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In vitro screening of antioxidant, free radical scavenging and antimicrobial potential of *Micrococca mercurialis* whole plant extracts

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ABSTRACT

Micrococca mercurialis is widely used for the treatment of sores, skin diseases, cold, fever, rheumatic pain, and infections. The present study aimed to study the antioxidant, free radical scavenging and antibacterial potential of different whole plant extracts (aqueous, ethanol, petroleum ether) of *Micrococca mercurialis* by *in vitro* methods. The amount of phenols, tannins, flavonoids and Vitamin C were estimated by conventional methods. Free radical scavenging potential was assessed by DPPH and FRAP assays. HRBC membrane-stabilizing study was done to determine its anti-inflammatory property by *in vitro* method. Activity against *Staphylococcus aureus* and *Bacillus subtilis* were determined in *Micrococca mercurialis* extracts. The phytoconstituents in the extracts of *Micrococca mercurialis* were explored by GC-MS analysis. The extracts of *Micrococca mercurialis* exhibited varied *in vitro* antioxidant, free radical scavenging, antibacterial and anti-inflammatory activities. The GC–MS results confirmed the presence of twenty-nine phytoconstituents totally responsible for the biological activities of *Micrococca mercurialis.* This pilot study has provided a scientific validation for the folkloric use of *Micrococca mercurialis* against many infections and diseases.

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INTRODUCTION

Free radicals contribute to the pathogenesis of many diseases, including earlier aging. Oxidative stress occurs due to the imbalance between the free radicals and their scavengers or neutralizers, the antioxidants. It was reported that around 10,000 human cells per second are targeted by oxidative hit by reactive oxygen species (Onkar *et al.*, 2012). Biological antioxidants prevents the formation of or scavenges / decomposes the free radicals. Sometimes there was a decrease in the efficiency of the antioxidant capacities due to some pathological conditions which could corrected by supplementing dietary antioxidants from plant sources.

Medicinal plants have always contributed significantly to the development of novel drugs as they ameliorate the damaging effects caused by reactive free radicals. The side effects caused by allopathic medicines and their cost have made the herbal drugs to gain importance. Nearly 80% of the world population relies on herbal medicines, and attention has to be paid to explore novel drugs for various ailments (WHO, 2005). Hence there is always a stipulation to obtain antioxidants from natural resources.

Micrococca mercurialis commonly called as "pulladi" or "kunuku thooki" belongs to the large flowering *Euphorbiacea* family. It is an annual herb grows well in saline soil and distributed throughout tropical Africa, Malaysia, North Australia and

India (Sutapa *et al.*, 2014). *Micrococca mercurialis* was found to be possess many important secondary metabolites, and traditionally it was used to treat sores, skin diseases, cold, fever, rheumatic pain, and infections (Kalaichelvi and Dhivya, 2017).The plant has immense medicinal properties and the leaves are consumed as food (Rao and Satyanarayanaraju, 1975). Despite its consumption by the local communities, no complete scientific validations about the bioconstituents were available to prove the therapeutic potential of *Micrococca mercurialis.*

So, in the present study, an attempt has been made to investigate the antioxidant, free radical scavenging and antibacterial potential of *Micrococca mercurialis* whole plant extracts.

MATERIALS AND METHODS

Chemicals

Gallic acid, quercetin, DPPH, ascorbic acid, DNPH were obtained from Sigma-Aldrich (St. Louis, M.O., USA) and Muller Hilton agar from Himedia. Analytical grade solvents and chemicals were used.

Collection of Plant materials

The plant material *Micrococca mercurialis* was collected in North Chennai, Tamil Nadu, India and was authenticated by Prof.P.Jayaraman, Senior Taxonomist, Plant Anatomy Research Centre, Tambaram, Chennai, India. The specimen was preserved in the Department for future references (PARC/2017/3544).

Plant Processing and Extraction

The whole plant, including root, was washed in tap water to remove the dirt and dust particles. It was shade dried and coarsely powdered and subjected to soxhlet extraction with different solvents (aqueous, ethanol and petroleum ether). Then the extracts were dissolved in the appropriate solvents for further analysis.

Qualitative Analysis

All the three extracts (Aqueous, Ethanol and Petroleum ether) of *Micrococca mercurialis* were subjected to preliminary qualitative phytochemical screening (Harborne, 1998; Kokate, 2001).

Quantification of phytoconstituents

Total Phenol content

The total phenolic content in the extracts were determined using Folin-Ciocalteau reagent (Mcdonald *et al.*, 2001) with Gallic acid as the standard. The colour was measured at 765 nm.

Total Flavonoid content

The flavonoid content was determined (Schanderl and Joslyn, 1970). The colour developed was measured at 415 nm, and quercitin was used as the standard.

Total Tannin content

The amount of tannins in the extracts was determined using Folin's phenol reagent (Schanderl and Joslyn, 1970). The optical density was measured at 640nm with Gallic acid as standard.

