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Phytochemcial Analysis and Biological Activities of Methanolic Extract of Redfruit Creeper, *Corallocarpus epigaeus*

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Abstract

Corallocarpus epigaeus is a rare tuberous medicinal creeper belonging to the Cucurbitaceae family that is native to India, Pakistan, and some parts of Africa. It has rich sources of medicinal properties and has been reported to have been widely used in Indian traditional medicine (Ayurveda, Siddha, and Unani) for centuries. As there has been scanty research on the phytochemical characterization and a biological activity of Redfruit creeper, the present study aimed to conduct phytochemical screening and evaluate the anti-bacterial, anti-oxidant and anti-inflammatory activities of the methanolic extract of leaves in Corallocarpus epigaeus. Phytochemical screening showed the presence of alkaloids, steroids, phenols, flavonoids, tannins, cardiac glycosides, coumarin and terpenoids in leaves extract. Twenty major chemical constituents were identified in the methanolic extract, among which the peak height concentrations of (i) 1,2-benzenedicarboxylic acid, bis (2-methylpropyl) ester, (ii) phytol, (iii) hexadecanoic acid and (iv) squalene were greatly high compared to the other compounds, all of which reportedly possess biological activity. Staphylococcus aureus and Bacillus marisflavi were significantly inhibited by methanolic leaves extract of Corallocarpus epigaeus, with inhibition zones of 22±0.1 and 23.01±0.1 mm, respectively. The methanolic leaf extracts showed promising anti-oxidant and anti-inflammatory effects in a concentration-dependent manner. The DPPH and H2O2 assays showed higher antioxidant activity with IC50 values of 149 and 150 mg/L, respectively than the control. According to heat induced, hypotonicity induced, and egg albumin denaturation assays revealed significant anti-inflammatory activity with respective percentage inhibitory values of 51.9±18.1, 36.11±2.79 and 97.09±0.19. Based on the phytochemical screening, anti-bacterial, anti-oxidant and anti-inflammatory activities, we conclude that the methanolic leaves extract of Redfruit creeper contains an abundance of bioactive compounds could serve as a potential source of natural anti-bacterial, anti-oxidants and anti-inflammatory agents. A further study will be required to identify the novel bioactive compounds in Redfruit creeper that may be more effective in different biological processes.

Keywords: Anti-Bacterial, Anti-Oxidant, Anti-Inflammatory, *Corallocarpus epigaeus*, Medicinal Plant. Phytochemical Screening

1. Introduction

As long as ancient times, plant parts such as leaves, flowers, stems, roots, rhizomes, seeds, fruit, bark, etc., have been utilized as herbal medicines. According to literature on Indian traditional medicine from Ayurveda, Siddha, and Unani, mentioned all herbal medicinal plants are used to treat a variety of human ailments [1,2]. India is home to over 45,000 different plant species, many of them have been discovered to have medicinal properties [2]. Since all medicinal plants are contain secondary metabolites such as glycosides, flavonoids, alkaloids, tannins, steroids, phenols, steroids etc., that may be provide new drug design opportunities [3,4]. The World Health Organization (WHO) reports that several medicinal plants possess a wide range of therapeutic agents, and over 80% of the population relies on conventional medical treatments derived from these plants. Also, plants can be used for remedy or as a precursor to the production of bioactive chemicals in the pharmaceutical industries [4].

Corallocarpus epigaeus is a rare /endangered medicinal plant from the Cucurbitaceae family, notably prescribed this plant has long been utilized in Indian traditional medicine as a treatment for acute dysentery, venereal diseases, skin diseases, snake bite [5,6]. In Tamil traditional medicine system this plant is known as “Agaya Garuda Kilangu” / kollankovai / pei mooli / peicheenthal and has been reported to be used to treat a variety of diseases such as mastitis, enteritis, arthritis, stomatitis, salivation from the mouth, wounding, and conjunctivitis in animals [6-8]. There

are fifteen genera of *Corallocarpus* species, which are native in tropical Africa, India, and Pakistan and have also been found in different states of India including Andhra Pradesh, Bihar, Punjab, Madhya Pradesh, Tamil Nadu, Telangana, West Bengal, and Uttar Pradesh [9]. Redfruit creeper is a perennial and monoecious tendril creeper with coiled tendrils and a climbing habit. This creeper is produces berries-like fruits with sweet and bitter taste and also has tuberous roots and rhizomes with bitter and sub-acid tastes that can be used as alterative and laxative [5,10]. According to the ethnobotanical information, *Corallocarpus epigaeus* is a popular folklore medicine in India used to treat a variety of medical issues. In the last few years, a few studies have been conducted on *Corallocarpus epigaeus* plant in different countries to prove its anti-fungal anti-microbial anti-cancer anti-diabetic anti-helminthics efficacy [10,5,11-13]. In the above investigation, there is unclear limited biological information available only on the Redfruit creeper, but detailed research on its phytochemical characterization and such biological activity is yet to be conducted. In the background of all these considerations, the present study aimed to investigate phytochemical screening and evaluate the anti-bacterial, anti-oxidant and anti-inflammatory activities of the methanolic extract of leaves in *Corallocarpus epigaeus.*

