

# Antimicrobial and Cytotoxicity Efficacy of *Commiphora swynnertonii* (Burt) Extracts

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**Abstract:** The *in vitro* antimicrobial activity of extracts from leaves, stem bark and root bark of *Commiphora swynnertonii* was assessed using ten microbes of human health importance namely *Vibrio cholerae* (clinical isolate), *Shigella flexneri* (clinical isolate), *Klebsiella oxytoca* (clinical isolate), *Klebsiella pneumoniae* (ATCC 700603), *Salmonella kisarawe* (clinical isolate), *Proteus mirabilis* (NCTC 1075), *Streptococcus pyogenes* (clinical isolate), *Staphylococcus aureus* (ATCC 25923), *Cryptococcus neoformans* (clinical isolate) and *Candida albicans* (ATCC 90028). Extracts were also evaluated for cytotoxicity activity against brine shrimps larvae. The antimicrobial bioassay was conducted using broth microdilution technique. Chloroform leaf extract demonstrated the highest antimicrobial activity with minimum inhibition concentration (MIC) range of 0.1953 – 1.5625 mg/mL against bacteria and 0.1953 – 0.7812 mg/mL against fungal species. All extracts exhibited low cytotoxicity activity against brine shrimp larvae with the exception of chloroform root extract which had  $LC_{50}$  of 4.5642 µg/mL. *Commiphora swynnertonii* is therefore a potential source of antimicrobial agents with relatively low cytotoxicity.

**Keywords:** Cytotoxicity, antimicrobial, *Commiphora swynnertonii*, plant extracts, Minimum Inhibition Concentration.

## 1. Introduction

The use of traditional medicines has contributed much in maintenance of human health care, about 80% of the world population relies on traditional use to heal different ailments [1], [2], [3]. According to the World Health Organization (WHO), poverty is a main factor leading to lack of access to commercial medicines in developing countries, and about 65-80% of the population use medicinal plants for primary health remedy as well as nutrition [4], [5].

The emergence of multi-drug resistant (MDR) pathogens is increasing to commercial medicines [6], [7]. Therefore, alternative ways of dealing with problems of microbial drug resistance, is the search for antimicrobial agents from medicinal plants that can inhibit microorganisms in different ways such as provision of alternative mechanism which previously the pathogen were not adapted to resist [7].

The genus *Commiphora* (Burseraceae) is distributed in tropical and sub-tropical parts of Asia, North-eastern Africa, and South America [8], [9]. The *Commiphora* species are characterized by shrubs or small trees with spine scent branches, pale grey barks that peel off in papery pieces and reddish brown resinous exudates [20]. The *Commiphora swynnertonii* has been used ethnomedicinally to treat diseases caused by bacteria, viral, inflammation and cancer [11], [12], [13]. Others have reported on this plant to treat fungi, cuts and wounds [14]. The secondary metabolites associated with antimicrobial activities in *Commiphora* species are terpenoids, flavonoid, steroids, sugars, and lignans [15].

This paper reports further the antimicrobial and cytotoxicity activity of *Commiphora swynnertonii* extracts so as to validate and document its efficacy and safety on human use.

## 2. Material and Method

### 2.1 Experimental site

The extraction process was done at the Institute of Traditional Medicine, Muhimbili College of Health Sciences and antimicrobial and brine shrimps lethality test were done at the Nelson Mandela Institution of Science and Technology.

### 2.2 Plant Material Collection

The leaves stem bark and root bark of *Commiphora swynnertonii* were collected from natural environment at Manyire Village in Meru district Arusha. Identification of a plant was done by Mr. Haji Selemani a botanist from the Department of Botany, University of Dar es Salaam and voucher specimen number CS 6872 is deposited in the herbarium at the Nelson Mandela African Institution of Science and Technology.