Total Vitamin C content

The ascorbic acid content in the extracts was estimated using the DNPH reagent (Omaye *et al.*, 1979). The yellow colour developed was read at 540nm, and the content of vitamin C was calculated by using standard ascorbic acid.

Radical Scavenging potential

DPPH radical scavenging assay:

The radical scavenging activity of *Micrococca mercurialis* extracts were determined using 1, 1- diphenyl-2-picrylhydrazyl (Shimada *et al.*, 1992). The absorbance was recorded at 517nm. The IC_{50} of DPPH radical scavenging activities of the extracts were calculated.

Ferric ion Reducing Antioxidant Power (FRAP) assay

The ferric ion antioxidant power of the extracts was determined using ascorbic acid as a positive control (Benzie and Strain, 1996) and the absorbance was read at 750nm.

In Vitro a nti-Inflammatory activity

Assay of Membrane stabilization (HRBC)

Human red blood cell (HRBC) membrane stabilization potential was done (Gandhisan *et al.*, 1991; Anosike *et al.*, 2012). Blood was collected from healthy individuals. To the collected blood, an equal volume of Alsevers solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride in distilled water) was added, and the solution was centrifuged at 3000 rpm activity for 10 mins to obtain the packed cells. The packed cells obtained was separated, washed with isosaline (0.85%, pH 7.2) and made up to 10% with isosaline. The mixture containing 1ml of the extract (Con. 0.25 - 1mg/ml), 0.5 ml of HRBC suspension, 2ml of hyposaline (0.36%) and 1ml of phosphate buffer (0.15 M, pH 7.4) was considered as the test. The above mixture replaced by 2 ml of distilled of water instead of hyposaline served as control. Diclofenac sodium was used as the reference drug. The mixture was incubated at 37°C for 30 minutes, cooled and centrifuged at 3000rpm for 10 min. The optical density of the supernatant was measured at 560nm. The

Table 1: Yield of different whole plant extracts of *Micrococca mercurialis*

Extract	Yield %
Aqueous	39.59%
Ethanol	13.10%
Petroleum ether	3.00%

Table 2: Preliminary analysis of *Micrococca mercurialis* extracts by qualitative method

Phytoconstituents	Aqueous	Ethanol	Petroleum ether
Phenol	+++	+++	++
Sugars	++	+++	+++
Flavones	++	+++	+++
Glycosides	+	+++	+++
Saponins	++	+	++
Anthraquinone	++	+++	+
Quinone	+	++	+++
Tannins	++	+	-
Steroids	+	+++	++
Cardiac glycosides	++	+++	+++
Tri-terpenoids	+	+++	+++

(+- Mild; ++ - Moderate; +++ - More; - Absent)

Table 3: Effect of different extracts of *Micrococca mercurialis* on radical scavenging and membrane-stabilizing activity

S.No	Extract/ Standard	DPPH (μg/10μl)	FRAP (mg/100ml)	Membrane Stabilisation potential (%)
1	Aqueous	222.89±13.85	55.82±5.47	50.56±3.55
2	Ethanol	131.23±10.41	67.83±3.40	68.05±3.90
3	Petroleum ether	767.22±60.36	62.95±5.78	57.28±5.31
4	Standard Ascorbic acid	71±3.86	82.15±0.40	—
	Diclofenac Sodium	—	—-	89.02±1.50

Values are expressed as Mean ± SEM (n=3)

protection percentage was calculated by using the formula,

Percentage protection (%) = [1- (OD of test / OD of control)] * 100

GC-MS analysis

The analysis of the three extracts (Aqueous, Ethanol, Petroleum ether) of *Micrococca mercurialis* was performed by using a GC-MS (Agilent: GC:(G3440A) 7890A. MS: 7000 Triple Quad GCMS,) with C-18 column (diameter length 30 mm; internal diameter 0.25-mm; thickness 0.25-μm. Initially, the oven temperature was kept stable at 50 _C for 1 min and then allowed to rise at a rate of 40ºC/min to 170ºC (isothermal for 4 min) then at the rate of 10ºC/min to 310ºC (isothermal for 10 min). The temperatures of injector and detector were set at 280 ºC and 260 ºC, respectively. Helium (99.999%) was used as carrier gas at a flow rate of 1 mL/min. 100μl of the sample was dissolved in 1ml of suitable solvents, vortexed for 10 seconds and filtered using microfilter and injected. The compounds were identified by matching GC-MS spectra of the sample with the mass spectra report provided by the library of National Institute of Standards and Technology (NIST05.LIB).

