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# Exploring the antimicrobial potential: *Chrozophora rottleri* battle against *Staphylococcus aureus* multidrug resistance pathogen

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**Abstract**
This have a look at targets to fill an essential hole in expertise the ability of medicinal plant as re-assets of opportunity remedies in opposition to multidrug-resistant (MDR) microbes. Despite the recognized variety of bioactive compounds in such medicinal plant, a complete exam in their antimicrobial pastime in opposition to MDR lines is lacking. Thus, the studies seek to assess each the antimicrobial efficacy and phytochemical composition of decided on medicinal plant. By systematically screening that medicinal plant, researchers desire to discover novel bioactive compounds with effective antimicrobial properties, doubtlessly supplying new avenues for fighting MDR microbes. This research is pivotal in addressing the urgent undertaking of antimicrobial resistance and will cause the invention of opportunity remedies which might be urgently wanted in scientific practice. The findings from this study have the potential to contribute to the improvement of new antimicrobial agents and strategies for addressing MDR infections, thereby benefiting global public health.

**Keywords:** *Chrozophora rottleri*, phytochemical analysis, *Staphylococcus aureus*, multidrug resistance (MDR)

## Introduction
*Chrozophora rottleri* one of the contributors of the three hundred genus and 5000–7500 species robust Euphorbiaceae family, that's divided into seven genera. The herbal conduct of Chrozophora medicinal plant improved attention of the want to find out novel lead compounds for the remedy of numerous diseases [1]. Monoecious or beneathneath shrubs are present. The genus is broadly allotted in the course of Asia, Africa, and Europe. It is an annual herb with silvery hairs that prospers in open waste regions obviously in the course of India [2]. A clinical plant known as *Chrozophora rottleri* occasionally known as Croton rottleri Geiseler and Chrozophora plicata var. rottleri (Geiseler). In Sanskrit, it's miles commonly known as Suryavarti. It is an natural plant with a lot of medicinal consequences. In India, stem powder is used to treatment jaundice in Sudan, even as in Nepal, the leafy elements of the plant are used to deal with colds and coughs. In order to treatment pores and skin situations which includes sunburn and sunstroke, leaf powder is used. Leucoderma is dealt with the usage of leaves. It is utilized in Ayurveda as a purgative, depurative, emetic, and cathartic. Concentrate of leaf well-knownshows phytotoxic consequences on rice, wheat, and mustard in addition to helmintic residences which can be averse to Pheritimaposthuma (Indian Earthworm). Laxatives and purgatives are crafted from seeds. The most powerful suppression of each Gram fantastic and Gram-bad microorganism is verified via way of means of plant extraction with methanol [3]. Our ancestors have trusted the natural treatment for as a minimum 5000 years as a type of remedy over the years [4]. In a few ways, the upward thrust of allopathic or cutting-edge remedy has decreased the significance of medicinal medicinal plant in desire of artificial medications. Even today, plenty of newly found pills have their roots in indigenous populations` use of medicinal medicinal plant [5]. The World Health Organization (WHO) estimates that because of their low chance of aspect consequences and affordability, kind of 80% of the world's populace is predicated on herbal merchandise for his or her health [6].

