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Phytochemical analysis, phenolic content and antioxidant activity of methanolic extract of *Caryopteris odorata* D.Don.Robin

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ABSTRACT

Background & Aim: *Caryopteris odorata* is an aromatic shrub of family verbenaceae growing in tropical to subtropical regions. Various traditional applications of this plant have been documented guiding us to investigate the pharmacological activities and to further investigate the chemical composition.

Experimental: Methanolic extract of aerial parts of *Caryopteris odorata* was investigated for antioxidant activity and the major phyto-constituents and total phenolic content were screened. The antioxidant activity was determined by measuring the scavenging activity using DPPH radical scavenging, NO radical scavenging, so radical scavenging activity, Metal chelating and reducing power assay. The antimicrobial efficacy was determined using agar well diffusion method against gram positive as well as gram negative bacteria. Sensitivity in terms of zones of inhibition and phytochemical composition of the extracts were also determined. *In vitro* anti-inflammatory activity was evaluated using albumin denaturation method. Diclofenec Sodium was used as a standard drug for the study of anti-inflammatory activity.

Results: The results revealed that, methanolic extracts possess broad spectrum antibacterial activity against three microorganisms. Phytochemical analysis revealed the presence of diterpenes carbohydrates, glycosides, sterols and triterpenoids, aliphatic acids , tocopherols and phenolics. The methenolic extract exhibit strong antioxidant activity (IC_{50} values for DPPH assay 164.60 ±0.37 mg/ml, for metal chelating 280.30 ± 3.28 mg/ml, for reducing assay 80.38 ± 0.79 mg/ml, respectively). The extract also showed *in vitro* anti-inflammatory activity by inhibiting the heat induced albumin denaturation with IC_{50} value as 497.22 ± 1.34 mg/ml, respectively. From the result, it is concluded that the *C.odorata* possess a rich amount of different class of compounds and further the phytochemicals present in the *C.odorata* extract may be responsible for the antimicrobial, antioxidant and anti-inflammatory activity.

Recommended applications/industries: The results obtained from various activities suggest *Caryopteris odorata* application as natural alternative antioxidant, anti-inflammatory and antimicrobial reagent.

1. Introduction

Caryopteris odorata D.Don.Robin is an aromatic shrub of Verbenaceae family and is well known to have a number of medicinal properties. The family Verbeneaceae consists of several important medicinal plants with broad range of biological activities and potent phytochemical constituents. This species has wide existence in tropical and subtropical climatic regions. It is used in traditional medicine to cure diabetic foot ulcers, tumors and wounds (Ajaib *et al.*, 2010). A number of iridoid glycosides have been isolated from species of Caryopteris genus having a wide range of bioactivities, including cardiovascular, anti-hepatotoxic, choleretic, hypoglycemic, analgesic, anti-inflammatory, anti-mutagenic, anti-spasmodic, antitumor, antiviral, immunomodulator and purgative activities (Shahzadi *et al*., 2012; Didna *et al*., 2007; Tundis *et al.*, 2008; Zhao *et al*., 2009).

The present study was done with the aim to investigate the phytoconstituents of *C. odorata* methenolic extract and to evaluate its *in vitro* antioxidant activity. The study was also designed to determine the antibacterial and anti-inflammatory efficiency of *C.odorata* extract. *Caryopteris odorata* was selected for the present study, based on its therapeutic value and the degree of research work which is not done mostly in earlier studies.

2. Materials and Methods

2.1. Collection of plant sample

The fresh aerial parts of *C. odorata* were collected from Garjiya village (345m) near Ramnagar. The plants herbarium was identified by Dr. D.S. Rawat, Professor, (Taxonomist), Dept. of Biological Sciences, G.B. Pant University and the voucher specimens deposited in the herbarium. Fresh leaves and flowers were collected for oil extraction.

2.2. Plant sample extraction

The whole aerial parts of *C.odorata* were shade dried and pulverized to powder in a mechanical grinder. Required quantity of powder was weighed and transferred to Stoppard flask and treated with methanol until the powder is fully immersed. The flask was shaken every hour for the first 6 hours and then it was kept aside and again shaken after 24 hours. This process was repeated for 3 days and then the extract was filtered. The extract was collected and evaporated to dryness by using a vacuum distillation unit. The final residue obtained was then subjected to GC-MS analysis.