***In Vitro* antimicrobial activity**

The antibacterial activities of the extracts were tested against *Staphylococcus aureus* (MTCC 96) and *Bacillus subtilis* (MTCC 441). The antibacterial activity was determined by well diffusion method (Holder and Boyce, 1994). Antibiotic

Table 4: Chemical constituents identified in the Aqueous extract of *Micrococca mercurialis* by GC-MS analysis

Sl.No	Rt time	Compound name	Area %
1	4.09	Paromomycin	9.88
2	4.16	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)-	17.29
3	4.49	2-Methyl-9-.beta.-d-ribofuranosylhypoxanthine	17.92
4	6.56	Benzeneethanamine, 2,5-difluoro-.beta.,3,4-trihydroxy-N-methyl-	9.85
5	7.14	Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-	12.64
6	9.53	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	5.82

Table 5: Chemical constituents identified in the Ethanol extract of *Micrococca mercurialis* by GC-MS analysis

Sl.No	Rt time	Compound name	Area %
1	4.09	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)-	6.1
2	7.46	Octadecanal, 2-bromo-	4.85
3	9.65	Phytol	18.52
4	10.12	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	2.85
5	12.59	Cyclobarbital	5.03
6	18.32	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	30.46

Azithromycin (30mg/well) served as positive and DMSO as a negative control. The plates were incubated at37°C, and the antibacterial activity was determined by measuring the diameter of the zone of inhibition (mm) after 24 hours of incubation. All the tests were performed in triplicates and results were expressed as mean ± SEM.

Statistical analysis

Statistical analysis was performed using SPSS software (Version 12.0), and the results were expressed as Mean ± Standard Error Mean (SEM).

RESULTS AND DISCUSSION

Percentage yields & Qualitative analysis

The percentage yield of the whole plant aqueous extract *Micrococca mercurialis* was found to be higher (39.59%) when compared with ethanol and petroleum ether extracts (Table 1). The qualitative analysis of the extracts confirmed the presence of phenols, reducing sugars, flavones, glycosides, saponins, anthraquinone, quinones, tannins, steroids, cardiac glycosides, anthocyanin and triterpenoids in variable quantities (Table 2). On comparing the three extracts, ethanol extract was found to contain more secondary metabolites. The variation in the solvent polarity and affinity of phytoconstituents towards specific solvent has made the difference in yield and qualitative analysis.

Antioxidants content

Phenolic content of ethanolic extract of *Micrococca mercurialis* was found to be more (155.12±10.86 μg/mg) when compared to aqueous (84.77±7.68 μg/mg) and petroleum ether (35.28±2.79 μg/mg of extract) extracts (Figure 1). Phenolic compounds donate hydrogen to free radicals, quench singlet oxygen and chelates metallic ions (Rice-Evans *et al.*, 1995). In congruence with the present results, different levels of phenolic compounds were reported when *Ceropegia thwaitesii* leaves were extracted

Table 6: Chemical constituents identified in the Petroleum ether extract of *Micrococca mercurialis* by GC-MS analysis

Sl.No	Rt time	Compound name	Area %
1	4.41	Eicosane, 7-hexyl-	2.59
2	4.82	Heptacosane	7.93
3	5.31	Tetraacetyl-d-xylonic nitrile	1.52
4	5.59	Dihydroxanthin	0.64
5	7.14	Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-	1.02
6	7.47	13-Heptadecyn-1-ol	1.05
7	7.52	Octadecanal, 2-bromo-	1.22
8	8.15	Pentadecanoic acid, 14-methyl-, methyl ester	3.21
9	8.70	Hexadecanoic acid, ethyl ester	0.99
10	9.52	9,12-Octadecadienoic acid (Z,Z)-	4.96
11	9.57	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	12.4
12	9.65	Phytol	7.59
13	9.79	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl] cyclopropyl] methyl]cyclopropyl] methyl]-, methyl ester	1.81
14	10.08	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-	1.87
16	12.59	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)-	2.09
17	18.32	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	20.78

Table 7: Antimicrobial activity of different extracts of *Micrococca mercurialis* against *Staphylococcus aureus*

Extracts	Zone of inhibition (ZOI -mm) Test sample (μg/well)				Azithromycin ZOI (mm) (30μg/well)
Concentration	25	50	75	100	
Aqueous (A)	–	–	–	14.9±0.17	
Ethanol (B)	8.93±0.12	12.03±0.08	14.86±0.18	15.9±0.15	20.16±0.12
Petroleum ether (C)	9.93±0.17	16.1±0.20	16.9±0.05	18.06±0.12	

Values are expressed as Mean ± SEM (n=3)

Table 8: Antimicrobialactivity of different extracts of *Micrococca mercurialis* against *Bacillus subtilis*

Extracts	Zone of inhibition (ZOI -mm) Test sample (μg/well)				Azithromycin ZOI (mm) (30μg/well)
Concentration	25	50	75	100	
Aqueous (A)	8.96±0.08	10.03±0..08	11.13±0.08	13.83±0.92	
Ethanol (B)	8.93±0.12	12.03±0.08	12.86±0.18	15.56±0.31	5.5±0.32
Petroleum ether (C)	9.6±0.34	10.76±0.53	13.23±0.28	13.4±0.26	

