replicates. Statistical significances were analyzed using two-tailed Student's *t*-test and means were compared at the level of $p \leq 0.05$.

RESULTS

Antimicrobial assay

The antibrucellosis activity was evaluated for different ethnomedicinal plants using disc diffusion method and represented in table (Table 1). *C. citrinus* showed excellent biocidal activity against *B. abortus B. melitensis and B. suis*, moderate activity was displayed by *A. nelotica* against *B. abortus*, while *T. arjuna* exhibited negligible activity against *B. abortus* and *B. suis*. Among the plant sources crude ethanolic *C. citrinus* (Figure 1) showed dose dependent inhibition against *Brucella* spp. such *as B. abortus, B. melitensis and B. suis*. The MIC concentration was observed according to Clinical and Laboratory Standards Institute (CLSI) for *B. abortus* (1.5 mm) at 300 mg/ml concentration, *B. melitensis* (1.7 mm) at 300 mg/ml and *B. suis* (1.7 mm) at 100 mg/ml concentration. This results were compared to the standard SM, GN, CIP –1.4 1.6, 1.3 respectively in the present study. The statistical significant was observed for *brucella* spp. P≤ 0.05 for the *B. Suis,* P≤ 0.05 for *B. melitensis* and P≤ 0.01 for *B. abortus*

Thin layer chromatography

The TLC plate was developed in respective mobile phase chloroform-methanol (99:1, v/v) for separations of *C. citrinus* ethanolic extract bioactive compound (Figure 3)**,** about over 8.5 cm, resulted in four bands, with four spots, spot A R_f value 0.27, spot B 0.39, spot C 0.83 and spot D 0.97 all the spots were scraped. Anti-microbial activity was evaluated for all the spots.[18] Only spot D was showed very good anti-bacterial activity at different concentration of 20, 50, 100, 200, 400, 800 and 1200 µg/ml against *Brucella* spp. The MIC observed was *B. abortus* (1.5 mm) at 800 µg/mL, *B. melitensis* (1.7 mm) at 800 µg/mL and *B. suis* (1.7 mm) at 800 µg/mL concentration. The MIC of *Brucella* spp. was compared to the standard GN –1.4 mm 1.6 mm, 1.2 mm respectively (Figure 2). Statistical significance was observed for *Brucella* spp. P≤ 0.05 for the *B. suis*, P≤ 0.05 for *B. melitensis* and P≤ 0.01 for *B. abortus*. Spot D showing good inhibitory activity was subjected to GC-MS analysis and also screened for antibacterial activity against other human pathogens (Table 2).

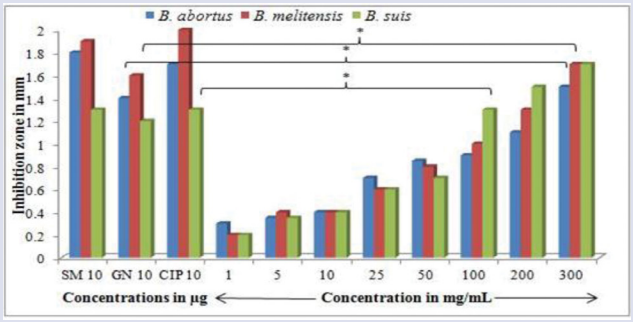

Figure 1: Zone of Inhibition from crude ethanolic extract of *C. citrinus* The crude ethanolic extract was evaluated in different concentrations of 1, 5, 10, 25, 50, 100, 200, 300 mg/mL against B. abortus, B. melitensis and B. suis compared to the standard antibiotics SM- streptomycin(10 µg); GN- Gentamycin(10 µg); CIP- Ciprofloxacin(10 µg). The MIC concentration was observed for B. abortus (1.5 mm) at 300mg/ml concentration, B. melitensis (1.7 mm) at 300mg/ml and B. suis (1.7 mm) at 100mg/ml concentration. The brucella spp. compared to the standard SM, GN, and CIP –1.4mm 1.6mm and 1.3mm respectively.

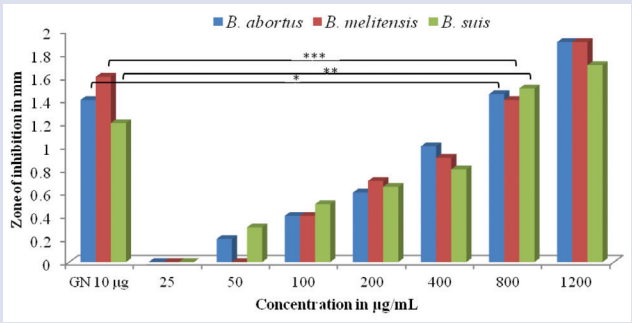

Figure 2: Zone of Inhibition from TLC purified ethanolic extract of *C. citrinus* Ethanolic extract of *C. citrinus* subjected for TLC and bioactive spot was identified and different concentration 25, 50, 100, 200, 400, 800 and 1200 µg/ml was tested against B. abortus, B. melitensis and B. suis. Compared to standard antibiotics GN- Gentamycin. The MIC concentration was found to be 800 µg/mL compared to standard GN(10 µg).