**Fig 1:** *Chorazophora rottleri* [7]

Ayurvedic medicines stored limitless lives earlier than current artificial medicinal drug reached the overall public, and the usage of merchandise for healing functions has an extended record in Indian medicinal drug relationship lower back to the Vedic Age. Additionally, there's a chronic marketplace call for for "natural" and/or "preservative-free" cosmetics and meals which might be microbiologically safe [8]. More than 10,000 medicinal flowers are hired in conventional medicinal drug in India, of which 1800 are applied in Ayurveda, 4700 in conventional medicinal drug, 1100 withinside the Siddha medicinal system, 750 in unani medicinal drug, three hundred in homeopathy, three hundred in Chinese medicinal drug, and one hundred withinside the allopathic system [9]. In negative international locations like India, infectious illnesses make up a huge proportion of fitness troubles. Antibiotic resistance amongst microorganisms has induced large medical troubles withinside the control of infectious illnesses. Scientists are pressured to search for new antimicrobial compounds from a whole lot of sources, which include medicinal flowers, because of the restricted availability and steeply-priced price of cutting-edge era antibiotics [10]. The bulk of the species on this own circle of relatives are determined in tropical America and the Indo-Malayan region, wherein they're usually determined withinside the tropics. Tropical Africa has a huge variety, however they're now no longer as not unusualplace or as assorted as in those different tropical areas. There are severa species of Euphorbia, though, in non-tropical areas such the Mediterranean Basin, the Middle East, South Africa, and the Southern United States [11]. The leaves have stipules and are alternating, not often opposite. Most of them are simple, however, they're usually palmate and by no means pinnate. Stipules can every so often be absent in succulent species or decreased to hairs, glands, or spines. Though every so often an adrupe, the fruit is often a schizocarp. This own circle of relatives is domestic to a huge variety of phytotoxins, in general diterpeneesters, alkaloids, glycosides, and pollution of the ricin-type [12]. Many participants of the Euphorbiaceae own circle of relatives of flowers are planted for his or her ornamental qualities, and sure species have proven promise in preventing genital herpes (HSV-2) [13]. The Euphorbiacea own circle of relatives consists of a whole lot of succulent and non-succulent flowers, consisting of herbs, shrubs, trees, and numerous forms of cacti. The milky juice that a lot of them incorporate is greater or much less hazardous, particularly for cold-blooded creatures. The culmination are normally three-celled tablets with an unmarried seed inner every cell. Some species of those culmination can offer poisonous, irritating, and vesicular seed oils. Croton, which has over seven-hundred species, and spurge or Euphorbia, which has approximately 1600 species, are the 2 biggest genera withinside the spurge own circle of relatives [14].

**Multiple drug resistance**

Multiple drug resistance (MDR) is an antimicrobial resistance Exhibited by some microorganisms to multiple antimicrobial drugs. MDR microorganisms are mostly threatening to public health Because they resist multiple antibiotics. Other MDR include those That are resistant to multiple antifungal, antiviral, and antiparasitic Drugs [15].

A broad range of biochemical and physiological mechanism may Be culpable for resistance [16]. In The specific case of antimicrobial agents, the complexity of the Processes that contribute to emergence and propagation of Resistance cannot be overestimated, and due to the lack of Elemental knowledge on these topics is one of the major reasons that there has been so limited significant fulfillment in the effective Prevention and control of resistance development [16].

There are specific classes of compounds of antimicrobial that are Capable of destroying or inhibiting microorganisms even in high Dilutions. [17]. Antibiotics are substances That are produced by the natural metabolic processes of some Microorganisms which can either inhibit or destroy other Microorganisms [18, 19]. The current root of antimicrobial drugs is diverse [20]. The Synthetic antimicrobial drugs in the laboratory are Derived from dyes or other organic compounds through chemical Reactions. For more than 60 years, Antibiotics have been critical in the fight against infectious Diseases which are caused by bacteria and another microorganism [21]. Nevertheless, disease causing microbes that have come to be resistant to antibiotic drug Treatment such as pneumonia, septicemia, gonorrhea, Tuberculosis, wound infections, and diseases that have become Difficult to treat with antibiotics remains an increasing public health Problem [22]. One side of the issue is that bacteria and Other microbes that because infections are exceptionally volatile and Have developed various ways to resist antibiotics and other form of antimicrobial drug [23]. Another side of the Issue is as a result of increasing use and misuse of current Antibiotics in human, veterinary medicine and in agriculture [24]. Microbes developing resistance, as well as due to Economic reasons have arisen in the research and development in the search for novel antibiotics in order to provide a wide range of effective drugs at all times [22].

**Materials and Methods**

**Preparation of plant extract**

The leaves of those plant life have been washed very well beneathneath going for walks faucet water after which dry at Room Temperature for two hours after which dried in an oven at 60 °C for eight hours. The dried plant cloth turned into pulverized to quality powder in a grinder, saved in air tight bottle, classified and stored in a darkish room [25].