2.3. GC-MS analysis

The analyses were performed on a Agilent 7890A chromatograph with Agilent 5975C mass detector, on a capillary non-polar column HP-5MS (30 m× 0.25 mm× 0.25 µm) at a helium flow rate of 1 mL/min. An injection of a 1-µL sample was performed using an Agilent 7693A autosampler. The injector worked in a split (1:10) mode at an injector temperature of 300°C. The initial column temperature was 50°C, rising to 325°C, at 3 °C/min; the final temperature was held for 10 min. The ion source and quadrupole temperatures were 230°C and 150°C, respectively. Electron ionization mass spectral (EIMS) was obtained at ionization energy 70 eV. The detection was performed in a full scan mode from 41 to 800 a.m.u. After integration, the content (%) of each component in the total ion current (TIC) was calculated. The identification of compounds was based on a comparison of MS spectra with computer mass library NIST 2012 along with the relative retention indices (RI, non-polar column). The experimental retention times (RTs) of the compounds were compared to the RTs of the *n*-alkane standard mixture (C_{10}-C_{40} Sigma Aldrich).

2.4. Estimation of phenols

The chemical assay of methanolic extract was studied quantitatively by spectrophotometer in terms of total phenols, flavonoids and orthodihydric phenols and the concentration of these samples were measured with the help of standard calibration curve by the relation between absorbance and concentration of the sample.

2.4.1. Total phenolics assay

The total phenolics were determined using the Folin-Ciocalteu method modified by Shetty *et al.* (1995). Briefly, 0.5 mL extract solution were dissolved in 1.0 mL of Folin-Ciocalteu reagent, 1.0 mL of aqueous solution of 7% Na_2CO_3 and 5 mL of distilled water. The solutions were mixed properly and were allowed to stand for half an hour. The absorbance was recorded at 765 nm. The standard curve was established using various concentrations of gallic acid, and results were

expressed as mg of gallic acid per gram of sample in dried weight.

2.4.2. Estimation of flavanols

Aluminum chloride colorimetric assay was applied for estimation of flavanols **(**Woisky and Salatino, 1998). Ten mg of extract were dissolved in 10 mL of solvent to prepare stock solution. 0.1 mL of stock solution was mixed with 1.25 mL water and 0.75 mL of 5% $NaNO_2$ in a test tube. The mixture was incubated for 5 min. After incubation, 0.15 mL of 10% $AlCl_3$ was added to the mixtures. After 6 min. 0.5 mL of 1 N NaOH and 275 µL of distilled water was added, after proper mixing of the solution the intensity of pink colour was obtained at 510. The flavanol content standard curve was established using various concentrations of catechin and the concentrations were calculated with the help of calibration curve and expressed in mg/ 100 gm of dry material material.

2.4.3. Estimation of orthodihydric phenols

10 mg of extract were dissolved in 10 mL of solvent to prepare stock solution, 0.1 mL of the extract solution was taken in a test tube and mixed with 0.4 mL water and 1 mL of 0.05N HCl, 1 mL of Arnow's reagent, 10 mL of water and 2 mL of 1 N NaOH. The solution were mixed thoroughly (pink colour was appeared) and absorbance at 515 nm was measured using a UV spectrophotometer (Thermo Scientific Evolution 201 series). To calculate the amount of ortho-dihydric phenols present in the sample standard curve was prepared with the help of working standard catechol solution at different concentrations. The concentration were calculated from the calibration curve and expressed in mg / 100gm of material (Sethi *et al.*, 2015).

2.5. Evaluation of antioxidant activity

To check the *in-vitro* antioxidant property, the Methanolic extract (COME) was subjected towards following methods practiced and reported earlier.

2.5.1. DPPH radical scavenging activity

DPPH radical scavenging activity was evaluated according to the method developed by Blois (1958) and described by Liu *et al*. (2008) and Lu *et al*. (2011) with slight modification. The assay mixture contained 5 mL of 0.004% methanol solution of DPPH and different amount of test sample solution of different concentrations. The solution was rapidly mixed and scavenging capacity was measured spectrophotometrically by Thermo Scientific Evolution 201 series by monitoring the decrease in absorbance at 517 nm. Ascorbic acid was used as positive control while reaction mixture (DPPH radical solution) minus extract solution was taken as control. Inhibition of free radical by DPPH in percent (IC %) was calculated by using the following equation.

$$IC\,\% = \frac{(A_0 - A_t)}{A_0} \times 100$$

Where, A_0 = Absorbance value of control sample; A_t= Absorbance value of test sample; IC = Inhibitory concentration

Percent inhibition was plotted against concentrations and the standard curve was drawn using standard antioxidant (ascorbic acid) to calculate the IC_{50} values for standard and different extracts. A lower IC_{50} value indicates more radical scavenging activity.

