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Characterization of *Commiphora caudata* and its ability to combat Methicillin-resistant *Staphylococcus aureus* (MRSA)

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This study examines the antibacterial properties ethanol extracts made from the dried leaves of *Commiphora caudata* (Wight & Arn.) Engl. (Burseraceae) *in vitro*. Phytochemical analyses, HPTLC, TLC, and gas chromatography-mass spectrometry (GC-MS) were used to investigate the leaf extract. A preliminary analysis of the plant's phytochemical composition revealed the presence of sugars, proteins, and amino acids, as well as flavonoids, saponins, tannins, terpenoids, phytosterol, and mucilage, whereas alkaloids, volatile oil, and fixed oil were not present. TLC and HPTLC profiles of the ethanolic extracts have been displayed. At 0.051% w/w, it revealed the existence of various pharmacologically significant and vital components, such as E-guggulsterone. The identification of 15 chemicals from leaf extract represents 100% to 99.97% of the total. Six-Octadecenoic Acid, Nine-Octadecenoic Acid, (E) n-Decanoic Acid, Two-Octadecanoic Acid, Tetradecanoic Acid, Oleic Acid, and Z (13,14-Epoxy) 2-tert-Butyl-5, Tetradec-11-en-1-...5-dimethyl-3-oxo-... Piperidine, Cyclo-3-Heptadecenal 3-isopropylcycloheptadecanone (Z)-2-Methyl-13-Octadecenal, Z-3-13-Octadecadienol Z and 13-octadecenal are the main elements. Methicillin-resistant *Staphylococcus aureus* was used to evaluate the plants' antibacterial abilities (MRSA). In comparison to the ethanol extracts, which had a MIC of 1.1 mg/mL, the ethanol extracts of *C. caudata* demonstrated moderate antibacterial activity with a MIC of more than 2.0 mg/mL against MRSA.

Keywords: *Commiphora caudata*, E-guggulsterone, GC-MS, HPLC, MRSA

INTRODUCTION

In order to live, plants manufacture unique compounds in their roots, leaves, blooms or seeds. In order to carry out crucial biological processes and protect themselves from predators like insects, fungus and herbivores, plants may synthesis a vast range of chemical compounds.

Plants and their extracts have been employed for their therapeutic powers since the beginning of time. When taken by humans, many of these phytochemical have positive health benefits and can be utilized to treat a variety of human ailments. So far, at least 12,000 of these compounds have been discovered, which is thought to represent less than 10% of the total. According to their therapeutic characteristics, numerous plant components, including roots, stems, leaves, buds, seeds, bark, exudates, etc., are utilized for a variety of diseases (Samy *et al.* 2007).*Commiphora caudata* Hill-mango, also known as Engl. (Syn. *Protium caudatum* Wight &Arn.) Engl., is a medium-sized (18 m tall), evergreen, fragrant tree (leaves 3-7 foliolate, leaflets elliptic-ovate, 3-10 1.5-6 cm, glabrescent, acute, base unequal). The South Indian subcontinent's arid or semi-evergreen woodlands are home to red blooms that are formed in axillary cymes with single seeds between March and October. Communities that live in the forest use the fruits and leaves to flavor meals in a way that resembles mango. Ingestion problems can be