Values are expressed as Mean ± SEM (n=3)

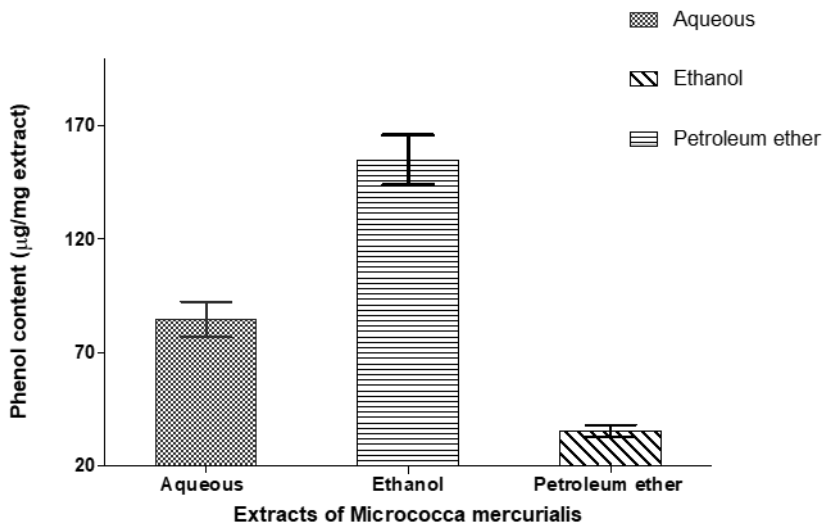

Figure 1: Phenol content of different extracts of *Micrococca mercurialis*

with different solvents (Muthukrishnan *et al.*, 2018). Phenolic compounds are responsible for the antioxidant activities of *Sapium sebiferum* leaves (Fu *et al.*, 2013).

In the present study, the flavonoid content of *Micrococca mercurialis ethanol* extract was abundant 14.29±0.95 μg/mg (Figure 2). The tannin content was least in aqueous extract (17.04±1.24 μg/mg) of *Micrococca mercurialis*, with the highest in petroleum ether and ethanol extracts (Figure 3). Flavonoids and tannins are the major class of phenolic compounds with strong antioxidant potential. The ethanol extract of *Micrococca mercurialis* contains more amounts of flavonoids and tannins due to its better-extracting capacity of the phenolic compounds. The position of hydroxyl groups and the number of double bonds in the carbon ring of the flavonoids influences the antioxidant power (Datta *et al.*, 2019). It was reported that phenolic compounds like flavonoids and tannins have a direct cor-