### 2.3 Chemical reagents and media.

Dimethyl sulphoxide (DMSO) was obtained from RFCL Limited (Haryana-India), Fluconazole and Gentamycin were purchased from Lincoln pharmaceutical ltd (Guj, India), Cyclophosphamide was brought from Khandelwa Laboratories Pvt Ltd (Mumbai), Iodonitrotetrazolium chloride was purchased from SIGMA (Sigma Aldrich, St Louis, USA). Nutrient agar and broth, Sabouraud dextrose agar and broth were all purchased from Hi Media Laboratories Pvt Ltd (Mumbai-India). Analytical solvents were brought from RFCL Limited (Haryana-India).

### 2.4 Plant Material Processing

The collected leaves were dried under the shade at room temperature 27-30°C till dryness. The root bark were washed

with clean running tap water to remove soil, and thereafter rinsed with distilled water. The stem and root bark were air dried before being pulverized into powders.

## 2.5 Extraction

The sequential extraction was done using solvents in the order of increasing their polarity namely; petroleum ether, chloroform, ethyl acetate, and methanol respectively. The powdered plant materials (1000g) were soaked in the extracting solvents for 24h. The extract was filtered through a Whatman No. 1 filter paper, and then concentrated *in vacuo* using Rotary evaporator. The obtained extracts were kept at 4°C until further use.

## 2.6 Test microorganism

The extracts were tested against sub cultured Gram negative and Gram positive bacteria namely; *Vibrio cholerae* (clinical isolate), *Shigella flexneri* (clinical isolate), *Klebsiella oxytoca* (clinical isolate), *Klebsiella pneumoniae* (ATCC 700603), *Salmonella kisarawe* (clinical isolate), *Proteus mirabilis* (NCTC 1075) *Salmonella typhi* (NCTC 8385), *Streptococcus pyogenes* (clinical isolate) and *Staphylococcus aureus* (ATCC 25923). The fungi species used in the assay include *Cryptococcus neoformans* (clinical isolate) and *Candida albicans* (ATCC90028).

## 2.7 Screening for antibacterial

Minimum Inhibitory Concentrations (MICs) were determined by micro dilution method [16], using 96-well micro titre plates with minor modifications. The plates were first preloaded with 50 µL of the nutrient broth in each well followed by an addition of 50 µL of the extract 100 mg/mL (prepared in DMSO) into the first wells of each row to make a total volume of 100 µL in the first wells. After thorough mixing 50 µL were drawn from each of the first row wells and put into the subsequent rows to the last wells where the drawn 50 µL was discarded. Thereafter, 50 µL of the bacterial suspension (0.5 Mac Farland standard turbidity- a suspension containing about 5×10<sup>7</sup> CFU mL<sup>-1</sup>) was then added in each well to make the final volume of 100 µL. The rows containing 100 mg/mL of Gentamycin (50µL) was used as a standard drug, the wells which contains DMSO, nutrient broth and bacteria only in triplicate were used as negative control. The plates were then incubated at 37°C for 24 h. For each extract, MICs were determined by adding 10 µL of 0.02% p-iodonitrotetrazolium (INT) chloride dye in each well followed by incubation for 1 h at 32°C. Bacterial growth was indicated by a change of color from purple to pink indicating active growth. The lowest concentration which showed no bacterial growth was considered as MIC. The MIC values were interpreted as follows 0.05-0.5mg/mL strong activity, 0.6-1.5mg/mL moderate activity and above 1.5mg/mL weak activity [17].

## 2.8 Screening for antifungal

The antifungal activity of the extracts was determined using the method described in 2.7. The test organisms in this assay

were; *Cryptococcus neoformans* (clinical isolate) and *Candida albicans* (ATCC90028).