2.5.2. Reducing power

The reducing power of extract (COME) was determined by the method developed earlier and recently used by (Arya *et al.*, 2015). Varying concentrations of tested sample (50-150 µg/mL) were mixed with 2.5 mL of phosphate buffer (200 mM, pH= 6.6) and 2.5 mL of 1% potassium ferricyanide, $K_3[Fe(CN)_6]$. After 20 minute incubation at 50±1ºC, 2.5 mL of trichloroacetic acid was added to the mixtures, followed by centrifugation at 650 RPM for 10 min. The upper layer (1 mL) was mixed with 5ml distilled water and 1 mL of 0.1% ferric chloride and absorbance of the resultant solution were measured at 700 nm using UV spectrophotometer (Thermo Scientific Evolution 201 series).

All the readings were taken as triplicate and gallic acid was used as the standard. The reducing power of samples was calculated using the following formula:

$$\text{Reducing Power}\,\% = \frac{(A_0 - A_t)}{A_0} \times 100$$

Where, A_0 = Absorbance value of control sample; A_t= Absorbance value of test sample

Percent inhibition was plotted against concentrations and the standard curve was drawn using standard antioxidant (Gallic acid) to calculate the RP_{50} values for standard and different extracts.

The lower RP_{50} value indicates greater reducing power ability.

2.5.3. Metal chelating activity

The metal chelating activity of Fe^{2+} by extracts was examined by spectrophotometric method recently used by Pal *et al.* (2011) based on the principle of the Fe^{2+} chelating ability of the antioxidant by measuring the absorbance of ferrous iron-ferrozine complex formed at 562 nm. 0.1 mL of 2mM $FeCl_2.4H_2O$, 0.2mL of 5mM ferrozine and 4.7 mL of methanol was added to different concentrations of tested sample (50-250 µg/mL). The solutions were mixed and incubated for 10 min. The absorbance of test sample was measured at 562 nm in a UV spectrophotometer (Thermo Scientific Evolution 201 series). All the readings were taken as triplicate, EDTA (0.01 mM) and Citric Acid was used as the standard. The metal-chelating activity of tested samples, expressed as percentage was calculated using the following formula:

$$IC\,\% = \frac{(A_0 - A_t)}{A_0} \times 100$$

Where, A_0 = Absorbance value of control sample; A_t= Absorbance value of test sample

The percent of chelating ability was plotted against concentrations and the standard curve was drawn using standard antioxidant (EDTA) to calculate the IC_{50} values for standard and different extracts. A lower IC_{50} value indicates greater metal-chelating ability.

2.5.4. Assay of nitric oxide scavenging activity

Nitric oxide (NO) was generated from sodium nitroprusside (SNP) and was measured by the Griess reagent. It is based on the principal that SNP in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated by the use of Griess Reagent. Scavengers of NO compete with oxygen leading to reduced production of NO. 2mL of SNP (10 mM) in phosphate buffer saline (PBS) pH 7.4 was mixed with different concentrations of extract (50–250 µg/mL) and incubated at 25°C for two and half hours. The samples were reacted with 1 mL of Griss reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dichloride and 2 ml orthophosphoric acid). Pink colour will arise. Absorbance was reset at 546 nm. Ascorbic acid was taken as standard (Naskar *et al.*, 2010). Nitric oxide scavenging activity was calculated by the following equation:

$$IC\,\% = A_0\text{-}A_s/\ A_0 \times 100$$

Where, A_0= Absorbance value of control sample A = Absorbance value of test sample IC = Inhibitory concentration.

Percent inhibition was plotted against concentrations. The standard curve was drawn using standard antioxidant (ascorbic acid) to calculate the IC_{50} values for standard and essential oils.