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treated using leaves, fruits used to make pickles and to treat injuries, gum resin to treat digestive issues. Researchers have examined the leaf extracts for its anti-bacterial, pharmacological, analgesic, anti-inflammatory and anti-lipid peroxidation (LPO) effects. In rat models, the anti-ulcer effects of bark and gum extracts were investigated. Plants have been utilized as a source of raw materials for cosmetics, medications, botanical pesticides, disinfectants, insect repellents, herbal teas, herbal beverages, etc. since ancient times. *C. caudata* extract contains a wide range of organic components, including sulfur compounds, several enzymes, various minerals, vitamins, and amino acids. One of *C. caudata's* most important and powerful biological compounds is e-guggulsterone. Although E-guggulsterone is thought to be the primary antioxidant component, additional substances, such as polar compounds of steroidal and phenolic origin, with a range of pharmacological characteristics, no smell, and thermal stability. Research has shown that *C. caudata* is effective and has broad-spectrum antimicrobial activity against a variety of bacteria, viruses, parasites and fungi, including *Staphylococcus aureus, Streptococcus pneumonia, Streptococcus fecalis, Enterobacter cloacae, Klebsiella pneumonia, Pseudomonas aeruginosa, Shigella* spp. (Jaishee, 2016) and also has antioxidant activities (Kumari *et al.* 2011). E-guggulsterones produced by the enzymatic activity of *C. caudata* have been primarily blamed for the plant's antibacterial properties. For both humans and animals, the rise of MRSA epidemics and multidrug-resistant strains of Gram-negative and Gram-positive bacteria is concerning. E-guggulsterone is therefore a well-liked alternative drug for the treatment of MRSA Determining the in vitro antibacterial activity of a crude *C. caudata* ethanol extract against a clinical isolate of methicillin-resistant *Staphylococcus aureus* was the goal of the current investigation (MRSA).

Survey of the literature reveals that there have been no studies on the phytochemical and pharmacological effects of *C. caudata* ethanol extract. The current experiment was carried out to examine the chemical makeup and anti-MRSA activity of *C. caudata* isolated from leaves utilizing *in vitro* tests in light of the aforementioned benefits. However, to the best of the authors' knowledge, this is the first report on the chemical profiles and biological activities of ethanol extract of *C. caudata.*

MATERIALS AND METHODS

Plant collection and authentification

Leaves of the plant *C.caudata* selected for the study was collected from T.kallipatti, Periyakulam (Tk), Theni (Dt), Tamil Nadu, India during the month of oct 2022 and was authenticated by Dr.V.Siva, PG & Research Department of Microbiology, V. H. N. Senthikumara Nadar college, Virudhunagar - 626 001.

Extraction

In the beginning, 200 g of the dried *Commiphora caudata* roots were packed into a thimble, and 2.5 lof the extraction solvent were put into a round bottom flask. The siphon tube gathered solvent appeared clear once the soxhlet extraction was completed, which took 18 to 24 hours. Later, to get dry extract, the extracted solvent was evaporated under decreased pressure. It was determined what the extraction's yield was.

Extraction yield (%) = (weight of the dried extract x 100) / (weight of the original sample)

Phytochemical screening

The ethanolic extracts of *C. caudata* leaves were tested chemically to identify the secondary plant components contained using a variety of techniques (Deepa *et al.* 2009), as follows:

Reducing sugars

To 2 ml of the extract, add 5 ml of a 1:1 combination of Fehling's solutions IA and II (B), and boil the mixture in a water bath for 5 minutes. The presence of free reducing sugars was indicated by a brick-red precipitate.

Anthraquinones

The filtrate after shaking 1ml of the extract with 10ml of benzene and adding a 10% ammonia solution to the filtrate. After shaking the mixture, anthraquinones might be detected by a pink, red, or violet color in the lower phase.

Saponins

One ml of the extract was dissolved in five ml of distilled water in a test tube, which was then corked, agitated erratically for 25 sec., and left to stand for 15 mins. The presence of saponins was detected by the appearance of foaming that persisted after heating.

Flavonoids

A little amount of the extract was mixed with a few drops of a 10% ferric chloride solution. Phenols were visible as a green or blue colour.

Steroids/Terpenes

Two ml of acetic anhydride were added to 1 ml of the extract, which was then thoroughly dissolved. Carefully applied sulfuric acid. A shift in hue from violet to blue to green meant that steroids were present.

Tannins

One ml of ethanol extract and a few drops of 10% ferric chloride were dissolved in water. If tannins are present, a blue-black, green, or blue-green precipitate would be present.

Alkaloids

On a steam bath, 1ml of ethanol extract and 2ml of hydrochloric acid were mixed. The filtrate from this mixture was then treated with a few drops of Mayer's reagent and another 1ml amount of Dragendorff's reagent. Alkaloids would be present if one of these reagents produced turbidity4.

