

Phytochemical Studies, GC-MS Investigation and Antibacterial Activity of Indian Medicinal Plant Extracts of *Coleus aromaticus* against Nosocomial Pathogens

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ABSTRACT

There is continuous discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and reemerging infectious disease. In recent years, drug resistance to human pathogenic bacteria has from all over the world. Plant-based therapies are an excellent alternative for antibiotics began to be offered in all the countries, the extracts of medicinal plants still used in traditional medicine. Plants have been the predominant source of medicine with eminence over millennia. Phytochemicals are the chemicals extracted from plants. Depends on the role in the plant metabolism the organic chemicals were classified as primary and secondary constituents. Phytochemical and GC-MS analysis act as an interesting tool in the evaluation of active principles in the extract obtained from the herbs utilized in the treatment against various clinical pathogens. The antibacterial activity of *Coleus aromaticus*, plant extract in different solvents such as ethanol, methanol, chloroform and petroleum ether of plant extracts against the *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* nosocomial pathogens.

Key words: *Coleus aromaticus*, Phytochemical studies, GC-MS analysis, Antibacterial activity, Nosocomial pathogens

Plants are emerging as a potential source of new drugs. Natural products derived from plants utilized as clinically effective potent and powerful drugs in the developing nations [1-2]. Plants gained more attention by the researcher due to the development of antimicrobial resistance nature of commonly used antibiotics. Over thousands of year's plant and plant extracts have been used in the treatment of skin disorders as antiseptics and antimicrobial agents [3-4].

Globally Four billion peoples of the world population still rely on herbal medicines as their primary source of health care in their treatment practice especially in economically developing nations [5]. A wide range of medicinal plants with its extract possessing various medicinal properties used as raw drugs. The raw drug was used by the local communities and folk healers in small quantity and it was traded commercially in larger quantities by many herbal industries [6-7].

Current research on natural molecules and its derivatives was focused on the plants with high ethno-medicinal values [8], the traditional medicine use plants that contain a wide range of substances used in the treatment of chronic infection as well as infectious diseases [9-10]. Clinical microbiologists paid more interest in the discovery of new therapeutics by screening medicinal plants. The main property of a natural drug extracted from plants is their secondary metabolites. The antimicrobial activity of plant is determined

by different components includes aldehyde and phenolic compounds [11-13]. Abuse and over usage of the antibiotics develop drug resistance in human pathogens has necessitated a search of new source includes plants or its products that possess antimicrobial nature. New compounds extracted from medicinal plants was Screened for antimicrobial activities is essential for therapeutic use [14].

A key source of indigenous pharmaceutical systems was Medicinal plants. WHO states, about 65–80% of the world's population in developing countries, due to the poverty and lack of access to modern medicine, depend essentially on plants for their primary healthcare [15]. The natural products are often selected for biological screening supported ethno-medicinal use of plants, because many infectious diseases are known to possess been treated with herbal remedies throughout the history of mankind. Even today, in many developing countries natural plant products pursue a lead role in primary early care as therapeutic remedies [16-17].

The most important herbs used in the Indian Auryedic medicine *Coleus aromaticus* which recently called as *Plectranthus amboinicus* (Lour) belongs to Lamiaceae family is a folkloric medicinal plant. The extract of leaf and stem exhibit phytochemical properties like antioxidant activity, antiosteoporosis activities, antidiabetic immunostimulatory, antitumor, anti-inflammatory and antimicrobial activity [18] possess Antimicrobial activity, Antifungal activity, Anti-inflammatory activity, Antibacterial activity, Antidiabetic activity, Anxiolytic activity, Diuretic activity, Antineoplastic activity, Respiratory disorder), Wound healing activity, Analgesic activity, Antiuro lithiatic activity, Antiplatelet

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aggregation activity, Antibiofilm efficacy and It is used to treat chronic cough, asthma, epilepsy, bronchitis, helminthiasis, colic, convulsions, dyspepsia, diarrhea, nervous tension, insect bites, toothache, earache, rheumatism, whooping cough, bronchitis, malarial fever, hepatopathy, renal and Rheumatoid arthritis [19-23].