2. Materials and Method

2.1. Plant Collection and Identification

Corallocarpus epigaeus plant species was collected from the Solavanthan village, Madurai in the month of December 2023. The plant specimens (ARIBASKBI-1) were identified by Dr. Karuppusamy (Plant Taxonomist) at Department of Botany, The Madura College, Madurai, Tamil Nadu, India. The plant specimen has been kept at the Botany Department with herbarium voucher number LSID-2PNI-20013498. The healthy and disease-free leaves of the plant were used to test its medicinal properties.

2.2. Preparation of Plant Extracts

The leaves were washed and air dried at 25°C for three weeks, ground into powder and stored in plastic bags. One hundred gram (100 g) of the powder was extracted with 500 mL of methanol in a rotary shaker at 200 rpm for 48 hrs. The extracts were filtered through Whatman filter paper 1, with a pore size of 11 µm and the filtrate was collected in a vacuum tube rotary evaporator. The filtrate was then freeze-dried in a lyophilizer and the dried extracts were stored in 100 mL centrifuge tubes maintained at 4°C for further use.

2.3. Preliminary Phytochemical Screening

The method of Harborne was used for the phytochemical analysis of methanolic extract of leaves in *Corallocarpus epigaeus* [14]. The tests were based on the visual observation of the precipitate formation or color change after the addition of specific reagents. The test performed includes alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, cardiac glycosides, reducing sugars, steroids and coumarins.

2.4. Gas Chromatography-Mass Spectrometry (Gc–Ms) for Phytochemical Analysis

The methanolic extract of leaves and gas chromatography-linked mass spectrometry was used to identify phytochemicals (GCMS-QP-5050, Schimadzu, Japan). The extract (2 µL) was injected into the GC-MS apparatus on a 30 m glass capillary column with a film thickness of 0.25 m (30 m □ 0.2 mm i.d. coated with UCON HB 2000) at the following temperatures: Initial oven temperature 40 degrees Celsius for 4 minutes, then escalated to 250 degrees Celsius at 15 degrees Celsius per minute for 15 minutes, and finally kept at 250 degrees Celsius for 10 minutes. The GC unit had a FID detector that was connected to an integrator. Each component's relative amount was expressed as the percentage of the total ion current. The GC-MS was controlled by a computer at 70 eV, and ammonia was used as the reagent gas at 95 eV. Probability-based matching with the computer library built within the NIST20 system identified the unknown compounds.

2.5. Antibacterial Assay

The agar diffusion method was used to test the antimicrobial activity of methanolic extract of *Corallocarpus epigaeus* plant leaves against the three bacterial strains like Staphylococcus aureus, Bacillus marisflavi, and Klebsiella pneumoniae as described by Irobi [15]. One hundred microliters of each active bacterial strain grown for 24 hrs at 37°C on brain Heart Infusion agar were spread on the surface Muller Hinton agar plates. The methanolic extract of *Corallocarpus epigaeus* was further diluted in methanol to a final concentration of 200 mg/mL and refrigerated overnight. Then, six holes were punched in the agar with a sterile cork borer with a diameter of 6 mm; four holes contained 100 µL of *Corallocarpus epigaeus* extract in different concentrations (25, 50, 75 and 100 µg/mL, respectively, while hole number five and six contained 100 µL of DMSO and 100 µg of gentamicin as a negative and positive control hole respectively. The agar plates were then incubated at 37°C for 24 hrs. The zone of inhibition of *Corallocarpus epigaeus* methanolic extract (mm) was measured against different bacterial strains. All experiments in this study were performed in triplicate, and the results were mean ± standard values.

2.6. Anti-Oxidant Assay

2.6.1. Ddph free Radical Scavenging Activity

Radical scavenging activity of methanolic extract of *Corallocarpus epigaeus* leaves was determined by Calorimetric assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a source of free radical according to the method of Blois with a slight modification [16]. An aliquot of 3 mL of 0.004% DPPH solution in 95% methanol and 0.1 mL of each plant extract at concentrations of 50, 100, 150, 200, 250, and 300µg/mL were mixed. The mixture was thoroughly mixed and allowed to sit for 30 min at room temperature. The procedure was repeated for the ascorbic acid (control). The decolorization of DPPH was ascertained by quantifying the absorbance at 517 nm. The control was prepared using 0.1 mL of each constituent and double-distilled water as a replacement for the plant extract or ascorbic acid.