Thin layer chromatographic analysis (TLC)

In the current work, silica gel 60f 254 pre-coated metal cards and TLC plates were used for the thin layer chromatography (0.2 mm thickness). Sharp cutters were used to create TLC plates with the necessary dimensions. For the application of the sample, plates were marked. By using a jet-tipped capillary tube, 5–10 L of material was spotted on the line designated at one side of the plate. Depending on the mobile phase concentration while collecting them through a column, multiple mobile phases were created for different elutions. Plates were positioned and given time to develop when the TLC tank developed pressure. The plates were taken off after the mobile phase reached its peak. Plates were dried after marking the solvent front. Both visible light and ultraviolet (UV long & short) light were used to view TLC plates and visualized spots were noted to get the Rf values (Dhivya *et al.* 2020). After measuring the lengths that mobile phases and substances covered, the Rf value was derived as follows :

$$RF = \frac{\text{Distance travelled by visualized spot R}}{\text{Distance travelled by solvent}}$$

GC-MS Analysis

Utilizing a 1.8 m capillary column (DB-5) filled with 5% phenyl dimethyl silicone and a 30 m 0.32 mm film thickness, the GC-MS (Agilent Technology: GC-MS) inside the electron impact (EI) mode at an ionizing potential of 70 eV, was used to investigate phytochemicals. The following GC/MS parameters were used for further analysis: The starting column temperature was set at 45°C and maintained for 4 mins; the temperature was then increased to 50°C, increased for 2 minutes at a rate of 100°C per minute up to 175°C, and then programmed to 240°C at a rate of 25°C per min., maintaining isotherm for 2 minutes. As a carrier gas, helium was employed at a flow rate of 1.491 ml/min with a split ratio of 1:10. During sample analysis the column oven temperature was maintained at 280°C (Zhang *et al*. 2011; Reddy *et al.* 2022).

Extract of leaves by HPTLC analysis

A more contemporary version of TLC with enhanced adaptability, separation effectiveness, and detection limits is high performance thin layer chromatography (HPTLC). Because each plant species creates a distinctive chromatogram and uses separate marker chemicals for the plant identification, HPTLC is a valuable method for identifying plant extracts. Since comparing the chromatograms of several lots may show the similarities and differences between test samples and their standard chemical markers, it is employed as a quality control tool. Even when present in complex configuration, HPLTC is a dependable approach for measuring nanogram levels. HPTLC fingerprint analysis is used for quick identification verification, drug purity monitoring, adulterant detection and establishing whether a substance is

originated from a certain botanical species and also to know whether the constituents are clearly characterized (Wagner *et al.* 1996). Details of procedure used are provided below :

Instrument used :	CAMAG TLC Scanner 3
Software :	win CATS Planar Chromatography Manager
Sample application:	Linomat 5
Detection:	at 254nm in TLC Scanner 3
Stationary phase:	HPTLC plates silica gel 60 F 254
Sample preparation:	100mg per ml of sample was prepared in ethanol solvent
Mobile phase:	Toluene: Acetone (9:1)
Sample solution:	5Ml
Drying device :	Oven
Temperature :	60°C
Volume : :	10.0ml
Time :	5minutes
Scanning speed :	20mm/s
Wavelength :	254 nm
Lamp :	D2&W
Measurement type :	Remission
Measurement mode :	Absorption

Collection of clinical samples

From the Government Hospital and Medical College in Virudhunagar, ten clinical samples (pus samples from wounds) were obtained.

Methicillin-resistant Staphylococcus aureus isolation

In addition to performing coagulase and catalase assays, the pus samples were cultivated on Nutrient and Mannitol Salt Agars (MSA).

Antimicrobial tests

By using a disc diffusion test, extracts' antibacterial activity was assessed (Kim *et al.*1995). On the surface of Hinton agar, a 100 L diluted bacterial solution (5 × 106 cfu mL-1) of test bacterial strains was applied. Then, Muller Hinton Agar was covered with a sterile blank paper disc containing 100, 200, 300, 400, and 500 g of extracts. Discs were coated with the appropriate solvent as a negative control. The widths of the inhibitory zones (mm) were measured after plates were incubated at 37°C for 24 hours.