**Maceration**

Extraction of leaves of respective flowers changed into finished through maceration technique. The solubility of pattern checked with distinctive solvents, the leaf powder changed into freely soluble within side the ethanol, methanol and ethyl acetate. 20 gm of dried powder changed

into macerated one by one in 250 ml of ethanol, methanol and ethyl acetate in take a look at tube. The flasks have been included with aluminum foil and allowed to face in a darkish for seventy-two hours for extraction. These extracts have been filtered and the filtrate changed into evaporated to dryness in heating plate [26]

**Preliminary phytochemical screening**

Ethyl acetate of *Chrozophora rottlerin* was subjected to qualitative chemical analysis. The various chemical test was performed on this extract for the identification of Mayer's, Wagner's, Molich's, Fehling's, Benedict's, Drangendorff's, Test for flavonoids, Test for acid, [27].

**Test for alkaloid**

**Mayer's test**

A small quantity of the extract was treated with Mayer's reagent. Cream color precipitate indicate the absence of alkaloids.

**Wagner's test**

A small quantity of the extract was treated with wagner's reagent. Reddish brown precipitate indicates the presence of alkaloids.

**Test for carbohydrates**

**Molisch's test**

The extract of the powdered drug was treated with 2-3 drops of 1% alcoholic naphthol and 2ml of concentrated sulphuric acid was added along the sides of the test tube. A purple colour indicating the absence of carbohydrates.

**Fehling's test**

The extract of the powdered leaf was treated with fehling's solution 1 and 2 and heated on a boiling water bath for half an hour. Red precipitate was obtain indicating the presence of free reducing sugar.

**Benedicts's test**

The extract of the powdered leaf was treated with equal volume of bendict's reagent. A red precipitate was formed indicating the absence of free reducing sugar.

**Test for anthraquinone**

**Glycosides borntrager's test**

The powdered drug was boiled with dilute sulphuric acid, filtered and to the filtrate benzene was added and shaken well. The organic layer was separated to which ammonia solution was added slowly. No pink colour was observed in ammonia layer showing the presence of anthraquinone glycosides.

**Test for cardiac glycosides**

**Keller kiliani test**

About 1gm of the powdered leaf was boiled with 10 of 70% alcohol for 2 minutes, cooled and filtered. To the filtered 10 ml of water and 5 drops of solution of lead subacetate were added and filtered, evaporated to dryness. The residue was dissolved in 3 ml of glacial acetic acid. To these 2 drops of ferric chloride solution was added. Then 3 ml of concentrated $H_2SO_4$ was added to the side of the test tube carefully and observed. No reddish- brown was observed indicating the presence of deoxysugars [27].

**Test for flavonoids**

**Lead acetate**

To the test solution add a mixture of 10% lead acetate in few drops added. It gives white precipitate presence of flavanoids.

**Test for acid**

To the small quantity of test solution, few drops of concentrated sulphuric acid were added. Yellow orange color was obtained indicates the absence of flavonoids.

**Fig 2:** Phytochemical Analysis of Ethyl Acetate.

**Multi Drug Resistance (MDR) methodology**

**Preparation of Plant Extracts**

Working concentrations of 100 mg/mL were prepared by dissolving the plant crude extracts in dimethyl sulfoxide (DMSO 100% v/v).

**Antimicrobial Assay**

Standardized broth cultures of the test bacterial isolates were aseptically spread onto Mueller Hinton Agar (MHA) plates using sterile cotton swabs. Following a drying period of approximately 5 minutes, agar wells, each with a diameter of 8 mm, were created using a sterile cork-borer. These wells were filled with 200 µL of the crude extracts (100 µg/mL) and controls. Post preparation, the plates were rested at room temperature for an hour to facilitate the diffusion of agents into the agar medium, before incubation. Controls and Incubation: Gentamycin (50 µg/mL) served as the positive control, while DMSO (100% v/v) was employed as the negative control for the antibacterial assay. Subsequently, the MHA plates were incubated at 37 °C for 24 hours, and the PDA plates were incubated at a temperature range of 25-27 °C for 3-5 days.

**Measurement of Inhibition Zones**

Post incubation, the inhibition zones' diameters (IZDs) were meticulously measured to gaugethe antimicrobial activity of the plant extracts.