‘Nosocomial’ or ‘hospital-acquired infection’(HAI) or ‘healthcare associated infections’ (HCAI) appear in a patient under medical care in the hospital or other health care facility which was absent at the time of admission and emerges after 48-72 hours or 3 days during the hospital stay. Bacteria, viruses and fungal parasites are the main cause of Nosocomial infections. WHO states, approximately 15% of all patients under hospitalization acquired HAI [24]. The most common microbial agents of HAI are *Staphylococcus aureus* [25], *Pseudomonas aeruginosa*, *Enterobacteriaceae* [26], *E. coli* [27], *B. cereus*, *M. tuberculi*, *Streptococcus spp*, *Acinetobacter spp*, *Legionell* [28], *Candida* [29], *Aspergillus* [30], *Fusarium*, *Trichosporon* and *Malassezia* [31]. HAI infections show variations in Epidemiological and etiological characteristics among various countries [32-33].

Nosocomial infections are the major causes of death and increased morbidity and mortality. Nosocomial infections can cause severe pneumonia and the major site of infection is surgical site, gastrointestinal tract, urinary tract, bloodstream and other parts of the body [34] (Magill *et al.* 2018). These types of infections are difficult to treat with previous generation antibiotics due to the transfer of antibiotic resistance to Gram negative bacteria and can infect people outside the hospital [35-36]. Hospital waste serves as a potential source of pathogens and about 20%-25% of hospital waste is termed as hazardous [28].

The most common etiological agent of nosocomial infection is bacteria like *S. aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *P. aeruginosa*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Acinetobacter*, *Proteus mirabilis*, and *Enterococci* and antibiotic resistant bacteria which is outstandingly present in hospitals like MRSA and VRSA [37-39]. The aim of the present research work was to study the Phytochemical, GC-MS analysis and antibacterial activity of *Coleus aromaticus* against common nosocomial pathogens.

MATERIALS AND METHODS

Collection and preparation of plant extract

The selected medicinal plant *Coleus aromaticus* were collected from different locations of Kollihills, Namakkal, Tamil Nadu, India. The collected plants were authenticated by Botanical Survey of India (BSI-Southern Circle) - Government of India, Coimbatore, Tamil Nadu. The plant Authentication reference letter No: BSI/SRC/5/23/2019-Tech/3135. The plant authenticated report and specimen was deposited in Microbiology Department, Muthayammal College of Arts and Science, Rasipuram, Tamil Nadu, India.

Processing and extraction

Garbling process

Garbling done by manually to separate a particular portion of a plant dried from other parts of the plant and other extraneous matter.

Drying process

After harvesting, all leaves had moisture content of 60–80% and could not be stored without drying to avoid break down of important compounds and contamination by

microorganisms. In room temperature the leaves collected from the plant was shadow dried. By processing proper drying the moisture content present in the plant product was reduced to 14%.

Grinding process

All the collected dried leaves were ground into fine powder by Mechanical shearing and sieving.

Extraction of active components

About 20g of the finely ground leaf powder was weighed and mixed with 100ml of ethanol, methanol, petroleum ether and chloroform in separate conical flasks and kept overnight. The filtrate was obtained by filtering these contents twice using No. 1 Whatman filter paper. The clear filtrate was condensed using rotary vacuum evaporator at 50°C for about 15 minutes.

Phytochemical analysis of plant extracts

The leaf extract that exhibited the maximum antimicrobial activity was assessed for the presence of phytochemicals. A preliminary phytochemical study was performed to seek out the presence of phytochemicals, like alkaloids, flavonoids, saponins, carbohydrates, proteins, phenols, steroids, glycosides, and tannins. The phytochemical analysis was performed supported color reactions with plant extracts, using the subsequent procedure described by [40-41].

Alkaloids (Meyor's test)

To 1 ml of the acidic aqueous extract, few drops of Meyor's reagent were added. Presence of alkaloids was confirmed by the formation of white or pale-yellow precipitate.

Flavonoids

In a test tube containing 0.5ml of aqueous extract, 5-10 ml of diluted HCl and a small amount of zinc or magnesium powder was added and the solution was boiled for few minutes. Presence of flavonoids was confirmed by the formation of reddish pink or dirty brown colour.

Saponins

In a test tube containing about 5ml of aqueous extract, a drop of sodium bicarbonate solution was added. The mixture was vigorously shaken for 3 minutes. Presence of saponins was confirmed by the formation of honey comb like froth appearance.