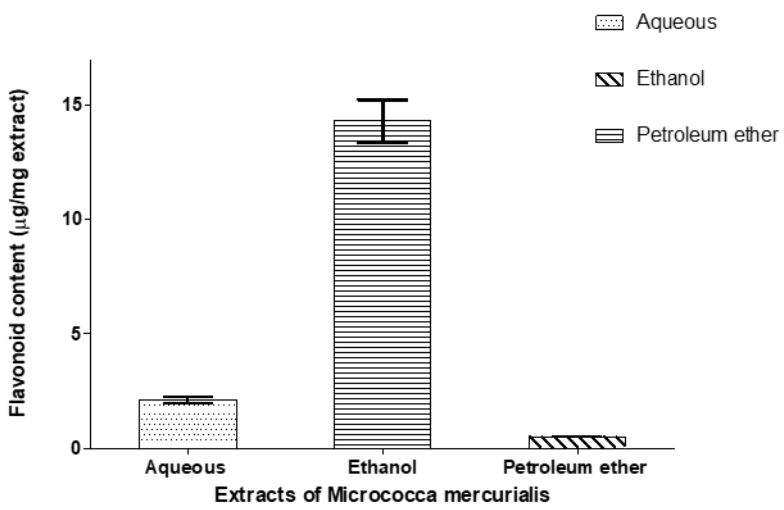

Figure 2: Flavonoid content of different extracts of *Micrococca mercurialis*

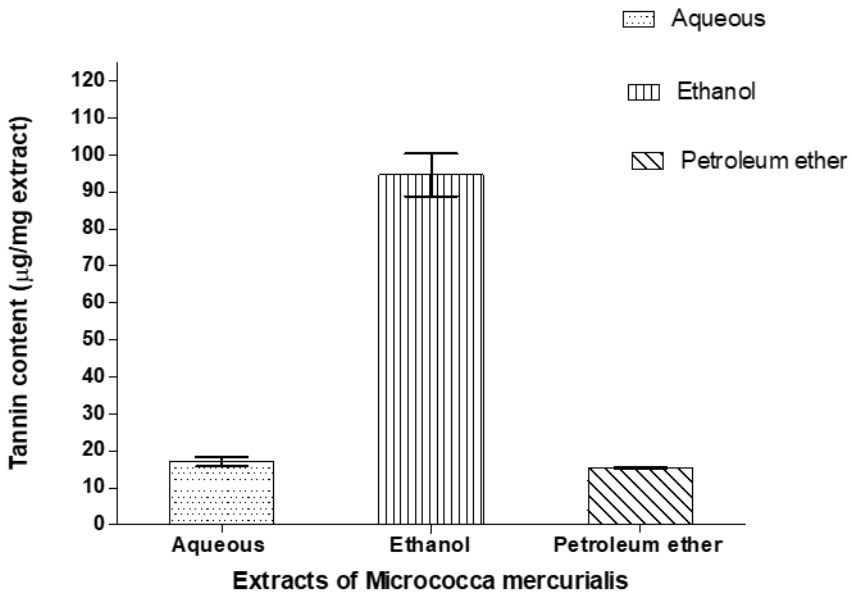

Figure 3: Tannin content of different extracts of *Micrococca mercurialis*

relation in inhibiting the death caused by oxidative damage (Panat *et al.*, 2016).

Compared to aqueous and petroleum ether extracts, the ethanol extract of *Micrococca mercurialis* has more amount of Vitamin C (Figure 4). The content of Vitamin C was found to more in ethanolic extract of *Micrococca mercurialis*, which would confer the reducing ability to the plant. Thus reductones donate hydrogen atom and break the radical forming reaction (Karuna *et al.*, 2018).

Radical Scavenging potential

In the present study ethanolic extract of *Micrococca mercurialis* plant was found to scavenge the DPPH radical better (IC_{50}=131.23±10.41 μg/10μl) when compared with aqueous (222.89±13.85 μg/10μl) and petroleum ether extracts (767.22±60.36 μg/10μl) (Table 3). The DPPH potential of standard ascorbic acid was found to be IC_{50}=55.71±3.86 μg/10μl). Discolouration of DPPH free radical from purple to yellow determines the hydrogen donation capacity of the antioxidants. In the present study, the observed better scavenging potential of ethanol extract was attributed to the higher amount of phenols and flavonoids when compared with other extracts. The scavenging role of methanolic extract of the *Moringa oleifera* leaves against DPPH radical confirms their antioxidant potential (Uzma, 2019).Thus the free radical scavenging potential of *Micrococca mercurialis* shows that it is a rich source of antioxidants.