The percentage inhibition and IC50 were calculated. All experiments in this study were performed in triplicate, and the results were mean ± standard values. The free radical scavenging activity (% inhibition activity) was calculated using the given equation:

$$\% \text{ inhibition activity} = \frac{\text{Control absorbance - Sample absorbance}}{\text{Control absorbance}} \times 100$$

2.6.2. Hydrogen Peroxide Scavenging Activity

Antioxidant activity of methanolic extract of *Corallocarpus epigaeus* leaves was evaluated by using hydrogen peroxide (H_2O_2) method [17]. 0.1 ml of sample added with 3.4 ml of 0.1 M phosphate buffer and 0.6 ml of 40 mM H_2O_2. This mixture was incubated 10 mints at room temperature. After incubation, absorbance was estimated at 230 nm in a spectrophotometer against a blank solution. Ascorbic acid was used as standard. The percentage inhibition and IC50 were calculated. All experiments in this study were performed in triplicate, and the results were mean ± standard values. The percentage scavenging of H_2O_2 was calculated using the equation:

$$H2O2\ \%\ \text{scavenging} = \frac{\text{Control absorbance - Sample absorbance}}{\text{Control absorbance}} \times 100$$

2.7. Anti-Inflammatory Assay

The standard methods were used for in vitro anti-inflammatory evaluation of methanolic extract of *Corallocarpus epigaeus* leaves [18].

2.7.1. Heat-Induced Hemolysis

5 ml of the isotonic buffer containing 100 µg/ ml, 200 µg/ ml, 300 µg/ml, 400 µg/ml and 500 µg/ml of an methanolic extract *Corallocarpus epigaeus* leaves was put into two duplicate sets of centrifuge tubes. The same amount of vehicles was added up in another tube as control. 50 µl of RBC suspension was contributed to each tube and mingled gently by inverting the test tube. One pair of tube was incubated at 54°C temperature, 20 min in water bath. Other pair was preserved at temperature 5°C in ice bath. Centrifugation of the mixture was done at 540 nm for 5 min at 5000 rpm and the absorbance was taken at 560 nm by using a spectrophotometer. For reference Aspirin (standard) at the different concentration of (100, 200, 300, 400 and 500 µg/ml) was used at similar absorbance. All experiments in this study were performed in triplicate, and the results were mean ± standard values. The percent inhibition of hemolysis was calculated according to the equation:

$$\% \text{ inhibition of hemolysis} = 1 - \left[\frac{OD_2 - OD_1}{OD_3 - OD_1}\right] \times 100$$

Where: OD_1: Test sample unheated; OD_2: Test sample heated; OD_3: Control sample heated.

2.7.2. Hypotonicity-Induced Hemolysis

The isotonic solution was made by composing 154 mM NaCl in 10 mM sodium phosphste solution and the buffer of this solution was 7.4 pH. Stock RBC suspension 50 µl was mixed with 5 ml of the hypotonic solution containing the methanolic extract *Corallocarpus epigaeus* leaves at concentrations of 100, 200, 300, 400 and 500 µg/ml. After incubating for 10 min at room temperature the whole mixture was centrifuged 5000 rpm for 5 min and at 540 nm, the absorbance of the supernatant was assessed using UV-spectrophotometer. For reference Aspirin (standard) at the different concentration of (100, 200, 300, 400 and 500 µg/ml) was used at similar absorbance. All experiments in this study were performed in triplicate, and the results were mean ± standard values. The percent inhibition of hemolysis was calculated according to the equation:

$$\% \text{ inhibition of hemolysis} = \left[1 - \frac{OD_2 - OD_1}{OD_3 - OD_1}\right] \times 100$$

Where: OD_1: Test sample in isotonic solution; OD_2: Test sample hypotonic solution and OD_3: Control sample in hypotonic solution.

2.7.3. Egg Albumin Denaturation Assay

The in vivo anti-inflammatory effects of methanol extract *Corallocarpus epigaeus* leaves were evaluated against denaturation of egg albumin. The reaction mixture (5 ml)

consisted of the 0.2 ml of egg albumin with saline of 2.8 ml phosphate buffer (PBS, pH 6.4) and 2 ml of changing concentrations of *Corallocarpus epigaeus* leaves extract so that terminal concentrations become 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml and 500 µg/ml. Incubation was done for 15 min at 37.2°C and after that it was heated for 5 min at 70°C. After that absorbance was measured at 660 nm. For reference Aspirin (standard) at the different concentration of (100, 200, 300, 400 and 500 µg/ml) was used at similar absorbance. All experiments in this study were performed in triplicate, and the results were mean ± standard values. Following formula is used for calculating inhibition of protein denaturation:

$$\% \text{ inhibition of egg albumin denaturation} = 1\left[-\frac{OD_2 - OD_1}{OD_3 - OD_1} x\right]100$$

Where: OD_1: Test sample unheated; OD_2: Test sample heated; OD_3: Control sample heated.