Carbohydrates (Fehling's test)

To a test tube containing 5 ml of aqueous extract about 1 ml of Fehling's solution was added, the contents were then boiled for few minutes. Presence of carbohydrate was confirmed by the formation of red or brick red precipitate.

Proteins (Biuret's test)

To 1ml of hot aqueous extract of the leaves, 5-8 drops of 10% sodium hydroxide solution, followed by 1 or 2 drops of 5% copper sulphate were added. Presence of proteins was confirmed by the formation of red or violet colour.

Phenols (Ferric chloride test)

To 1ml of alcoholic extract, 2ml of distilled water followed by few drops of 10% aqueous ferric chloride solution was added. Presence of phenols was confirmed by formation of blue or green colour.

Steroids (Solkowsky's test)

To the sides of the test tube add 2ml of chloroform extract and 1 ml of concentrated sulphuric acid. Formation of red colour in the chloroform layer confirms the presence of steroids.

Glycosides

To a small amount of aqueous extract, a few drops of aqueous NaOH solution was added. Presence of yellow colour confirmed glycosides.

Resins

To 2 ml of chloroform or alcoholic extract, about 5-10 ml of acetic anhydride was added and dissolved by gentle heating. The solution was cooled and about 0.5 ml of concentrated H₂SO₄ was added. Appearance of bright purple colour which immediately changed into deep violet colour confirmed the presence of resins.

Tannins (Ferric chloride test)

To 1-2ml of an alcoholic extract, few drops of 5% aqueous ferric chloride solution were added. A bluish black colour, which disappeared on addition of few ml of dilute sulphuric acid, was followed by the formation of a yellowish-brown precipitate which indicated the presence of tannins.

Test organisms

The four test bacteria used in the study such as, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were isolated from nosocomial sample. The clinical Nosocomial isolates obtained from the Microbiology Laboratory of at Namakkal. Processing of the specimen was started immediately once it reaches the laboratory of microbiology. 50 ml of sterile nutrient broth was prepared, the samples containing cotton swab was inoculated into the broth and incubated at 37°C for 24 hours. After incubation the samples were inoculated on the nutrient agar medium and incubated at 37°C for 24 hours. The organisms *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Serratia marcescens* fresh subcultures were made before use.

Antibacterial activity of *Coleus aromaticus* against isolated Nosocomial pathogens

The antimicrobial activity of the leaf extracts was evaluated by agar disc diffusion method test organisms, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* with 0.5 McFarland standards were spread over on the Muller Hinton agar plates by using separate sterile cotton swabs. The sterile disc was prepared and coated with 30 µg concentration of plant extracts. The prepared extracted discs were placed on medium with standard Tetracycline disc with 30 µg concentration. At 37°C the inoculated plates was kept for 24 hours incubation. The zone of inhibition of bacterial growth was measured and compared with standard antibacterial agents [42-43].

Gas chromatography-mass spectroscopy (GC-MS)

Identification of the main component was carried out by the comparison of both the GC retention times and MS data against those of the reference standards. A Hewlett Packard GC-MS system were used for research work, a model 5890A gas chromatograph, a model 5970B mass selective detector, a HP 5970C MS chemstation, and a HP 7946-disc drive. The fused-silica capillary column coated with HP-5 cross-linked with 5% phenylmethylsilicone was used. The GC temperature

program was as follows: initial temperature was 100°C, held for 1 min, increased to 130°C at a rate of 2°C/min, then to 200°C at a rate of 3°C/min, and finally to 280°C at a rate of 6°C/min and held for 10 min. The split ratio was 1:12, injection temperature was 250°C, transfer line temperature was 270°C, and ion source temperature was 200°C. The mass spectrometer was operated at 70 eV in the electron impact mode with SCAN or SIM. In the present research, the bulk herbal extract was analyzed for their chemical constituents by GC-MS [44-45].

RESULTS AND DISCUSSION

Phytochemical analysis of *Coleus aromaticus*

Phytochemical analysis of *Coleus aromaticus* was carried out by the following solvents Methanol, Ethanol, Petroleum ether and Chloroform. In Methanol extract of *Coleus aromaticus* contain all the components, CHO, Alkaloids, flavonoids, tannin, terpenoids, glycosides, phenolic compounds and steroids are present. Whereas the Ethanol extract of *Coleus aromaticus* contain all the constituents except CHO. The presence of Alkaloids, flavonoids, tannin, terpenoids, glycosides, phenolic compounds and steroids are noted. In Chloroform extract of *Coleus aromaticus* contain CHO, Alkaloids, flavonoids, terpenoids, glycosides, and steroids are present. Tannin and phenolic compounds are absent. Petroleum ether extract of *Coleus aromaticus* contains Steroids, flavonoids, tannin and Sugars are present. The predominant components present in all the extracts of *Coleus aromaticus* are flavonoids, Terpenoids and steroids (Table 1).