2.5.5. Super oxide radical scavenging activity

1 mL of Nitroblue terazolium (156 Mm), 1 mL Nicotinamide adenine dinucleotide (reduced) (468 Mm) and 0.1 mL of phenanzine methosulphate solution (PMS) in 0.1 M of phosphate buffer solution (pH7.4) were added to different concentrations of essential oil (5- 25 µg/ml) then incubated at 25°C for 5 min and absorbance was read at 560 nm against blank containing all reagent except PMS. Ascorbic acid was taken as standard (Wei *et al.*, 2010). Super oxide radical scavenging activity was calculated by the following equation:

$$\text{Superoxide radical scavenged (\%)} = IC\,\% = A_0\text{-}A_s/\ A_0 \times 100$$

Where, A_0= Absorbance value of control sample A = Absorbance value of test sample IC = Inhibitory concentration.

Percent inhibition was plotted against concentrations. The standard curve was drawn using standard antioxidant (ascorbic acid) to calculate the IC_{50} values for standard and essential oil.

2.6. Statistical analysis

All experiments were performed thrice and the results averaged data were expressed as mean ± SD. Linear regression analysis was used to calculate IC_{50} for each essential oil sample.

3. Results and discussion

3.1. Phytochemical examination

The results pertaining GC-MS analysis of the extract are given in Table 1. The bioactive principles identified

were Diterpenes (phytol & other) (13.87%), Glycosides (1.78%), Sterols and triterpenoids (1.78%), aliphatic acids(5.8%), tocopherols (0.21), phenolics (including phenylpropanoids) (2.23%), etc. The major bioactive compounds detected were coumarine (1.29%), Z-p-coumaric acid (0.47%), palmitic acid (1.68%), E-caffeic acid (0.14%), Phytol (1.60%), linoleic acid (0.25%), α-linoleic acid (1.34%), α-tocopherol (0.21%), stigmasterol (0.58%), sterol (0.59%),α-amyrin (0.22%), β-amyrin(0.12%), ursolic acid(0.11%).

Table 1: Chemical composition of *Caryopteris odorata* methenolic extract**.**

S.N.	Compound	RI^{Exp}	RI^{Lit}	% Contribution
1	1,2-Propanediol	1012	1011	0.11
2	Lactic acid	1072	1073	0.05
3	Glycolic acid	1086	1083	0.16
4	2-Hexenoic acid	1125	1123	t
5	β-Lactic acid	1155	1155	t
6	Malonic acid	1216	1216	0.06
7	Benzoic acid	1248	1246	0.00
8	Glycerol	1294	1293	2.93
9	Succinic acid	1325	1324	0.16
10	Glyceric acid	1352	1348	0.59
12	Fumaric acid	1359	1357	t
13	β-Caryophyllene	1416	1417	t
14	Coumarine	1429	1430	1.29
15	*trans*-Dihydro-3,4-dihydroxy-2(3H)-furanone	1441	-	0.10
16	Eugenol	1477	1477	t
17	Malic acid	1512	1512	0.64
18	Salicylic acid	1518	1521	0.06
19	Cinnamic acid,	1544	1546	0.15
20	2,3,4-Trihydroxybutyric acid	1577	1575	0.62
21	Trihydroxybutyric acid	1596	1597	0.11
22	2-Hydroxyphenylpropanoic acid	1690	-	0.31
23	Methyl ester of tetradecanoic acid	1726	1726	0.17
25	Ribitol	1762	1763	4.29
26	Vanillic acid	1775	1776	t
27	(*Z*)-*p*-Coumaric acid	1787	1794	0.47
29	Galactonic acid, γ-lactone	1923	1924	0.45
30	Methyl palmitate	1926	1926	0.60
31	(*E*)-*p*-Coumaric acid	1945	1947	0.12
32	Methyl caffeate	2016	2015	t
33	Hexadecanoic (palmitic) acid	2049	2052	1.68
34	Methoxycyclohexane-1,2,3,4,5-pentol	2076	2080	0.12
35	Methyl linoleate	2093	2097	0.08
36	Methyl linolenate	2100	2101	0.41
37	(*E*)-Caffeic acid	2153	2154	0.14
38	Phytol	2184	2187	1.60
39	Linoleic acid	2114	2214	0.25
40	α-Linolenic acid	2221	2219	1.34
41	Octadecanoic (stearic) acid	2249	2249	0.13
42	α-Tocopherol	3148	3150	0.21
43	Stigmasterol	3284	3285	0.58
44	Sterol	3328	3285	0.59
45	β-Amyrin	3345	3347	0.12
46	α-Amyrin	3380	3378	0.22
47	Ursolic acid	3618	3619	0.11

3.2. Phenol content

The phenolic content was estimated in the methenolic extract of *C.odorata*. The estimated value for total phenol was 35.12±0.01 mg/g gallic acid equivalent, total flavanoid was 18.09±0.02 mg/g catechin equivalent (CNE) and orthodihydric phenol was 31.88±0.00mg/g catechol equivalent.