## 2.9 Brine Shrimps Lethality Test

The artificial sea water required for shrimps to hatch was prepared using sea salt. The 3.8g of sea salt was dissolved in 1 Liter of distilled water to make a concentration of 3.8g/L, an appropriate concentration for hatching brine shrimps eggs. The glass container was used as a hatching container and was partition into two compartments with a piece of holed glass in between. One compartment was covered by a black paper while the other one was left to be illuminated. The prepared sea water was poured in a glass container, then eggs were spread on dark compartment of a container and the light was illuminated on other sides of larger compartment. The light will attract hatched shrimps to move onto illuminated side through holes in a separating glass placed in between compartments. The shrimps took 36 hours to hatch into larva. After 36 h the nauplii (brine shrimps larva) were picked by pasteur pipette from the lightened side into beaker with salt water. 5mL of salt water was poured into each universal bottle followed by addition of ten brine shrimp larvae. Extract solutions prepared at concentrations of 8, 24, 40, 80, 120, 240 µg/mL were added to the universal bottles. The same procedure was for the standard (cyclophosphamide) and negative control which was the solvent used to dissolve extracts. After 24 h the surviving nauplii were counted and the LC<sub>50</sub> values of extracts were calculated at 95% Confidence Interval and the concentration for 100% mortality were calculated using regression equation for each extract.

## 2.10 Data analysis

The Fig P computer software was used to obtain regression equation, and from which LC<sub>50</sub>, LC<sub>16</sub>, and LC<sub>84</sub> were calculated. The 95% Confidence Interval was then calculated using method reported by Litchfield & Wilcoxon (1949)[18]. The results were used to document safety and cytotoxicity activity of plant extracts (Table 2). The LC<sub>50</sub> greater than 100 µg/mL was considered non-toxic and below it as toxic [19].

# 3. Results

## 3.1 Antimicrobial Activity

Results show that chloroform extract of leaves was very active to most of microbes tested registering lowest MIC values than other extracts (Table 1). The results imply that the medium polar secondary metabolites from the leaves extract may be responsible for the observed efficacy. The methanolic leaf, stem and root bark extracts had MIC values ranging from 0.3906 to 0.7813mg/mL. On the other hand, the petroleum ether root and stem bark extracts exhibited no activity with the MIC values above 25mg/mL for *K. oxytoca*, *S. flexneri*, and *P. mirabilis*. All extracts exhibited appreciably higher antifungal activity against *C. neoformans* with the MIC values of 0.1953mg/mL.

**Table 1:** Minimum Inhibition Concentration (MIC) mg/mL of *Commiphora swynnertonii* against human pathogenic microbes

| Plant part | Microorganisms     |                    |                   |                     |                    |                     |                   |                  |                    |                      |
|------------|--------------------|--------------------|-------------------|---------------------|--------------------|---------------------|-------------------|------------------|--------------------|----------------------|
|            | <i>V. cholerae</i> | <i>S. flexneri</i> | <i>K. oxytoca</i> | <i>K. pneumonia</i> | <i>S. kisarawe</i> | <i>P. mirabilis</i> | <i>S. pyogens</i> | <i>S. aureus</i> | <i>C. albicans</i> | <i>C. neoformans</i> |
| LC         | 0.1953             | 0.1953             | 0.3906            | 1.5625              | 0.3906             | 1.5625              | 0.7813            | 0.7813           | 0.7813             | 0.1953               |
| SM         | 1.5625             | 0.3906             | 0.7813            | 0.7813              | 0.78125            | 12.5                | 3.125             | 0.5625           | 1.5625             | 0.1953               |
| RP         | 12.5               | 12.5               | >25               | 6.25                | 1.5625             | >25                 | 12.5              | 0.5625           | 6.25               | 3.125                |
| RM         | 1.5625             | 0.3906             | 1.5625            | 0.7813              | 0.7813             | 12.5                | 1.5625            | 0.5625           | 6.25               | 0.1953               |
| RC         | 3.135              | 12.5               | 3.125             | 3.125               | 3.125              | >25                 | 6.25              | 3.125            | 6.25               | 0.1953               |
| LM         | 0.7813             | 1.5625             | 0.3906            | 0.3906              | 0.3906             | 0.7813              | 6.25              | 3.125            | 6.25               | 0.3906               |
| SE         | 6.25               | 0.7813             | 0.7813            | 0.7813              | 0.7813             | 6.25                | 1.5625            | 1.5625           | 3.125              | 0.1953               |
| SP         | 12.5               | >25                | >25               | 6.25                | 1.5625             | >25                 | 6.25              | 0.7813           | 6.25               | 0.1953               |
| DMSO       | 12.5               | 12.5               | 12.5              | 12.5                | 12.5               | 12.5                | 12.5              | 12.5             | 12.5               | 12.5                 |
| GC         | 0.00153            | 0.00153            | 0.00153           | 0.00153             | 0.00153            | 0.00153             | 0.00153           | 0.00153          | NT                 | NT                   |
| FC         | NT                 | NT                 | NT                | NT                  | NT                 | NT                  | NT                | NT               | 0.00625            | 0.00625              |