Table 1 Preliminary phytochemical screening of various extracts of the leaves of *Coleus aromaticus*

Constituents	Ethanol	Methanol	Petroleum ether	Chloroform
Alkaloids	+++	+	-	+
Flavonoids	+++	++	+	+
Tannin	+	++	+	-
Carbohydrate	-	+	-	+
Terpenoids	++	+	++	+
Glycosides	+++	+	-	+
Steroids	++	+	+	++
Phenols	+	+	-	-

+++Abundantly present, ++Moderately present, +Present, -Absent

The phytochemicals found in the *Coleus aromaticus* are carvacrol, thymol, α- humulene undecanal, P-cymene, caryophyllene oxide, α- terpineol and β- selinene [46-48]. Another analysis obtained thymol, carvacrol, 1,8-cineole, eugenol, caryophyllene, erpinolene, α- pinene, β- pinene, methyl eugenol, and β- phellandrene. The variations can be attributed to the methodology used in the extraction process, seasonal variations, soil type, climate, genetic and geographical variations of the plants [49]. It also contain Xanthophylls [50].

Antibacterial activity of *Coleus aromaticus*

The antibacterial effects of plant extract of *Coleus aromaticus* had been investigated against isolated *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. The disc prepared with 30µg concentration of the plant extract was placed on Muller-Hinton agar plates with standard antibiotic tetracycline disc. The standard antibiotic tetracycline disc (30µg) impregnated

on the Muller-Hinton agar plate exhibit the antibacterial activity with the zone of inhibition (22mm), against *Staphylococcus aureus*. The antibacterial activity of the ethanol extract of *Coleus aromaticus*, at the 30µg concentration was high (15mm and 11mm) against *S.aureus* and *Klebsiella pneumonia* respectively. Minimum activity was recorded (10mm, 10mm) against *Escherichia coli*, *Pseudomonas aeruginosa*. The antibacterial activity of chloroform extracts of *Coleus aromaticus*, at 30µg concentration the inhibition zone was high (11mm) against

Pseudomonas aeruginosa. Whereas minimum activity (08mm) against *Staphylococcus aureus*. The antibacterial activity of methanol extracts of *Coleus aromaticus*, at 30µg concentration the inhibition zone was high (10mm) against *S. aureus*. Whereas minimum activity (2mm) against *Pseudomonas aeruginosa*. In petroleum ether extracts of *Coleus aromaticus*, not inhibit the growth of bacteria. (Table 2). The results plainly reveal that the growth of the bacteria was inhibited by the ethanol and chloroform extracts of *Coleus aromaticus* (Fig 1).

Table 2 Antibacterial activity of *Coleus aromaticus* extracts against isolated organisms

Organisms	Concentration of extract and zone of inhibition (mm)				
	Tetracycline	Methanol extract	Ethanol extract	Chloroform extract	Petroleum ether extract
<i>Staphylococcus aureus</i>	22	10	15	08	-
<i>Klebsiella pneumoniae</i>	-	-	11	10	-
<i>E. coli</i>	-	06	10	10	-
<i>Pseudomonas aeruginosa</i>	-	02	10	11	-