3.3. Antioxidant activity

Antioxidants are used as food additives, to stabilize foods that undergo significant loss in quality due to rancidity from oxidation of unsaturated fats resulting in off-odours and off-flavours and discoloration from oxidation of pigments or other components of the food. In this study, we investigated the in vitro antioxidant potential of *C.odorata*, a traditionally important plant from verbenaceae family, and the results were listed in Table 2.

3.3.1. Reducing power activity

The reducing power represent as a significant indicator of its potential antioxidant activity. It can be seen that the reducing power percentage values of methenolic extract and the positive control (BHT) were concentration related and increased with the increase in sample concentration in the range of the tested concentrations. The IC_{50} values were calculated and listed in Table 2. The results revealed that methenolic extract of *C.odorata* is a potent reducing agent with IC_{50} as 80.38 ± 0.79 mg/mL compared to BHT (IC_{50}=59.34±0.47 mg/mL).

3.3.2. DPPH radical scavenging activity

The DPPH (1,1-Diphenyl-2-picrylhydrazyl radical) assays have been widely used to determine the free radical-scavenging activity of various plants and pure compounds. The DPPH are stable free radical producer which dissolve in methanol or ethanol, and their colors show characteristic absorptions at 517 nm respectively. When an antioxidant scavenges the free radicals by hydrogen donation, the colors in the DPPH assay solutions become lighter. The results revealed that the DPPH inhibition percentage values were dose dependent, whereby they increased in the range of the tested concentrations. Catechin and Gallic acid was used as positive control. The methenolic extract of *C.odorata* exhibited strong DPPH radical scavenging activity with IC_{50} as 164.60±0.37 mg/mL compared to gallic acid (IC_{50}=144.07 ± 0.40 mg/mL).

3.3.3. Metal chelating activity

Chelating activity of the methenolic extract was determined by the ferrozine assay. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of other chelating agents, the complex formation is disrupted with the result that the red color of the complex is decreased. Measurement of the rate of red color reduction therefore allows estimation of the chelating activity of the coexisting chelator. The methenolic extract of *Caryopteris odorata* inhibited the formation of ferrozine-Fe^{2+}complex in a dose dependent manner with the IC_{50} values of 280.30 ± 3.28 mg/mL compared to the positive control EDTA with IC_{50} value as 215.39 ± 2.76 mg/mL.

3.3.4. Nitric oxide radical scavenging activity

Nitric oxide ($NO^{\cdot}$) released from SNP has a strong NO^{+} character which can alter the structure and function of many cellular components. The methanolic extract of *C.odorata* exhibited good $NO^{\cdot}$ scavenging activity leading to the reduction of the nitrite concentration in the assay medium. The $NO^{\cdot}$ Scavenging capacity was concentration dependent showing highest activity at 250 µg/mL. The *C.odorata* extract in SNP solution significantly inhibited the accumulation of nitrite, a stable oxidation product of $NO^{\cdot}$ liberated from SNP in the reaction medium with time compared to the standard ascorbic acid (Table 2). The IC_{50} for methenolic extract was found to be 150.74± 0.11 mg/mL whereas that for positive control was 60.13±0.07 mg/mL.