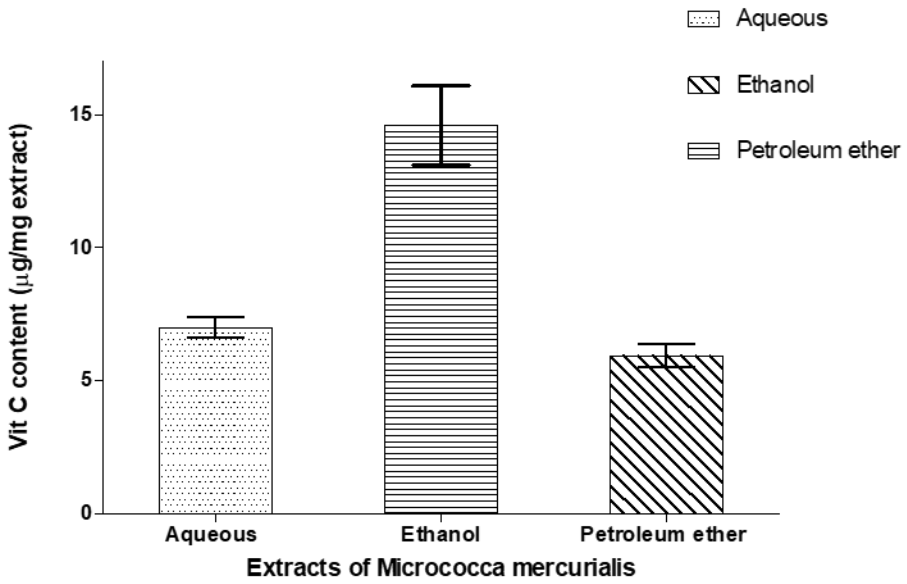

Figure 4: Vitamin C contentof different extracts of *Micrococca mercurialis*

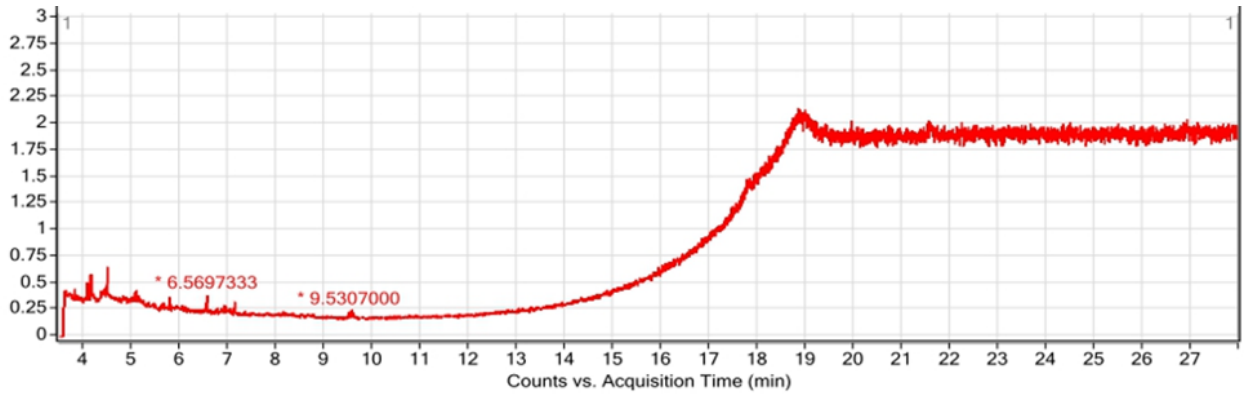

Figure 5: GC-MS chromatogram of aqueous extract of *Micrococca mercurialis*

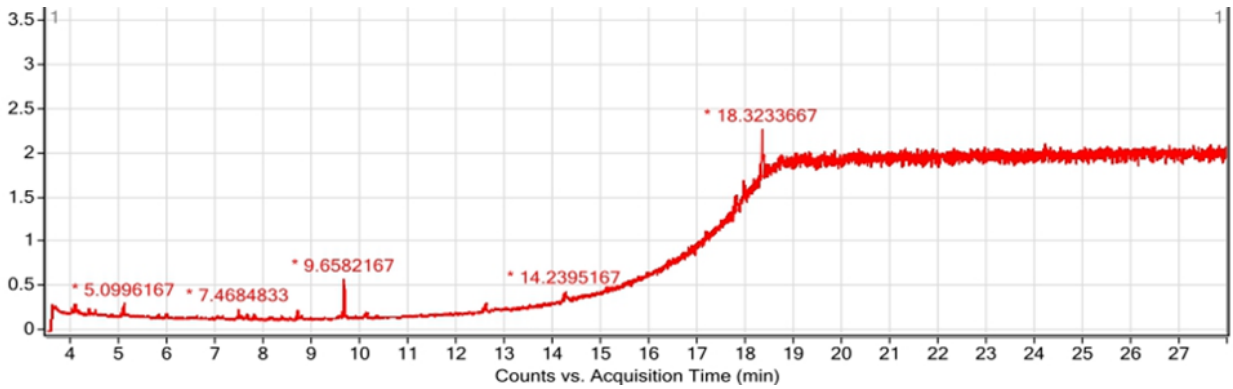

Figure 6: GC-MS chromatogram of ethanol extract of *Micrococca mercurialis*

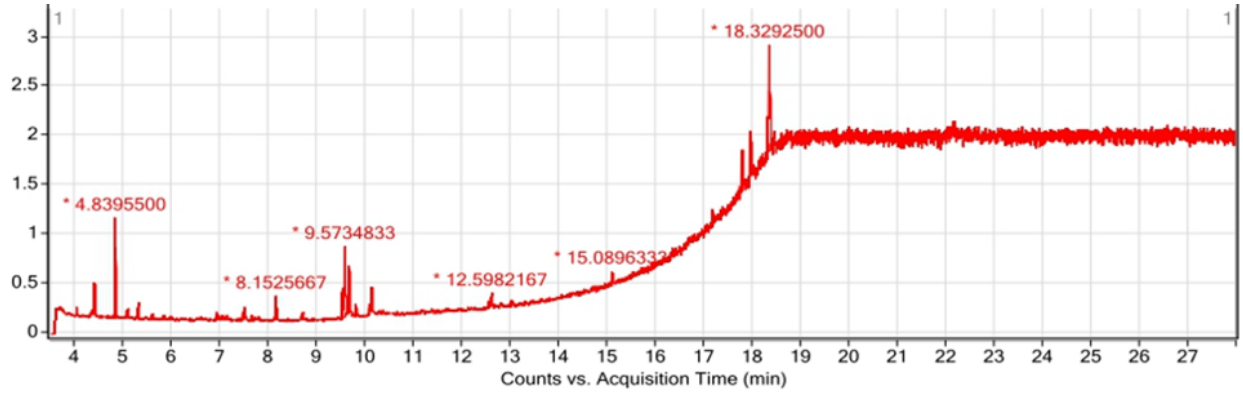

Figure 7: GC-MS chromatogram of petroleum ether extract of *Micrococca mercurialis*