Table 3 Analysis of active ingredient of *Coleus aromaticus* extract by GC-MS

R.time	Compound name	Molecular formula	Molecular weight	Area %
7.10	Monobenzylidene-d-glucose	C ₁₃ H ₁₆ O ₆	268	1.76
30.28	Prosta-5,13-dien-1-oic acid, 9,11,15-tris[(trimethylsilyl)oxy]-, trimethylsilyl ester, (5Z,9à,11à,13E,15S)-	C ₃₂ H ₆₆ O ₅ Si ₄	642	2.58
30.69	Quinine, trimethylsilyl ether	C ₂₃ H ₃₂ N ₂ O ₂ Si	396	1.37
31.03	Estra-1,3,5(10)-trien-6-one, 3,16,17-tris(acetyloxy)-, 6-(O-methyloxime), (16à,17à)-	C ₂₅ H ₃₁ NO ₇	457	4.92
31.43	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	C ₁₆ H ₅₀ O ₇ Si ₈	578	3.94
31.64	Dipyridamole	C ₂₄ H ₄₀ N ₈ O ₄	504	2.19
32.22	Aspidospermidine-1-ethanol, 17-hydroxy-16-methoxy-à-methyl-	C ₂₃ H ₃₄ N ₂ O ₃	386	6.18
33.05	7-Chloro-3-[3,4-dichlorophenyl]-1-[[3-[dimethylamino]propyl]imino]-10-hydroxy-2-methyl-1,2,3,4,9,10-hexahydro-9-acridinone	C ₂₅ H ₂₆ C ₁₃ N ₃ O ₂	505	1.54
33.32	Stearic acid, 3-(octadecyloxy)propyl ester	C ₃₉ H ₇₈ O ₃	594	2.30
33.60	1,2,8-Trihydroxy-6-methoxy-3-methylanthraquinone, O,O',O"-tris(trimethylsilyl)	C ₂₅ H ₃₆ O ₆ Si ₃	516	2.31
33.78	3,9à;14,15-Diepoxy-pregn-16-en-20-one, 3,11à,18-triacetoxy-	C ₂₇ H ₃₄ O ₉	502	2.38
34.41	Octadecane, 1,1'-[1,3-propanediylbis(oxy)]bis-	C ₃₉ H ₈₀ O ₂	580	5.78
34.98	Hydromorphone pfp	C ₂₀ H ₁₈ F ₅ NO ₄	431	4.56
35.17	(5à)Pregnane-3,20à-diol,14à,18à-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-,Diacetate	C ₂₈ H ₄₃ NO ₆	489	1.35
35.69	2-Benzo[1,3]dioxol-5-yl-8-methoxy-3-nitro-2H-chromene	C ₁₇ H ₁₃ NO ₆	327	2.72
36.11	2à,4a-Epoxy-methylphenanthrene-7-methanol,1,1-dimethyl-2-methoxy-8-(1,3-dithiin-2-ylidene)methyl-1,2,3,4,4a,4b,5,6,7,8,8a,9-dodecahydro-, acetate Acetic acid,	C ₂₇ H ₃₈ O ₄ S ₂	490	1.83
36.19	9-Desoxy-9x-chloroingol 3,7,8,12-tetraacetate	C ₂₈ H ₃₉ ClO ₉	554	3.40
36.76	1-Monolinoleoylglycerol trimethylsilyl ether	C ₂₇ H ₅₄ O ₄ Si ₂	498	3.12
37.43	3-Bromopiperidin-2-one	C ₅ H ₈ BrNO	177	23.42
38.13	3,9à;14,15-Diepoxy-pregn-16-en-20-one, 3,11à,18-triacetoxy-	C ₂₇ H ₃₄ O ₉	502	3.41
38.47	1,3-Dioxane, 5-(hexadecyloxy)-2-pentadecyl-, trans-	C ₃₅ H ₇₀ O ₃	538	2.59
38.82	1,1,2,2-Tetrakis(adamant-1-yl)ethane	C ₄₂ H ₆₂	566	3.27
39.20	Prost-13-en-1-oic acid,9-(methoxyimino)-11,15-bis[(trimethylsilyl)oxy]-, trimethylsilylester, (8.xi.,12.xi.)-	C ₃₀ H ₆₁ NO ₅ Si ₃	599	1.88
39.43	9,10-Anthracenedione,1-(methylamino)-4-[(4-methylphenyl)amino]-	C ₂₂ H ₁₈ N ₂ O ₂	342	4.36
39.83	5-Carbethoxysuccinylacetone, O,O,O-tris(trimethylsilyl)-	C ₁₉ H ₃₈ O ₆ Si ₃	446	4.32
40.04	4-(9-Acridinyl)-N,N-dimethylbenzenamine	C ₂₁ H ₁₈ N ₂	298	2.52

The leaf extracts of *Coleus aromaticus* have shown to have antibacterial activity [51]. Methanolic extract of *Coleus*