GC-MS study

GC-MS analysis was carried out for the TLC separated spot D (Figure 3) of the ethanolic extract of *C. citrinus*. Bioactive compounds were characterized and tabulated (Table 3). The total ion chromatograph (TIC) showing the peaks and identity of the compounds is given in Figure 4. Chromatographs of the individual identified compounds are provided in the supplementary file.

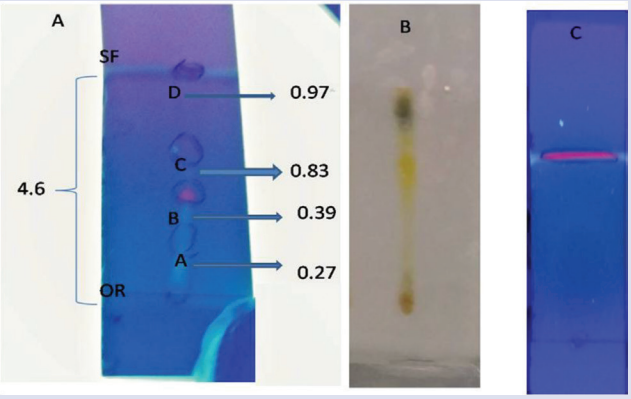

Figure 3: Purification of active spot from ethanolic extract of fusing TLC The ethanolic extract of *C. citrinus* was subjected for identification and separation of active spot using chloroform-methanol (99:1, v/v) as mobile phase. The retention factor (R_f) value of separated spots was determined by calculating the distance migrated by the solvent between the origin (OR) and solvent front (SF) is indicated. The separated spots were identified by exposing plate under UV lamp at 254 nm and calculated R_f value for the ethanolic extract of *C. citrinus*.

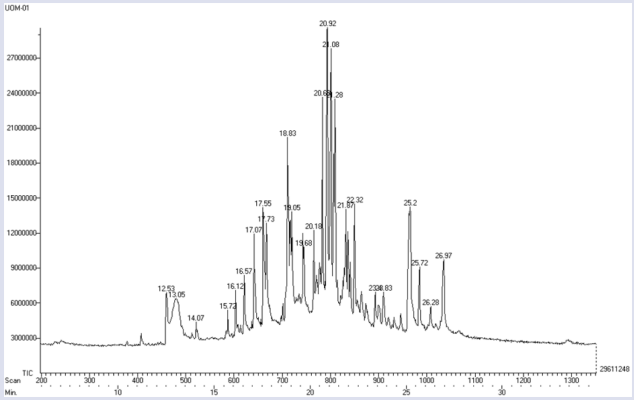

Figure 4: GC-MS Chromatogram of TLC purified ethanolic extract of *Callistemon citrinus* showing the bioactive metabolites. The total ion chromatograph (TIC) showing the peak identities of the compounds identified.

Table 1: Screening of ethnomedicinal plants for antibrucellosis activity Different medicinal plants were collected from Western Ghats of Karnataka, India. The ethanolic extracts of different medicinal plants were assessed against different Brucella spp. such as B. abortus, B. melitensis and B. suis. "-" indicates No inhibition "+" indicates low Inhibition "++" medium inhibition "+++" indicates strong inhibition. *C. citrinus* ethanolic extract showed very strong inhibition and against B. abortus, B. melitensis, and B. suis.

Ethanolic extract of medicinal plants screened	*Brucella abortus*	*Brucella melitensis*	*Brucella suis*
Acacia nilotica	++	-	-
Withania somnifera	-	-	-
Eugenia jambolana	+	++	+
Callistemon citrinus	+++	+++	+++
Clerodendrum inerme	-	-	-
Terminalia arjuna	-	+	+
Lucas aspera	-	-	-
Thevetia peruviana	-	-	-
Hemidesmus indicus	-	-	-
Gloriosa superba	-	-	-
Cymbapogan citratus	-	-	-
Acorus calamus	-	-	-
Rhamnus cathartica	-	-	-
Cinnamon	-	-	-
Thuja occidentalis	-	-	-
Santalum album	-	-	-