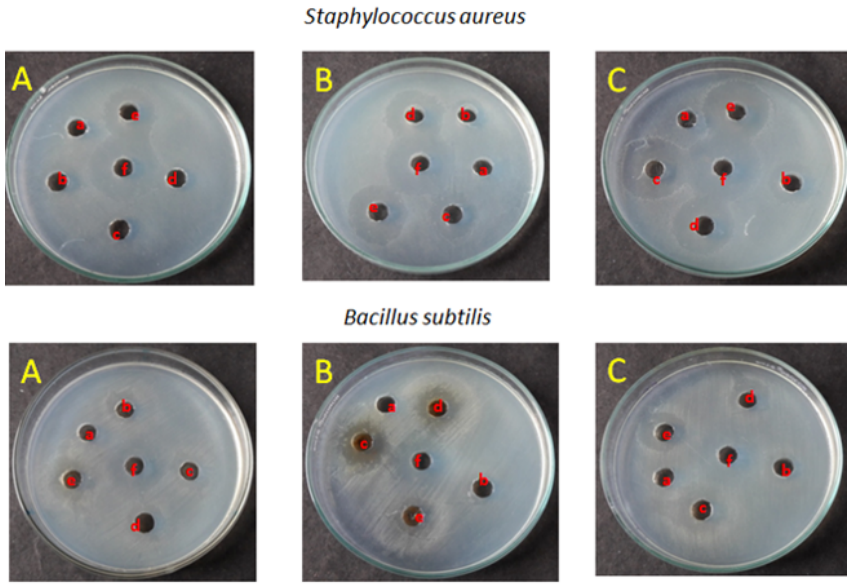

Figure 8: Antibacterial potential of *Micrococca mercurialis* wholeplant extracts against *Staphylococcus aureus* and *Bacillus subtilis* (Aqueous (A), Ethanol (B), Petroleum ether (C))

Table 3 shows the reducing potential of *Micrococca mercurialis extracts* on ferric tripyridyltriazine [Fe3+-TPTZ] complex. The reducing power of ethanolic extract of *Micrococca mercurialis was* found to be 67.83±3.40% followed by petroleum ether and aqueous extracts. The antioxidants provide the electrons to reduce 2, 4, 6- triperidyl-s-triazine complex in acidic medium to blue coloured ferrous complex in FRAP assay. The better reducing potential of ethanolic extract of *Micrococca mercurialis* is accordant with the high phenols and flavonoids in ethyl acetate extract of *Ferula caspica* (Cigdem *et al.*, 2019). In the present study, the presence of antioxidants like phenols, flavonoids and tannins in *Micrococca mercurialis* confirms the health benefits and its potential to combat various diseases caused by ferric ion radical.

HRBC stabilization potential

The ethanol extract offers maximum protection of HRBC membrane (68.059±3.90%), followed by petroleum ether extract 57.28±5.31% and minimum protection by aqueous extract 50.56±3.55% (Table 3). Diclofenac sodium used as standard reference drug showed 89.02±1.50 % protection. The anti-inflammatory property of *Micrococca mercurialis* extracts was best studied by HRBC membrane stabilization method as it mimics the lysosomal membrane. Activated nueutrophils release the lysosomal contents like bactericidal enzymes and proteases leading to the tissue damage on the extracellular site resulting in acute and chronic inflammation. The membrane stabilizing protection exhibited by *Micrococca mercurialis* extracts shows that it could stabilize the lysosomal membrane and could serve as an anti-inflammatory agent. This activity will be corroborated with the flavonoids present in the ethanolic extract of *Micrococca*. The role of flavonoids in attenuating the inflammatory responses that leads to diseases was reported (Kim *et al.*, 2004).

GC-MS analysis of extracts

In the present study, a total of 29 compounds were reported in all the extracts. Six in aqueous and ethanol extracts and seventeen in petroleum ether extract (Tables 4, 5 and 6). The chromatograms of different extracts of *Micrococca mercurialis* were shown in Figures 5, 6 and 7.

In an aqueous extract of *Micrococca mercurialis,* six compounds were present. Among them, Paramomycin (9.98%) serve as an antibacterial agent (Huda, 2015), 5,8,11,14-Eicosatetraenoic acid methyl ester (5.82%) is an unsaturated fatty acid ester with cardioprotective, hypocholesterolemic, anticoronary and anticancer potential (Prabakaran *et al.*, 2014). Cyclobarbital (5.03%) found only in the ethanolic extract has antimicrobial and anticancerous effects (Rajendran *et al.*, 2017). Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl] is an alkaloid with antimicrobial and anti-inflammatory (Dhayabaran and Thangarathinam, 2016) was observed in aqueous (9.85%) and petroleum ether extracts (1.02%) of *Micrococca mercurialis*. Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl is an volatile organic compound reported in both ethanol (30.46%) and petroleum ether (20.78%) extracts possess antimicrobial property (Venkatesh *et al.*, 2014).