**Results and Discussion**

**Preliminary phytochemical screening**

**Table 1:** Preliminary Phytochemical Screening.

| S. No | Phyto chemical tests | Present / absent |
|---|---|---|
| 1 | Mayer's test | Absent |
| 2 | Wagner's test | Present |
| 3 | Molish test | Absent |
| 4 | Fehlings solutions A & B | Present |
| 5 | Benedict's test | Absent |
| 6 | Borntrager's test | Present |
| 7 | Killer Killani test | Present |
| 8 | Test for flavonoids | Present |
| 9 | Test for acid | Absent |

**(MDR) Multi Drug Resistance**

**Table 2:** Shown Zone of Inhibition Against *Staphylococcus aureus* (MDR)

| Culture | Name of sample | Zone of Inhibition |
|---|---|---|
| *Staphylococcus aureus*(MDR) | +VE (Gentamycin) | 20MM |
| | EA | 6MM |
| | Ethanol | 13MM |

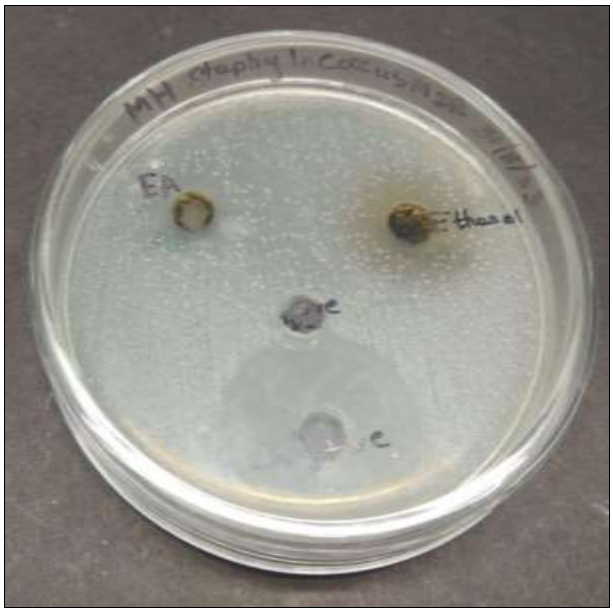


**Fig 3:** *Staphylococcus aureus*

**Table 4:** Shown Multi Antibiotic Resistance of *Staphylococcus Aureus*

| Organism | Antibiotic Resistance | Antibiotic Sensitivity |
|---|---|---|
| *Staphylococcus aureus* | NX, C, CXM, CIP, CPZ, CAZ, RO, CLR, COT | GM, NET, CF, CTX, CFR, AZM, AX, P, AK, SPX, A/S |

**Note:** *AK – Amikacin*; *AC – Amoxicillin*; *CF – Cefalotin*; *COT – Co – trimoxazole*; *GM - Gentamicin*; *PT – Pristinamycin*; *NF – Nafcillin*; *CE – Cefalexin*; *CAZ – Ceftazidime*; *AO – Amoxicillin*; *CPM – Cefepime*; *IM – Imipenem*; *LE – Levofloxacin*; *PB – Polymixin B*; *MRP – Meropenem*; *OF – Ofloxacin*; *TE – Tetracycline*; *FO - Fosfomycin*; *TG – Tigecycline*; *COL – Colistin*; *NX – Norfloxacin*; *C – Chloramphenicol*; *CXM – Cefuroxime*; *CIP – Ciproflaxacin*; *CPZ – Cefoperazone*; *RO – Roxithromycin*; *CLR – Clarithromycin*; *NET – Netillin*; *CTX – Cefotaxime*; *CFR – Cefadroxil*; *AZM – Azithromycin*; *AX – Ampicillin/ Coxacillin*; *P – Pencillin – G*; *SPX – Sparofloxin*; *A/S – Ampicillin / Sulbaclam*.

## Conclusion

In our research, we're actively fighting the rather multidrug-resistant *Staphylococcus aureus* pressure through harnessing the powerful homes of our *Chorazophora rottleri* plant extract. Through meticulous research making use of a multidrug resistance (MDR) assay, we've as compared the efficacy of our extract in opposition to the same old antibiotic gentamicin, extensively to be had withinside the market. Notably, our consequences monitor tremendous inhibition zones: 20mm for Gentamicin, 6mm for our *Chorazophora* rottleri plant extract with ethyl acetate (EA), and 13mm for *Chorazophora rottleri* with Ethanol. These values denote the high-quality quantity of bacterial boom inhibition surrounding the respective antibiotic or disinfectant discs. Encouraged through those findings, we're poised to increase our task with heightened enthusiasm and medical rigor.

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