DISCUSSION

Currently, the treatment of brucellosis remains a major public health concern, especially in developing countries.[19] In order to increase the treatment efficacy and avoid disease relapse, a classic combination of synthetic tetracycline and aminoglycoside antibiotics has been used. But due to the microbial resistance, multiple drug resistant strains of *Brucella* have developed. Unfortunately, bacteria have the ability to transmit and acquire resistance to drugs.[20] Plants produce secondary metabolites in order to protect themselves from microorganism, herbivores and insects. Even though antimicrobial activities of various medicinal plants have been discovered, very little target compounds have been characterized for activity against *Brucella* spp.[21]

The natural plant sources were evaluated to explore antibacterial compounds against Gram negative bacteria *B. abortus, B. melitensis* and *B. suis* which are found to be highly pathogenic to human beings. The result of this study showed that the ethanolic extract of *C. citrinus* exhibited excellent antimicrobial activity against the tested organism including

Table 2: Antibacterial activity of TLC purified ethanolic extract The ethanolic extract of *C. citrinus* was purified using TLC and the bioactive spot D having R_f 0.97 is tested in different concentration against different human pathogens such as B. cereus, V. cholera, E. aeroginosa, E. Coli, S. typimurium, S. flexineri, B. subtilis, V. parahaemolyticus and compared to the standard antibiotic Gentamycin(10 µg).

Concentration in µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml	800 µg/ml	1200 µg/ml	GN Std 10 µg
B. cereus	2±0.04	3±0.04	5±0.02	8±0.2	9.5±0.06	11±0.1	12±0.06	8±0.2
V. cholera	3±0.04	5±0.02	6±0.06	7±0.03	9.5±0.06	11±0.4	13±0.06	10±0.09
E. aeroginosa	2±0.04	3.5±0.04	5±0.02	6±0.06	7.5±0.03	9±0.06	12±0.06	9±0.06
E. Coli	3±0.04	4±0.05	5.5±0.04	7±0.03	8±0.2	9.5±0.06	11±0.1	9±0.06
S. typimurium	-	3±0.04	4±0.05	5±0.02	7±0.03	8.5±0.5	11±0.1	10±0.09
S. flexineri	3±0.04	5±0.01	5.5±0.03	7.5±0.03	8.5±0.2	10±0.09	13±0.02	11±0.1
B. subtilis	-	-	3±0.04	4±0.05	6±0.07	8±0.2	10±0.09	8±0.4
V. parahaemolyticus	4.5±0.05	6±0.06	8±0.2	10±0.09	11±0.1	14±0.08	18±0.05	13±0.06

Table 3: The bioactive metabolites present in TLC purified ethanolic extract of *Callistemon citrinus*.

Sl NO	Retention Time	Peak Area	Peak area %	COMPOUND NAME
01	12.53	10303672	1.06	Durohydroquinone
02	13.05	30261244	3.129	Germacr-4-en-12-oic acid, 6a-Hydroxy, c-lactone(11S).
03	14.07	5613736	0.581	9-acetoxy-1-propyl-3, 6-diazahomoadamantane
04	15.72	7439848	0.770	Cyclohexane, 1-methyl-5-(1-methyletheyl)-, (R).
05	16.12	10339848	1.071	11, 13-Dimethyl-12-tetradecen-1-ol acetate.
06	16.57	13544548	1.403	Cyclopentanecarboxylic acid, 2-acetyl-5-methyl.
07	17.07	29596452	3.067	Hexadecanoic acid, methyl ester.
08	17.55	43310600	4.488	1, 2-Benzenedicarboxlic acid, butyl 2-methylpropyl ester
09	17.73	4331060	0.448	1-pentadecene, 2-methyl
10	18.83	83748952	8.679	10-Octadecenoic acid, methyl ester
11	19.05	88786768	9.201	Heptadecanoic acid, 16-methyl-, methyl ester
12	19.68	24135528	2.501	14. hydroxy-15-methylhexadec-15-enoicacid, ethyl ester
13	20.18	16702744	1.731	2-Cyclohexen-3-ol-1-0ne, 2-(9-phennonanoyl)
14	20.60	34961840	3.623	Acetic acid, 10, 13-dimetyl-2-oxo-2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-tetradecahydro-1H-cyclopentane(a)phenanthren-17-yl ester
15	20.92	114157520	11.830	11-ketopropogesterone
16	21.08	114157526	11.830	Pregnan-20-one,5,6-epoxy-3, 17-dihydroxy-16-methyl, (3a, 5a, 6a, 16a)
17	21.28	117747992	12.203	11-0xo-9-thiocyanato-testosterone
18	21.87	49011904	5.079	2-Demethylthiodemecolcine
19	22.32	14828080	1.536	1,2-benzenedicarbolxylic acid, 6-methylheptyl 8-methylnonyl ester
20	23.60	12407884	1.285	16-hydroxymethyleneandrost-5-en-3-ol-17-one
21	24.83	9918716	1.027	B(9a)-Homo-19-nortregna-9(11), 9a-dien_20-one, 3-(dimethylamin)-4, 4, 14-trimethyl, (3a, 5a)
22	25.2	73646104	7.632	3H-cyclopenta(d)anthracene-8, 11-diol, 3-isopropyl-6-oxo-1, 2, 3a, 4, 5, 6, 6a, 7, 12, 12a-decahydro-diacetate
23	25.72	15937504	1.651	Docosa-2, 6, 10, 14, 18-pentaen-22-al, 2, 6, 10, 15, 18-pentamethyl-,al-trans
24	26.28	9358504	0.969	Cholesta-4, 7-dien-3-ol.4-methyl, (3a)
25	26.92	30725384	3.184	a-Homocholest-4a-en-3-one