2.7.4. Statistical Analysis

The results are expressed as mean ± SD using Graph Pad Prism (version 7). The data for the anti-bacterial, anti-oxidant and anti-inflammatory activities of methanolic extract *Corallocarpus epigaeus* leaves were analysed using the one-factor analysis of variance (ANOVA - with SPSS statistical software 16th version) followed by post hoc Duncan multiple rank test (DMRT) to compare experimental and control across different concentration doses. In all cases, a significant level of $p < 0.05$ was used.

3. Results and Discussion

An analysis of the phytochemical screening of the methanolic extract of *Corallocarpus epigaeus* leaves revealed the presence of different phytochemicals constituents such as alkaloids, flavonoids, phenols, tannins, terpenoids, cardiac glycosides, reducing sugars, steroids and coumarins, but saponins were absent (Table 1). Our findings are consistent with those reported by EL-Kamali and AL-Amir Ashok and Mustafa. [19-21]. The previous literatures also supported that the analysis of *Corallocarpus* species showed the presence of alkaloids, coumarin, phenols, glycosides, flavanoids, triterpenoids, amino acids [12,22]. It is reported that the majority of herbal plants contain alkaloids with various chemical configurations in different plants parts (i.e., organs) and have been reported to have analgesic, anti-inflammatory, and adaptogenic properties that aid in the relied of pain, development disease resistance, development of stress tolerance, cure asthma, snake bite and skin diseases [23-26]. In addition, phenols are reported antitumour agents and to exhibit antiviral and antimicrobial activities [26]. The findings of the present study clearly indicate that the extract of *Corallocarpus epigaeus* leaves contains abundant phytochemicals with diverse biological properties, such as anti-bacterial, anti-viral, anti-fungal, anti-inflammatory, antioxidant activities etc.

S.No.	Phytochemicals	Name of Test	Reaction is characterized by	Result
1	Alkaloid	Mayer's test	brown colour precipitate	++
2	Flavanoid	Lead acetate test	formation of yellow colour	++
3	Phenol	FeCl3 test	precipitates of green/blue/violet	++
4	Tannins	Ferric chloride test	black precipitate	++
5	Saponins	Foam test	froth formation	--
6	Terpenoids	Salowski's test	reddish Brown precipitate	++
7	Cardiac Glycosides	Keller-Killiani Test	formation of a brown ring	++
8	Reducing Sugar	Fehling's or Benedict's test	brick-red precipitate at the bottom	++
9	Steroids	Lieberman-Burchard test	greenish precipitate	++
10	Coumarin	NaOH test	appearance of a fluorescent colour	++
Abbreviation: ++: strong reaction intensity; -: non-detected.				

Table 1: Phytochemical Screening of Methanolic Leaf Extract of Redfruit Creeper *Corallocarpus epigaeus*

Phytochemical constituents of the methanolic extract of leaves were quantitatively analyzed by GC-MS, and it was revealed that 20 phytochemicals were identified (Fig 1 and Table 2). In the present study, more number of carboxylic acids compounds has been excreted as compared to other constituents such as alcohol, phenol, aldehyde, alkane, alkene, ester, ketone, amin etc. Carboxylic acid compounds probably play a significant role in biological activities like anti-microbial, anti-fungal, anti-viral, anti-oxidant, anti-inflammatory etc. For example, plant-derived natural carboxylic acids like benzoic acid, cinnamic acid, p-coumaric acid, caffeic acid, rosmarinic acid, and chicoric acid are known to possess a several biological activities like Anti-oxidant, anti-cicrobial and cytotoxic [27]. The phenolic acids often found in fruits, vegetables, and herbs, were recognized for their medicinal properties (anti-cancer and anti-oxidant) and used in traditional healing practices [28]. It is noteworthy that all 20 phytochemicals have already been proven to have biological properties in various biological sources (Table 3), in this regards, *Corallocarpus epigaeus* plants naturally have a rich amount of biological active compounds that can be used in other biological processes.