**Key:**LC-Leaf chloroform extract, SM-Stem methanolic extract, RP-Root petroleum ether extract, RM-Root methanolic extract, RC-Root chloroform extract, LM-Leaf-methanolic extract, SE-Stem-ethyl acetate extract, SP-Stem petroleum ether extract, GC-Gentamycin, FC- Fluconazole, DMSO-Dimethyl sulphoxide, NT-Not tested.

### 3.2 Brine Shrimps Lethality Test

The lethality Concentration (LC<sub>50</sub>) of plant extracts ranged from 4.5642 to 410.2134 µg/mL (Table 2). The chloroform

root bark extract was very toxic with the LC<sub>50</sub> value of 4.5642 µg/mL, followed by stem bark extract of ethyl acetate, methanol leaf and methanol root bark extracts with LC<sub>50</sub> values ≤ 100 µg/mL. The chloroform leaf and petroleum ether root bark extracts had LC<sub>50</sub> values ≥ 100 µg/mL. Since the LC<sub>50</sub> value of less than 100 µg/mL is considered toxic and LC<sub>50</sub> value of greater than 100 µg/mL is non-toxic [20], this implies that chloroform leaf, petroleum ether root bark, petroleum ether stem bark and petroleum ether leaf extracts are therefore non-toxic.

**Table 2:** Brine Shrimps Lethality Test results of *Commiphora swynnertonii* extracts

| Plant extract | LC <sub>50</sub> µg/mL | 100%Mortality µg/mL | Regression equation | Regression coefficient (R <sup>2</sup> ) | 95% CI            |
|---------------|------------------------|---------------------|---------------------|--|-------------------|
| LC            | 111.9013               | 2124.4577           | Y=39.111logx-30.132 | 0.8765                                   | 67.4551-185.633   |
| SM            | 93.0245                | 1485.1286           | Y=41.557logx-31.809 | 0.9745                                   | 57.7720-149.7880  |
| RP            | 101.1229               | 1566.3013           | Y=42.032logx-34.287 | 0.934                                    | 63.1426-161.9483  |
| RM            | 74.8720                | 1325.5170           | Y=40.062logx-25.089 | 0.9591                                   | 45.6815-122.7152  |
| RC            | 4.5642                 | 136.0476            | Y=33.914logx+27.638 | 0.9606                                   | 2.4080-8.6509     |
| LM            | 60.8078                | 840.4201            | Y=43.839logx-28.207 | 0.9458                                   | 38.7138-95.5108   |
| SE            | 54.2477                | 337.2736            | Y=63.004logx-59.273 | 0.925                                    | 39.6228-74.2705   |
| SP            | 410.2134               | 15,419.1740         | Y=31.745logx-32.95  | 0.9232                                   | 207.1994-812.1404 |
| LP            | 101.7609               | 3204.7791           | Y=33.373logx-16.999 | 0.9706                                   | 56.2308-184.1567  |
| CP            | 16.409                 | 85.1951             | Y=69.9logX-34.936   | 0.9949                                   | 12.006-22.305     |
| DMSO          | -                      | NM                  | -                   | -  | -                 |

**Key:**LC-Leaf chloroform extract, SM-Stem methanolic extract, RP-Root petroleum ether, RM-Root methanolic extract, RC-Root chloroform extract, LM-Leaf-methanolic extract, SE-Stem-ethyl acetate extract, SP-Stem petroleum ether, LP-Leaf petroleum ether, CP- Cyclophosphamide, DMSO-Dimethyl sulphoxide, NM- No mortality.