RESULTS AND DISCUSSION

A plant's secondary metabolites, such as alkaloids, tannins, glycosides, phytosterols, flavonoids, and phenols, are what give it its therapeutic effects. Alkaloids, coumarins, tannins, glycosides, phytosterols, flavonoids, phenols, and saponins were discovered in the ethanol extracts from *C. caudata* leaves. The identification of bioactive chemicals and subsequent drug discovery and development benefit from qualitative phytochemical investigation (Varadarajan, 2008). The presence of glycosides and saponins demonstrates the cardioprotective quality. The biological effects of the phenolic compounds include anti-aging, anti-inflammation, and cardiovascular protection (Kumar *et al*. 2014). Numerous studies demonstrate that medicinal plants with antioxidant properties are abundant in flavonoids (Krings and Berger, 2001). Table No. 1 displays the findings of a preliminary phytochemical investigation. 52 chemicals were found in the ethanol extract of *C. caudata* leaves according to a GC-MS investigation on the bioactive components. In Table 2 and Fig.1, the major and minor compounds are included

Table 1: Presence of phytochemical compounds in leaf extracts of *Commiphora caudata*

Alkaloids	+
Amino Acids	+
Anthraquinones	-
Flavonoids	+
Glycosides	+
Gums and Mucilage	-
Proteins	+
Reducing Sugars	+
Saponins	-
Starch	+
Steroids	+
Tannins	+

(+present -absent)

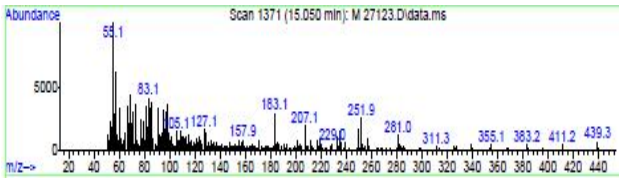

Fig. 2: Phytocomponents in ethanol extract of *Commiphora caudata* leaf by GC-MS

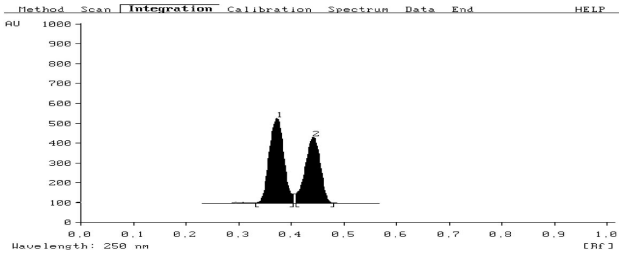

Fig. 3 :TLC analysis of extracts of *C.caudata*

Table 2: Compounds detected in the leaf extracts by GC-MS

Sr. No.	RT	Name of the Compound	MW g/mol	MF	Area (%)
1.	55.10	6-Octadecenoic Acid	282.5	C18H34O2	59.10%
2.	81.1	9-Octadecenoic Acid,	282.5	C18H34O2	49.19%
3.	109.2	Oleic Acid	282.5	C18H34O2	47.38%
4.	137.0	n-Decanoic acid	172.26	C10H20O2	62.86%
5.	159.0	Octadecanoic acid	2282.5	C18H34O	62.31%
6.	183.1	Tetradecanoic acid	228.37	C14H28O2	56.21%
7.	219.0	Oleic Acid	282.5	C18H34O2	51.74%
8.	251.9	Z-(13,14-Epoxy) tetradec-11-en-1-...	268.39	C16H28O3	61.77%
9.	281.0	2-tert-Butyl-5,5-dimethyl-3-oxo-...	-	-	43.02%
10.	311.3	Cycloheptadecanone	252.4	C17H32O	40.73%
11.	355.1	3-Heptadecenal	252.4	C17H32O	37.55%
12.	383.2	Piperidine, 3-isopropyl-	127.23	C8H17N	69.45%
13.	411.2	13-Octadecenal, (Z)-	266.5	C18H34O	41.83%
14.	439.3	2-Methyl-Z,Z-3,13-octadecadienol	280.5	C19H36O	41.43%
15.	83.81	cis-7,cis-11-Hexadecadien-1-yl a...	280.4455	C18H32O ·	38.50%