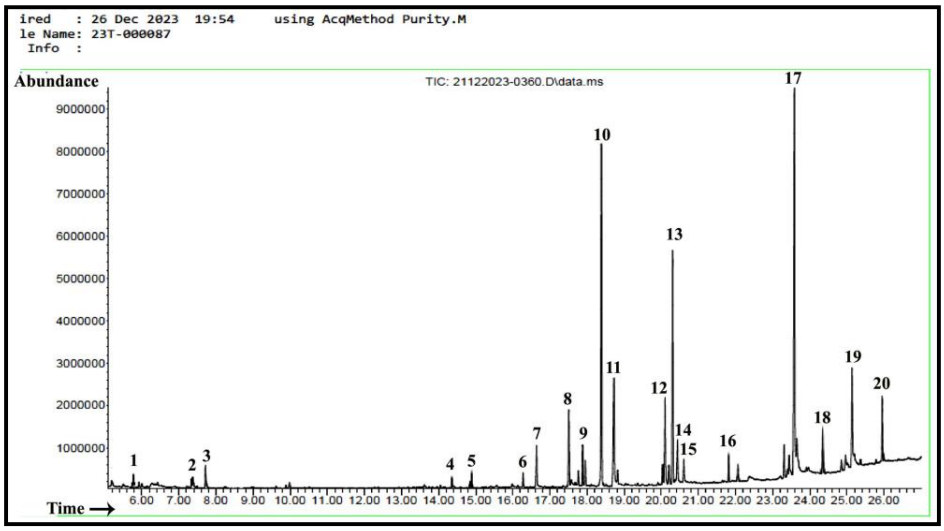

Figure 1: Gas Chromatographic Profiles of Phytochemicals in Methanol Extract of *Corallocarpus epigaeus* for Details, Please Refer Table 2

Peak No.	RT (min)	Name of the compound	Chemical Group	MF	MW
1	5.77	N-Methylmaleimide	Amine	$C_5H_5NO_2$	111.1
2	7.37	1-Nethyl-2-pyrrolidinone	Ketone	C_5H_9NO	99.13
3	7.71	Hexamethyl- Cyclotrisiloxane	Alkane	$C_6H_{16}O_3S_1$ 3	222.46
4	14.35	Dodecanoic acid	Carboxylic acid	$C_{12}H_{24}O_2$	200.32
5	14.88	Diethyl phthalate	Aldehyde	$C_{12}H_{14}O_4$	222.24
6	16.27	Tridecanoic acid, 12-methyl-methyl ester	Ester	$C_{15}H_{30}O_2$	242.4
7	16.63	Tetradecanoic acid	Carboxylic acid	$C_{14}H_{28}O_2$	228.37
8	17.57	2-Isopropyl-5-methyl-1-heptanol	Alcohol	$C_{11}H_{24}O$	172.31
9	17.75	Neophytadiene	Alkene	$C_{20}H_{38}$	278.50
10	18.38	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	Ester	$C_{16}H_{22}O_4$	278.34
11	18.82	Dibutyl phthalate	Aldehyde	$C_{16}H_{22}O_4$	278.34
12	20.19	3-Pyrrolidin-2-yl-propionic acid	Carboxylic acid	$C_7H_{13}NO_2$	143.18
13	20.20	Phytol	Alcohol	$C_{20}H_{40}O$	296.5
14	20.42	3,4-Dimethyl-1H-pyrrole	Pyrrole	C_6H_9N	95.14
15	20.60	Octadecanoic acid	Carboxylic acid	$C_{18}H_{36}O_2$	284.5

16	21.81	Glycidyl palmitate	Aldehyde	$C_{19}H_{36}O_3$	312.49
17	23.64	Hexadecanoic acid	Carboxylic acid	$C_{19}H_{38}O_4$	330.5
18	24.34	Silane, diethylheptadecyloxy (2-methoxyethoxy)	Alkane	$C_{24}H_{52}O_3Si$	416.8
19	25.36	Squalene	Terpenoids	$C_{30}H_50$	410.7
20	25.99	1-hexadecyl-2,3-dihydro-1H-Indene	Alkene	$C_{25}H_{42}$	342.60

Table 2: Phytochemical Compounds Identified in the Methanolic Leaf Extract of *Corallocarpus epigaeus* by Gcms Analysis

S.No.	Name of the compound	Biological property	Reference
1	N-Methylmaleimide	Anti-microbial, Cytotoxic, Anti-hypotension, Inhibition of platelet aggregation	Tamaoki et al. [39]
2	1-Methyl-2-pyrrolidinone	Anti-fungal, Anti-bacterial, Anti-convulsant, Anti-cancer	Hosseinzadeh et al. [40]
3	Hexamethyl-Cyclotrisiloxane	Antioxidant, Anti-inflammatory, Ant-imicrobial, Anti-cancer	Benila et al. [41] Chetehouna et al. [42]
4	Dodecanoic acid	Anti-tumor, Anti-viral, Anti-fungal, Anti-inflammatory, Anti-mycobacterial, Anti-bacterial, Ant-imicrobial	Kato et al. [43] Villamor et al. [44] Calder et al. [45] Saravana kumar et al [46]
5	Diethyl phthalate	Antimicrobial, Insecticidal	Huang et al. [47]
6	Tridecanoic acid, 12-methyl-methyl ester	Ant-imicrobial, Anti-inflammatory, Anti-tumor	Aravindakshah et al. [48]
7	Tetradecanoic acid	Anti-fungal, Anti-virulence, Anti-oxidant Anti-larvicidal	Sivakumar et al. [49] Sokmen et al. [50]
8	2-Isopropyl-5-methyl-1-heptanol	Anti-microbia Anti-bacterial	Jeon et al. [51] Uyan et al. [52]
9	Neophytadiene	Anti-inflammatory, Cytotoxic, Ant-ioxidant, Cardio-protective	Selmy et al. [53] Bhardwaj et al. [54]
10	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	Anti-microbial Anti-oxidant Anti-bacterial Anti-fungal Anti-nematicidal	Sholkamy et al. [55] Kumar et al. [56]