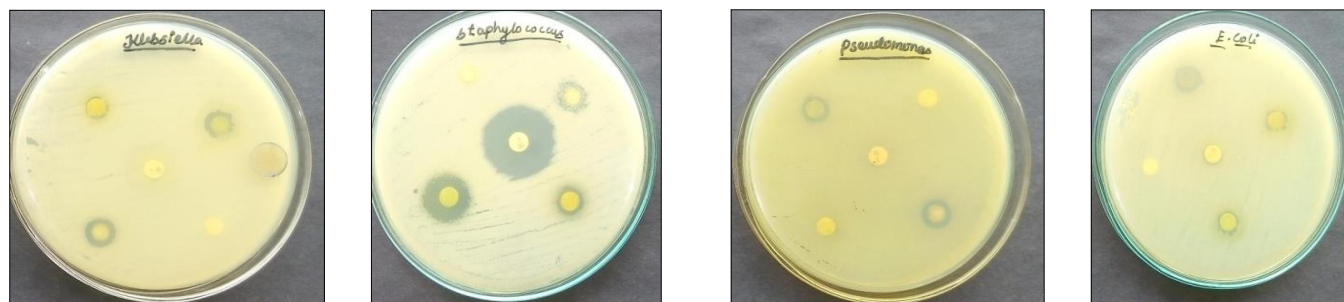
amboinicus leaves shows antibacterial activity against Gram negative pathogens includes *Escherichia coli*, *Klebsiella*

pneumoniae, *Citrobacter divergens*, *Shigella flexneri*, *Salmonella paratyphi* A, *Salmonella paratyphi* B, *Proteus mirabilis* and *Pseudomonas aeruginosa* and Gram-positive *Staphylococcus aureus*, Methicillin resistant *Staphylococcus aureus* and Enterococci and [52].

Antibacterial activity of aqueous methanol extract of *Coleus amboinicus* against the *Bacillus subtilis* and *Staphylococcus aureus* and the *Escherichia coli* and *Pseudomonas aeruginosa* have reported by [53]. The plant extracts as well as the leaf oil of *Coleus amboinicus* exhibited antibacterial activity against clinical isolates of *Proteus mirabilis* and *Pseudomonas aeruginosa*. The *Coleus aromaticus* leaf extract inhibited human pathogenic

microorganisms, like *Bacillus subtilis*, *Escherichia coli*, Methicillin Resistant *Staphylococcus aureus* (MRSA), *P. vulgaris*, *S. marcescens*, *Staphylococcus aureus* and *S. epidermidis* [54-55].

Plectranthus amboinicus essential oil alters the membrane permeability and anti-bacterial effect on its double concentration of MIC against *Klebsiella pneumoniae*. In addition, *Plectranthus amboinicus* leaf oil potentialized the antibiotic activity against *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris* and *Bacillus cereus* with standard antibiotics like amikacin, kanamycin and gentamicin. Methanol extract using 1µml using antibiotic activity for this 5 bacteria's, and 1mg of Amikacin drug is used [56-58].



Klebsiella pneumoniae

Staphylococcus aureus

Pseudomonas aeruginosa

E. coli

Fig 1 Antibacterial activity of *Coleus aromaticus* extracts against nosocomial pathogens