The presence of diterpene phytol in ethanol (18.52%) and petroleum ether (7.59%) extracts has significant antimicrobial, anti-inflammatory, anticancer and diuretic potential (Dhayabaran and Thangarathinam, 2016). Octadecanal,2-bromo with anti-inflammatory and antiapoptotic effects (Huda, 2015) was found in ethanol (4.85%) and petroleum ether (1.22%) extracts of *Micrococca mercurialis*. Hexadecanoic acid, ethyl ester (0.99%) present only in petroleum ether extract has antioxidant, hypocholesterolemic, nematicide, pesticide, anti-androgenic flavor, hemolytic 5-Alpha reductase inhibitor and antimicrobial properties (Musa *et al.*, 2015; Nishanthini *et al.*, 2014). Ethanol, 2-(9-Octadecenyloxy) (1.87%) found uniquely in petroleum ether extract was reported to possess anticancer, antigonorrheal, and antireverse transcriptase (Huda, 2015).

The unsaturated fatty acids 9,12-Octadecadienoic acid (Z, Z)-linoleic and 9,12,15-Octadecatrienoic acid, (Z, Z, Z)- linolenic were detected only in petroleum extracts of *Micrococca mercurialis.* Linoleic acid possess hypocholesterolemic, nematicide, 5-Alpha reductase inhibitor, antihistaminic, anticoronary, insectifuge, antieczemic and anti-acne properties (Nishanthini *et al.*, 2014).Palmitic acid methyl ester, Pentadecanoic acid 14-methyl-, methyl ester (3.21%), present in the petroleum ether extract was found to exhibit antioxidant, antifungal and antimicrobial activities (Elaiyaraja and Chandramohan, 2016). Alpha-glucosidase inhibitor activity (Elaiyaraja and Chandramohan, 2016) was shown by 5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)- (5.82%) present in the aqueous extract of *Micrococca mercurialis.*

9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)- (2.85%) reported only in ethanol extract exhibits analgesic, antipyretic, anticonvulsant and antiseptic properties (Srivastava *et al.*, 2015). In the petroleum ether extract, the presence of Tetraacetyl-d-xylonic nitrile (1.52%) with antimicrobial and antioxidant potential and 13-Heptadecyn-1-ol (1.05%) with anti-inflammatory and antifungal properties (Al-Rubaye *et al.*, 2017) were reported. Heptacosane (7.93%), the alkane was present in the petroleum ether extract acts as an antioxidant (Marrufo *et al.*, 2013). No activities were reported for the phenolic amine compound benzeneethanamine, 2,5-difluoro-.beta.,3,4-trihydroxy-N-methyl (9.85%) found in the aqueous extract and for 9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)- found in all three extracts. Thus GC-MS results have confirmed the presence of bioconstituents in aqueous, ethanol and petroleum ether extracts of *Micrococca mercurialis*. The presence of these phytocompounds is the possible reason for the exhibited pharmacological activities by *Micrococca mercurialis.*

Antibacterial activity

The petroleum ether extract was found to be very effective in inhibiting both the strains *Staphylococcus aureus* and *Bacillus subtilis*. The zone of inhibition against *Staphylococcus aureus* was found to be maximum (18.06±0.12mm) at 100μg/well with when compared with ethanol extract (15.9±0.15mm) and aqueous extract showed the minimum zone of inhibition (14.9±0.17 mm) (Tables 7 and 8 and Figure 8). The positive control Azithromycin at 30μg/well showed the maximum zone of inhibition (20.16±0.12 mm) against *Bacillus subtilis.*

In the present study, different extracts of *Micrococca mercurialis* exhibited the varied extent of antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*. Ethanol and petroleum ether extracts of *Micrococca mercurialis* showed maximum inhibition. The hexane extract of *Avicennia alba* leaves were able to inhibit the growth of *S.aureus* and

B.subtilis (Eswaraiah *et al.*, 2019). Medicinal plants are a surfeit of phytochemicals with varied pharmacological potentials. In the present study, the better antimicrobial properties of the ethanol and petroleum ether extracts of *Micrococca mercurialis* can be directly corroborated with their phytoconstituents like phenol, tannins, flavonoids and other bioactive constituents identified by phytochemical and GC-MS analysis. Many investigators have reported the direct relationship between the phytoconstituents and antimicrobial potential of medicinal plants (Bhat and Rajanna, 2017).

CONCLUSION

The present results indicated that the presence of important phytoconstituents with antioxidant and antibacterial potential in different extracts of *Micrococca mercurialis*. The results confirm their potential application in biomedical and pharmacological studies. This pilot study has provided a scientific insight for the folkloric use of *Micrococca mercurialis* against many infections and diseases. *In vivo* study is warranted to evaluate its potential against inflammatory diseases due to oxidative stress.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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