11	Dibutyl phthalate	Anti-fungal, Anti-microbial	Roy et al. [57] Anantharaman et al. [58]
12	3-Pyrrolidin-2-yl-propionic acid	Anti-microbial	Veilumuthu et al. [59]
13	Phytol	Anti-microbial, Anti-tumor, Anti-oxidant, Anti -mutagenic, Anti-convulsant, Anti-depressant, Anti-inflammatory, Cytotoxic, Anti-diabetic, Anxiolytic, Anti-dandruff, Lipid-lowering, Hair fall defence Anti-spasmodic, Anti-teratogenic, Chemotherapeutic, Hair growth facilitator, Immunoadjuvant	Lee et al. [60] Olofsson et al. [61]
14	3,4-Dimethyl-1H-pyrrole	Anti-bacterial, Anti-cancer, Anti-oxidant, Anti-fungal	Astakhina et al. [62] Rasal et al. [63]
15	Octadecanoic acid	Anti-viral, Anti-bacterial, Anti-inflammatory, Anti-microbial	Pu et al. [64] Sivasamy et al. [65] Manivannan et al. [66]
16	Glycidyl palmitate	Anti-microbial, Anti-diabetic, Anti-oxidant	Jannat et al. [67] Arya et al. [68] Wong et al. [69]
17	Hexadecanoic acid	Anti-inflammatory, Anti-bacterial, Anti-fungal, Anti-cancer	Daniels et al. [70] Thejashree et al. [71] Christiana et al. [72]
18	Silane, diethylheptadecyloxy (2-methoxyethoxy)	Anti-viral, Anti-microbial	El et al. [73] Mohorcic et al. [74]
19	Squalene	Anti-oxidant, Anti-inflammatory, Anti-cancer Drug carrier, Detoxifier, Skin hydrating	Huang et al. [47] Kim and Karadeniz [32] Güneş [75]
20	1-hexadecyl-2,3-dihydro-1H-Indene	Anti-microbial Anti-inflammatory Anti-cancer	Bukhari et al. [37] Mohamed et al. [76]

Table 3: Twenty Phytochemical Already Been Documented with their Biological Characteristics in Various Biological Sources

It is important to note that the peak area and peak height of the certain phytochiemcials (i) 1,2-benzenedicarboxylic acid, bis (2-methylpropyl) ester, (ii) phytol, (iii) hexadecanoic acid and (iv) squalene were noticed. This suggests that the these compounds may be maximally produced *Corallocarpus epigaeus* plants as a defense mechanism against herbivores

and pathogens, acting as deterrents due to their toxicity. It is remarkable to note that the phytochemcial 1,2-benzenedicarboxylic acid, bis (2-methylpropyl) ester isolated from plants have bioactive properties, such as antifungal, antimicrobial, and cytotoxic activities against cancer cells [29]. Phytol, a natural diterpene alcohol, is a phytochemical that plays an important role in synthesis of plant metabolites and has a variety of biological properties, including antioxidant, anti-inflammatory, and anticancer properties [30]. Hexadecanoic acid, a common saturated fatty acid can be involved in the formation of plant waxes, oils, and other structural lipids, and has also been reported to exhibit anti-inflammatory, antioxidant, and antibacterial properties [31]. Phytochemcial Squalene is a natural compound that is mainly involved in sterols synthesis of and has a variety of biological properties, including antioxidant, anti-inflammatory, anti-cancer activity, and skin health [32]. This finding suggests that the phytochemicals found in the methanolic extract of *Corallocarpus epigaeus* leaves might have an impact on the biological properties. The results of anti-bacterial activities of *Corallocarpus epigaeus* leaf of various extracts tested against three pathogens are depicted in Table 4. Maximum inhibition was observed with methanolic extract of *Corallocarpus epigaeus* leaves against Bacillus marisflavi (23.01±0.1) and Staphylococcus aureus (22±0.1 mm) while minimum activity against Klebsiella pneumoniae (12.01±0.1 mm). The present investigation is in contrast to Chowdhury (2008), who reported strong antibacterial potential against Klebsiella pneumoniae as compared to Staphylococcus aureus. Our results are in agreement with several studies that have demonstrated that leaf extracts from *Corallocarpus* plants exhibit antimicrobial properties against various pathogens [11,12, 22]. According to Sasidharan. The solvent has a significant impact on the extraction of plant bioactive materials from plant parts [33]. This study clearly demonstrates that the methanol solvent extracts the maximum bioactive compounds from *Corallocarpus epigaeus* leaves and has greater anti-bacterial activity.