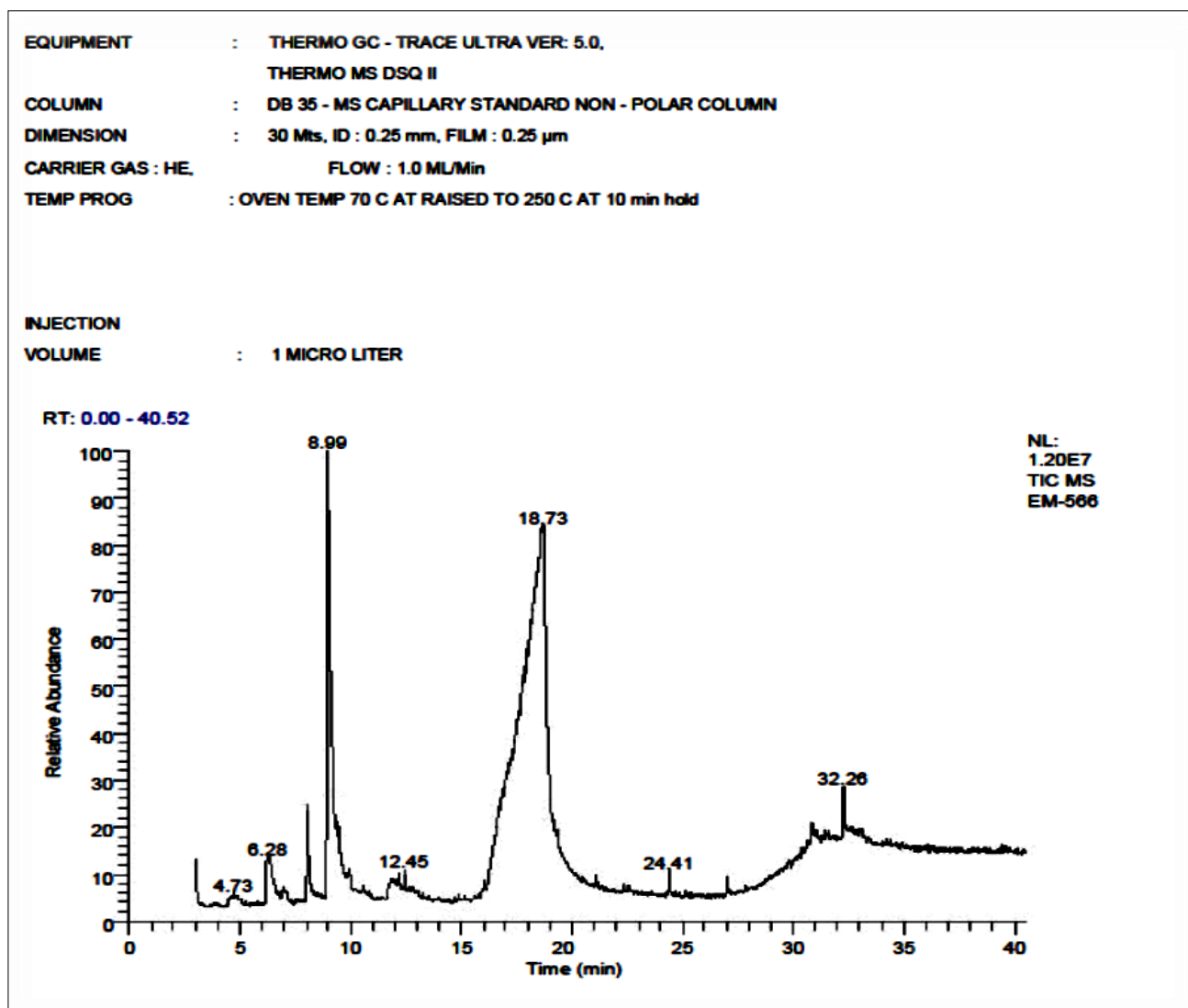


Fig 2 Analysis of active ingredient peak of *Coleus aromaticus* by GC-MS

GC – MS Analysis of *Coleus aromaticus*

The multifaceted applications of Plant-derived substances make them as an expertise one. From the ancient time the use of herbal medicine for the treatment of diseases and infections is in practice. The selected plant *Coleus aromaticus* was selected on the basis of ethnobotanical information collected from folk medicine and was subjected to GC-MS analysis (Fig 2).

The present research work, the GC-MS analysis indicated that the *Coleus aromaticus* extract consist the following compounds, Monobenzylidene-d-glucose, Prosta-5,13-dien-1-oic acid, 9,11,15 tris[(trimethylsilyl)oxy]-, trimethylsilyl ester, 5-Carbethoxysuccinylacetone, (5Z,9à,11à,13E,15S), Quinine, trimethylsilyl ether, Estra-1,3,5(10)-trien-6-one, 3,16,17-tris(acetyloxy), O,O,O'-tris(trimethylsilyl), 6-(O-methyloxime), (16à,17à)-, Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl, Dipyrindamole, Aspidospermidine-1-ethanol, 17-hydroxy-16-methoxy-à-methyl, 9,10-Anthracenedione, 1-(methylamino)-4-[(4-methylphenyl)amino], 7-Chloro-3-[3,4-