3.3.5 Super oxide radical scavenging activity

Superoxide anions (O_2^{-}) are the most common free radicals whose concentration increases under conditions of oxidative stress and are generated either by autooxidation processes or by enzymes and produces other cell damaging free radicals and oxidizing agents (Liu and Ng, 2000). The ability of the extract to scavenge O_2^{-} radical generated from the photochemical reduction of riboflavin resulted in a decrease in the absorbance of the blue formazan solution at 560 nm. The scavenging activity was dose-dependent. The IC_{50} value of the methanolic extract of *C.odorata* was 65.74 ± 0.25 µg/mL compared to the standard antioxidant Ascorbic acid 62.26 ±0.76

mg/mL. The O_2^- radical scavenging effect of the extracts could culminate in the prevention of $OH^·$ radical formation since O_2^- and H_2O_2 are required for $OH^·$ radical generation. The scavenging potential will depend on the number and locations of the hydroxyl groups in the phenolic compounds present in the extract. The radical scavenging activity is also consistent with the quantity of phenolic compounds observed in the plant extract. Since phenolic compounds such as flavonoids are known to posses high $O_2^{·-}$ anion scavenging abilities (Robak and Gryglewski, 1988). Collectively, these results suggest that the extract effectively scavenges ROS and could protect against oxidative damage.

Antioxidants are tremendously important substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. The antioxidant potential of *C. odorata* methanol extracts was investigated in the search for new bioactive compounds from natural resources. It became clear that *C.odorata* methenolic extract present the strong antioxidant activity compared with reference antioxidants for DPPH, metal chelating, reducing power and super oxide radical scavenging activity. The obtained results from various antioxidant assays are in agreement with the phenol contents determined. Plant polyphenols act as reducing agents and antioxidants by the hydrogen-donating property of their hydroxyl groups (Aberoumand and Deokule, 2008). Hence, we could conclude that these polyphenols are responsible for the observed antioxidant activity in this study. Previously, Shahzadi *et al.* (2011) studied the *in vitro* antioxidant potential of the different fractions of *Caryopteris odorata* (Ham. ex Roxb.) and concluded strong antioxidant activity. Our findings are in lane with that of earlier reports.

Table 2: IC_{50} values for different antioxidant assay.

Assay	IC_{50}(mg/ml)	
	C. odorata	**Positive control**[a]
DPPH Radical Scavenging Activity	164.60 ±0.37[b]	144.07 ± 0.40
Metal chelating activity	280.30 ± 3.28	215.39 ± 2.76
Reducing power activity	80.38 ± 0.79	59.34±0.47
Super Oxide Radical Scavenging Activity	65.74 ± 0.25	62.26 ±0.76
Nitric Oxide Radical Scavenging Activity	150.74± 0.11	60.13±0.07

[a:] The positive controls for DPPH inhibition, metal chelating activity, reducing power, SO Radical scavenging and NO radical scavenging assays were gallic acid , EDTA, BHT, ascorbic acid and ascorbic acid, respectively. [b:] Mean ± standard deviation for triplicate experiments.

4. Conclusion

In conclusion, the present study revealed the phytoconstituents, *in-vitro* antioxidant, anti-inflammatory and anti-microbial activity of methanol extract of *C. odorata*. Phytochemical investigations on the *C. odorata* revealed the presence of various phytoconstituents such as diterpenes (phytol and other), glycosides, sterols and triterpenoids, aliphatic acids, tocopherols, phenolics (including phenylpropanoids) and other compounds. These phytochemicals have various health benefits such as antioxidant, anti-microbial, anti-inflammatory, cancer preventive, anti-diabetic and anti-hypertensive effect. The secondary metabolites (phytochemicals) and other chemical constituents of this medicinal plant account for their medicinal value. Inflammation is a complex biological response of vascular tissue to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling and pain. Denaturation of proteins is a well-documented cause of inflammation. The alcoholic extract of *C. odorata* showed significant anti-inflammatory activity at increasing concentration. It may due to the presence of active principles of phytocompounds such as flavonoids and diterpenes and related polyphenols may also be responsible for this anti-inflammatory activity. Hence, *C.odorata* can be used as an anti-inflammatory agent. The investigation is based on the need for anti-inflammatory agents from natural sources with potent activity and lesser side effects as substitutes for chemical therapeutics. The *C.odorata* methanolic extract represent a broad range of antimicrobial action inhibiting both gram positive and negative bacteria's. The therapeutic value of medicinal plants lies in the various chemical constituent's presents in it. The bioactivity of plant extracts is attributed to bioactive constituents.

Further investigations are required to find active compo nent of the extract and to confirm the mechanism of act ion. The preliminary phytochemical screening tests may be useful in the detection of the bioactive phytocompounds and may lead to the discovery and development of drugs.

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