Gram positive and Gram negative bacteria, which is comparable to standard antibiotic effect in table (Table 2). The result of antibacterial activity are in the agreement with the findings of Seyydnejad *et al.*[22] and salem *et al.*[23] but there is no clear evidence of bioactive compounds has been explored for this plant till now. This literature gap prompted us to carry out characterization and test the bioactive compounds against *Brucella* spp. The result revealed that ethanolic extracts of *C. citrinus* has promising antibacterial activity.

The *C. citrinus* extracts exhibited potent antimicrobial activity. TLC is a widely used technique for separation of natural substances and possesses applications in analyzing biologically important compounds, identification and characterization.[24] The retention factor values for the plants extracted with ethanol ranged from R_f value of 0.27, 0.39, 0.83 to 0.97. The R_f value 0.97 spot showed antibrucellosis activity and antibacterial activity against other human pathogens. This indicates presence of bioactive metabolites were concentrated in spot D. The individual

compounds were screened for the antimicrobial activity but the activity was not observed matching the results of Minqing *et al.*[25] This might be due to the separation of the constituents, which were showing activity at the synergistic level. The antibacterial activity showed by the TLC purified ethanolic extract of *C. citrinus* could be attributed to the presence of bioactive metabolites. The overall result of the study can be considered as very promising in perspective of new drug discovery from the unknown rare ethnomedicinal plant source, especially because of their medical importance against both bovine and human brucellosis.

CONCLUSION

Different medicinal plants from the Western Ghats were screened for antibrucellosis activity. Based on the result of this study it can be said that *C. citrinus* is an effective antimicrobial plant that can be used in biomedical, pharmaceutical field and will be a good source for finding new antimicrobial agents in order to treat and control infections. For the first time we are reporting the antibrucellosis activity in plants *Acacia nelotica, T. arjuna, E. jambolana* and *C. citrinus. C. citrinus* showed strong antibrucellosis activity. The bioactive metabolites identified by GC MS were found to have strong antibacterial activity against human pathogens. More studies concerning about the molecular basis of this interaction is important. In future *C. citrinus* can be assigned as the source of antimicrobial compounds for the treatment caused by the human pathogens.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

ABBREVIATION USED

TLC: Thin layer chromatography; **GC-MS:** Gas chromatography-mass spectrometry; **MIC:** Minimum inhibitory concentration; **CLSI:** Clinical and Laboratory Standards Institute.

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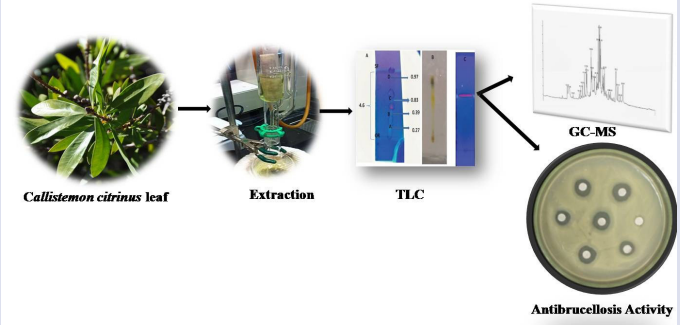
GRAPHICAL ABSTRACT



SUMMARY

- Brucellosis is an infectious disease caused by gram-negative bacteria Brucella species *B. abortus, Brucella melitensis, B. suis*, and *B. canis*. For management, a combination of antibiotics that penetrate the macrophage should be used.
- However, because of high rates of treatment failure or relapses due to emerging resistance, the natural resources drugs have fewer side effects than chemical drugs are used as natural therapy.
- In the present study different medicinal plants have been screened for antibrucellosis activity and characterized the bioactive component.
- The results indicated that ethanolic extract of *Callistemon citrinus*, showed potent antibrucellosis activity and further identification of its bioactive component against antibrucellosis candidate was warranted for future.

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