### 4. Discussion

The antimicrobial activity of *Commiphora swynnertonii* extracts were found to be higher against tested bacteria and fungi species. The *Commiphora* species are known for their antimicrobial, anti-inflammatory, antiproliferative, hepatoprotective and cardiovascular properties [8]. The chloroform leaf extract showed the highest activity against tested microbes with a MIC value of 0.1953mg/ml to *V. cholerae*, *S. flexneri* and *C. neoformans*. The MIC values of extracts against other microorganisms varied from medium to low activity as shown in Table 1. Study conducted by Bakari *et al.*, (2011) [12], revealed that, the ethanolic extract of

resins of *C. swynnertonii* had appreciably higher activity against *S. pyogens* with the inhibition zone of 23.3±3.9 mm, while *Candida albicans* and *S. aureus* were moderately inhibited with the inhibition zones of 14.4±4.7 and 11.5±2.9 mm respectively. In this study the chloroform leaf and ethyl acetate stem bark extracts inhibited the growth of *S. pyogens* with the MIC values of 1.5625 and 0.7813µg/mL respectively.

The cytotoxicity assay using brine shrimps larvae revealed that chloroform root extract is very toxic compared to other extracts with LC<sub>50</sub> 4.5642µg/mL (Table 2). These results corroborate a report made by Bakari *et al.*, (2011) [12], who reported the ethanolic root extract to be toxic with the LC<sub>50</sub> value of 3.5µg/mL. According to Meyer *et al.*, (1982)[20], the extracts with LC<sub>50</sub> not above 20µg/mL may be potential anticancer agent; therefore the root extract of *C. swynnertonii* needs further investigation to see if it can offer a new anticancer agent. Other findings on antimicrobial efficacy of *Commiphora* species were revealed when the crude

methanolic resin extract from *C. mukul* showed significance activity with MIC value of 62.5mg/mL against *Mycobacterium aurum* [22]. The study done in South Africa on Commiphora species, namely *C. schimperi* (stem), *C. neglecta* (stem), *C. tenuipetiolata* (stem and leaf), and *C. edulis* (stem), evaluated the LC<sub>50</sub> of extracts of these species and found ranging between 7.31 µg/mL and 10.81 µg/mL [11]. In another study, about 83.3% of the standard bacteria and clinical isolates showed some degree of growth suppression due to the vapor of methanol extract of *C. molmol* [23], also the plant extract is used to cure *Schistosomiasis haematobium* using concentration of 600mg [11]. Furthermore, the genus Commiphora including *Commiphora molmol* are used as antimicrobial agent to heal sore throat and gingivitis [11]. *Commiphora mukul* is used as a stimulant, also as a hypolipidaemic agent [24]. *Commiphora myrrh* is used to treat cuts and wounds, this application made Greek soldier not to go into battle without poultice of myrrh in their pockets to put on their wounds [15]. Strong inhibition activity of Gram negative and Gram positive bacteria has been shown in *Commiphora mukul* [25]. The muscanone from *C. wightii* was found to be active against *Candida albicans* [26].

## 5. Conclusion

*Commiphora swynnertonii* leaves, root bark and stem bark extracts have demonstrated potency against pathogenic bacteria and fungal species which support traditional use of a plant for the management of bacterial and fungal infections. Root bark chloroform extract exhibited high cytotoxicity activity against brine shrimp larvae suggesting the presence of secondary metabolites which can be evaluated for the development of anticancer agents. Thus, further study to characterize secondary metabolites with the displayed activities is hereby proposed.

## 6. Future Scope

Antimicrobial and cytotoxicity efficacy of extracts from *Commiphora swynnertonii* is reported in the study. Characterization of secondary metabolites with antimicrobial activity from this plant will be investigated. Antimicrobial secondary metabolites with low cytotoxicity will act as drug templates for development of antimicrobial agents.

## 7. Acknowledgement

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