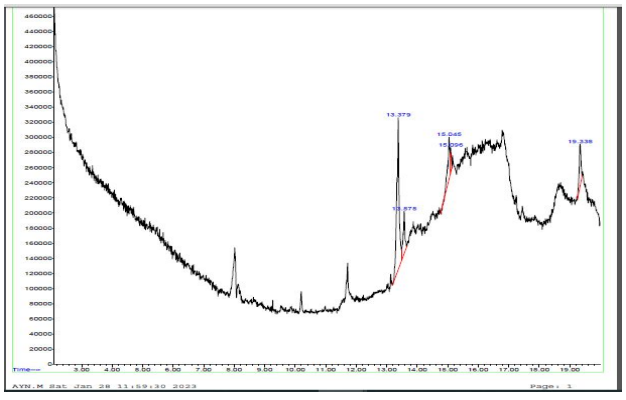

Fig.1: GC-MS analysis of *Commiphora caudata*

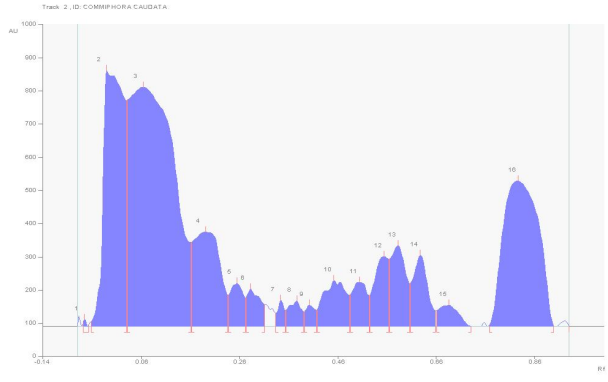

Fig. 4 :HPTLC analysis of extracts of *C.caudata*

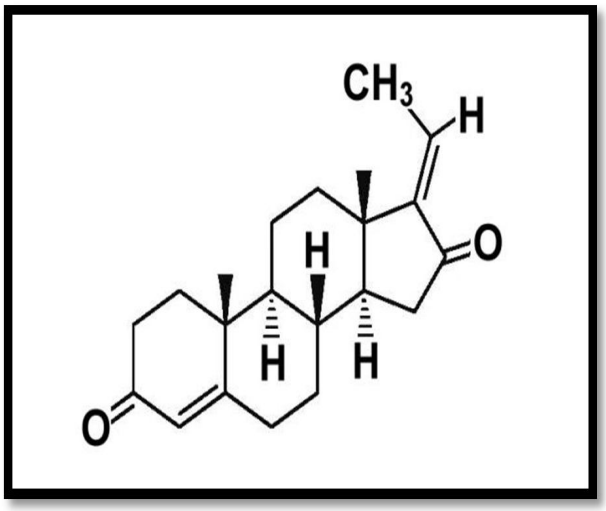

Fig. 5 : Structure of E-Guggulsterone

Fig:6: Methicillin resistant *Staphylococcus aureus* from clinical sample

together with their retention durations (RT), molecular formulas, and molecular weights (MW).

Preliminary phytochemical screening

The phytochemical screening showed that the presence of alkaloids, amino acids, flavonoids, glycosides, proteins, reducing sugars, starch, steroids, tannins, terpenoids.

Chemical composition of leaf extract

The GC-MS study on bioactive principles of hexane, ethyl acetate and methanol extract of stem bark of *Commiphora caudata* showed the presence of 52 compounds. The major and minor compounds with their retention times (RT), molecular formulae, molecular weights (MW) are presented in Table 2, and Figs. 1, 2 and 3. The leaves of *C. caudata* produced 2.7 mL/kg pale-yellow coloured extract with mango-like odour. Fifteen constituents were identified and listed in Table 1. Monoterpene hydrocarbons (51.54) Six-Octadecenoic Acid, Nine-Octadecenoic Acid, (E) n-Decanoic Acid, Two-