dichlorophenyl]-1-[[3-[dimethylamino]propyl]imino]-10-hydroxy-2-methyl-1,2,3,4,9,10-hexahydro-9-acridinone, Stearic acid, 5-(hexadecyloxy)-3-(octadecyloxy)propyl ester, Prost-13-en-1-oic acid, 1,2,8-Trihydroxy-6-methoxy-3-methylanthraquinone, 3,9à;14,15-Diepoxyregn-16-en-20-one, 3,11à,18-triacetoxy, 5-Carbethoxysuccinylacetone, Octadecane, 1,1'-[1,3-propanediylbis(oxy)]bis, Hydromorphone pfp, 2-pentadecyl, (5à)Pregnane-3,20à-diol,14à,18à-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, Diacetate, 9-(methoxyimino)-11,15-bis[(trimethylsilyl)oxy]-, 2-Benzo[1,3]dioxol-5-yl-8-methoxy-3-nitro-2H-chromene, 2à,4a-Epoxyethylphenanthrene-7-methanol, 1,1-dimethyl-2-methoxy-8-(1,3-dithiin-2ylidene)methyl-1,2,3,4,4a,4b,5,6,7,8,8a,9-dodecahydro-, acetate, trimethylsilylester, (8.xi.,12.xi.), Acetic acid, 9-Desoxy-9x-chloroingol 3,7,8,12-tetraacetate, 1-Monolinoleoylglycerol trimethylsilyl ether, trans, 1,1,2,2-Tetrakis(adamant-1-yl)ethane, 3-Bromopiperidin-2-one, 3,9à;14,15-Diepoxyregn-16-en-20-one, 3,11à,18-triacetoxy, 1,3-Dioxane, O,O',O"-tris(trimethylsilyl) (Table 3-4).

Table 4 Activity of phyto-components identified in the extracts of the *Coleus aromaticus* leaves by GC-MS

Name of the compound	Compound	Biological activity
4-(9-Acridinyl)-N,N-dimethylbenzenamine	Acridine compound	Antimicrobial activity [59]
9,10-Anthracenedione,1-(methylamino)-4-[(4-methylphenyl)amino]-	<i>Anthraquinone</i>	Antimicrobial activity [60]
Prost-13-en-1-oic acid,9-(methoxyimino)-11,15-bis[(trimethylsilyl)oxy]-, trimethylsilylester	Alcoholic Compound	Anti-ulcer agent and antimicrobial activates [60]
1,3-Dioxane,5-(hexadecyloxy)-2-pentadecyl-, trans	Phenolic compounds	Antimicrobial, Anti-Inflammatory and Cytotoxic Activities [61]
3-Bromopiperidin-2-one	Terpene alcohol	Antibacterial, bactericidal, fungicidal and antiinflammatory activities [62]
1-Monolinoleoylglycerol trimethylsilyl ether	Steroid	Antimicrobial, Antioxidant Activities [63]
Monobenzylidene-d-glucose	Acylated derivatives	Antibacterial and antifungal activities [64]
Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	Volatile organic compounds	Anti-microbial activity [65]
Dipyrindamole	Diterpene compound	<i>Antibacterial property</i> [66]

Previous study was reported by [67], the presence of following compounds 1,2-benzenedicarboxylic acid, diethyl ester, phytol, octadecenal, dibutyl phthalate, 2- hexadecen-1-ol, 3,7,11,15-tetramethyl, hexadecanoic acid, methyl ester, oleic acid, 9,12,15-octadecatrienoic acid, (z,z,z), 9,12,15-octadecatrienoic acid, ethyl ester, (z,z,z) and solanesol. (Table 2) represents the active phyto-components identified in the methanolic extracts of the *Coleus aromaticus* leaves by GC-MS. The GC-MS study was carried out by [68], to identify the chemical compounds of *P. amboinicus*. The results revealed that the presence of similar volatile constituents, though the parent plants and root cultures contained 21 compounds in comparison to only 15 compounds noticed in the *Coleus aromaticus*.

CONCLUSIONS

The present research concluded that the methanol extract of traditionally using medicinal plants showed the maximum antibacterial activity against the selected nosocomial pathogens. The results of this study showed that

different plants belonging to various families have the ability to inhibit the growth of some nosocomial pathogens. This work may furnish the necessary information in the selection of plants and its extraction for the isolation of constituents possesses antibacterial effect against the selected species. The present study may also provide a scientific basis on the use of crude plant extracts and oil on herbal medicine. The investigation culminates that methanol with its stronger extraction capacity produce more active constituents that own many biological activities. So that those might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants used to treat many incurable diseases.

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