Bacterial strain	Zone of inhibition (mm)					
	Different concentration methanolic extract (µg/ml)				NC (-)	PC (+)
	25	50	75	100		
Staphylococcus aureus	NA	NA	17.03±0.1^{b}	22±0.1^{a}	NA	15 ± 0.1^{bc}
Bacillus marisflavi	NA	NA	20.04±0.1^{ab}	23.01±0.1^{a}	MA	15 ± 0.1^{c}
Klebsiella pneumoniae	NA	NA	NA	12.01±0.1^{b}	NA	15 ±0.1^{a}

Values are expressed in Mean ± SD. Means in the same vertical column that are not marked with the same superscript (alphabets) letters are significantly different at p<0.5 level, comparison by DMRT.

Abbreviation:

NA: No activity; ***PC:*** Positive control (Gentamicin – 100 µg); ***NC:*** Negative control (DMSO – 100 µL).

Table 4: Zone of Inhibition (Mm) of Methanolic Extract of Redfruit Creeper, *Corallocarpus epigaeus* Against Some Bacterial Strains

DPPH and H_2O_2 were employed to investigate the antioxidant activity of the methanolic leaf extracts of *Corallocarpus epigaeus* (Tables 5 and 6). The results showed that the methanolic leaf extracts has potent antioxidant activities as evidenced by the IC50 values obtained in DPPH (149 µg/ml) and Hydrogen peroxide (150 µg/ml) scavenging assays. In the present study, the extracts were capable of scavenging DPPH and H2O2 dose dependently. Amin and Tan reported that the percentage inhibition of DPPH was strongly dependent on the concentration of the plant extract, especially the availability of bio compounds. Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) groups [34,35,39,40]. According to similar findings reported by Iqbal the methanolic extract of A. annua leaves showed the higher scavenging activities, indicate that the phytochemcial constituent with anti-oxidant properties was extracted efficiently using a methanol solvent [36,41-49].

Concentration (µg/mL)	A	%	IC_{50}	PC (%)	IC_{50}
50	0.063^{cd}	86.049^{d}	149	93.001^{cd}	150
100	0.070^{c}	90.586^{c}		95.050^{c}	
150	0.076^{bc}	95.556^{bc}		96.330^{bc}	
200	0.082^{b}	96.967^{b}		97.000^{b}	
250	0.089^{ab}	97.078^{ab}		97.979^{ab}	
300	0.095^{a}	97.097^{a}		98.006^{a}	

Values are expressed in Mean ± SD. Means in the same horizontal column that are not marked with the same superscript (alphabets) letters are significantly different at p<0.5 level, comparison by DMRT.

Abbreviation:

A: Absorbance; ***%:*** Percentage of Inhibition; ***PC:*** Positive control - ascorbic acid: ***IC:*** Inhibitory concentration.

Table 5: Dpph Radical Scavenging Activity of Methanolic Leaf Extract of Redfruit Creeper, *Corallocarpus epigaeus*

Concentration (µg/mL)	A	%	IC_{50}	PC %	IC_{50}
50	0.048^{d}	89.2857	150	93.001	150
100	0.054^{cd}	92.633		94.921^{c}	
150	0.060^{c}	96.468		96.375^{bc}	
200	0.066^{bc}	97.559		97.009b	
250	0.072^{b}	97.622		98.544^{ab}	
300	0.132^{a}	95.967		98.784^{a}	

Values are expressed in Mean ± SD. Means in the same horizontal column that are not marked with the same superscript (alphabets) letters are significantly different at p<0.5 level, comparison by DMRT.

Abbreviation:

A: Absorbance; ***%:*** Percentage of Inhibition; ***PC:*** Positive control - ascorbic acid: ***IC:*** Inhibitory concentration.

Table 6: Hydrogen Peroxide Radical Scavenging Activity of Methanolic Leaf Extract of Redfruit Creeper, *Corallocarpus epigaeus*