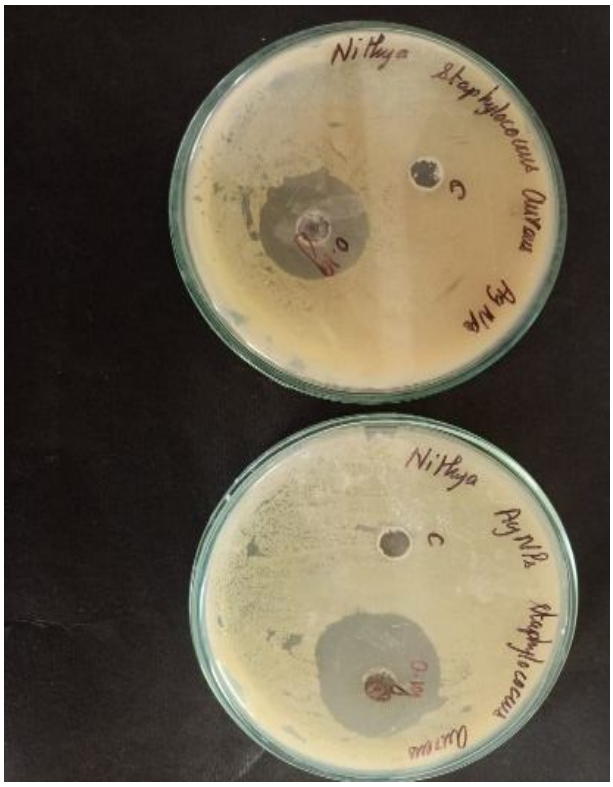

Fig. 7: Inhibition of growth of *S. aureus* by ethanol extract of *Commiphora caudata* (at 500 µg concentration)

Octadecanoic Acid, Tetradecanoic Acid, Oleic Acid, and Z- (13,14-Epoxy) 2-tert-Butyl-5, Tetradec-11-en-1-... 5-dimethyl-3-oxo-... Piperidine, Cyclo-3-Heptadecenal 3-isopropylcycloheptadecanone (Z)-2-Methyl-13-Octadecenal, Z-3 13-Octadecadienol Z and 13-octadecenal of the ethanol extract of *Commiphora caudata*.

Identification of Compound present in the EECCL by HPTLC & TLC analysis

Ethanol-based C. caudate leaf extract Using a CAMEG Linomat sample applicator, 5 l of standard quinine and 5 l of EECCL were put as a band on aluminum sheets that had been previously coated with silica gel 60 GF 254 HPTLC plates, which were employed as the stationary phase. The plates were produced in a CAMAG trough glass chamber using the mobile phase toluene and acetone (9:1) at a distance of 80 mm. The WinCATS 1.43 software was used to scan the tracks at a wavelength of 254 nm. A presentation of the fingerprint profiles was made and recorded. Following the mobile phase of development, the plot displayed 12 locations.

HPTLC profile of standard guggulsterone mixture

Chromatogram of standard guggulsterone mixture (1000ng/spot); peak 1is of E-guggulsterone (*R*f:0.38 ± 0.02), peak of Z-guggulsterone (*R*f:0.46 ± 0 . 0 2); mobile phase: toluene-acetone (9.0:1.0,v/v).

The TLC and HPTLC profile of EECCL have been presented in Figs. 3 & 4. It showed the presence of some pharmacologically active important component is E –guggulsterones (Fig.5) as 0.051%w/w.

Anti MRSA activity

Staphylococcus aureus were isolated from the collected pus samples and three was identified as methicillin resistant *Staphylococcus aureus* (MRSA) (Fig.6). The antibacterial activity of MRSA was shown in Fig.7. MRSA was highly sensitive to ethanol extract of *Commiphora caudata* at 500 ìg concentration (27mm). Mun *et al.* (2014) also reported the mechanism of antimicrobial activity of sophoraflavanone B against Methicillin-resistant *Staphylococcus aureus.*

CONCLUSION

Plants contain phytochemicals, which are used as natural medicines to treat a variety of illnesses. Multiple secondary metabolites are present, according to the current investigation. Additional research on these chemicals, as well as its isolation, purification, and characterisation, will be instructive in advancing the field of plant-based medicine.

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