The present study shows that the methanolic extract of *Corallocarpus epigaeus* leaves has greater anti-inflammatory effects (Tables 7, 8, and 9). This effect can be demonstrated by heat-induced membrane stabilization method, hypotonicity induced HRBC membrane stabilization method and egg albumin denaturation method. Anti-inflammatory effects are a concentration-dependent process, and protection increases with increasing the concentration of the leaves extract (i.e., sample). Aspirin a standard anti-inflammatory drug showed the maximum inhibition of 78.33% at the concentration of 500 µg/ml, whereas methanolic extract of *Corallocarpus epigaeus* leaves showed 97.09% at that concentration. According the heat induced hemolysis, hypotonicity induced hemolysis, and egg albumin denaturation assays revealed significant anti-inflammatory activity with respective percentage inhibitory values of 71.9±18.1, 36.11±2.79 and 97.09±0.19 as compared to control. Bukhari and Abdulrashid reported that the hemolysis occurs when excessive fluid accumulates in the cells, causing the RBC membrane to rupture [37]. When the cell membrane is damaged, it becomes more prone to secondary damage, which is caused by free radicals induced lipid peroxidation. Ethanolic leave extract of *Corallocarpus epigaeus* might be stabilizing the membrane of RBC by precluding the discharge of lytic enzymes and other active inflammatory mediators. In the anti-denaturation assay, egg albumin denatures due to heat treatment [50-60]. Ahmad reported that some antigens are expressed by denatured proteins [61-69]. These antigens trigger hypersensitive reactions (type-III) associated with some diseases, such as glomerulonephritis and serum sickness [38]. The present investigation has demonstrated that the leaf extract possesses significant anti-inflammatory properties that regulate auto-antigen synthesis [70-76].

Sample	Concentration (µg/mL)	Percentage Inhibition (Mean ± SD)
Positive control (aspirin)	100	14.6 ± 2.63^{e}
	200	25.66 ± 0.61^{d}
	300	46.9 ± 11.50^{c}
	400	69 ± 1.53^{b}
	500	78.33 ± 1.69^{a}
Methanolic extract of *C. epigaeus*	100	40.67 ± 15.79^{d}
	200	50 ± 0.58^{c}
	300	56 ± 1.49^{b}
	400	66.1 ± 3.7^{a}
	500	71.9 ± 18.1^{bc}
Values are expressed in Mean ± SD. Means in the same horizontal column that are not marked with the same superscript (alphabets) letters are significantly different at p<0.5 level, comparison by DMRT.		

Table 7: Effect of Methanolic Leaf Extract of *Corallocarpus epigaeus* on Heat-Induced Hemolysis of Rbc Membrane

Sample	Concentration (µg/mL)	Percentage Inhibition (Mean ± SD)
Positive control (aspirin)	100	13.6 ± 0.56^{d}
	200	23.55 ± 0.56^{c}
	300	37.66 ± 0.49^{bc}
	400	$40\pm0.\ 56^{b}$
	500	60 ± 0.98^{a}
Crude extract of *C. epigaeus*	100	12.7 ± 0.56^{c}
	200	26.99 ± 0.56^{bc}
	300	28.65 ± 0.57^{b}
	400	31.11 ± 0.58^{ab}
	500	36.11 ± 2.79^{a}
Values are expressed in Mean ± SD. Means in the same horizontal column that are not marked with the same superscript (alphabets) letters are significantly different at p<0.5 level, comparison by DMRT.		

Table 8: Effect of Methanolic Leaf Extract of *Corallocarpus epigaeus* on Hypotonicity Induced Hemolysis of Rbc Membrane

Sample	Concentration (µg/ml)	Percentage Inhibition (Mean ± SD)
Positive control (aspirin)	100	3.7 ± 0.56^{d}
	200	20.98 ± 2.29^{cd}
	300	24 ± 0.2^{c}
	400	50.98 ± 0.56^{b}
	500	59 ± 6.8^{a}
Crude extract of *C. epigaeus*	100	73.31 ± 0.03^{cd}
	200	79.98 ± 0.21^{c}
	300	87.01 ± 0.06^{b}
	400	96.01 ± 1.09^{ab}
	500	97.09 ± 0.19^{a}
Values are expressed in Mean ± SD. Means in the same horizontal column that are not marked with the same superscript (alphabets) letters are significantly different at p<0.5 level, comparison by DMRT.		

Table 9: Effect of Methanolic Leaf Extract of *Corallocarpus epigaeus* on Egg Albumin Denaturation

4. Conclusion

The investigation indicated that the methanolic leaf extract of *Corallocarpus epigaeus* shows abundance of various plant metabolites, further GC-MS analysis detected 20 major phytochemcial constituents, among these four compounds (i) 1,2-benzenedicarboxylic acid, bis (2-methylpropyl) ester, (ii) phytol, (iii) hexadecanoic acid and (iv) squalene peak height concentrations were greatly high compared to the other compounds. Anti-bacterial, anti-oxidant and anti-inflammatory effects are a concentration-dependent process, and protection increases with increasing the concentration of the leaves extract. Based on the phytochemical screening, anti-bacterial, anti-oxidant and anti-inflammatory activities, we conclude that the methanolic leaves extract of Redfruit creeper contains an abundance of bioactive compounds could serve as a potential source of anti-bacterial, anti-oxidants and anti-inflammatory agents. A further study will be required to identify the novel bioactive compounds in Redfruit creeper that may be more effective in different biological processes.

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