REVIEW ARTICLE

Effect of Alcoholic Extract, Polyphenol, Tannin, and Flavonoid of *Cynomorium coccineum* L Plant on Pathogenic Bacteria: A Comparative Study

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ABSTRACT

Cynomorium coccineum L is one of the plants belonging to the family of Balanophoraceae. The antibacterial activity was evaluated for the groups of compounds isolated from the *C. coccineum* L plant. At the beginning for the alcoholic extract, polyphenols, tannins, and flavonoids were extracted from the plant with a yield of (10.211%w/w, 8.6%w/w, 6.4%w/w, 1.2%w/w) respectively. The activity of these groups was evaluated against gram-positive and gram-negative bacteria. Results showed that the four isolated groups significantly differed at $p \leq 0.05$ between *C. coccineum* L extract concentration and control for (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Staphylococcus aureus, Staphylococcus saprophyticus*, and *Streptococcus agalactiae*) as these isolated groups demonstrated efficacy against bacteria in multiple concentrations.

Keywords: Antibictrial, *C. coccineum* L, Pathogenic bacteria, Ultrasound-assisted extraction.

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INTRODUCTION

Cynomorium coccineum L. is a species of *Cynomorium coccineum* L. It is a perennial herbaceous plant available in the western Mediterranean, western China, northern Africa, and the Arabian Peninsula, in addition to other places.[1] The melanophore family includes *C. coccineum* L. (family Balanophoraceae). It is a plant that can not photosynthesize and can be found in Mediterranean countries' dry sandy and rocky soils.[2] It possesses a combination of compounds that can be useful for treating some chronic diseases that are related to the diet. The most important diseases are cancer, obesity, type II diabetes, and multiple inflammatory conditions.[3,4] The cultivation of such plants, especially if they are endemic, can represent a considerable source of income in developing countries. Similarly, the discovery of natural remedies has also gained a lot of attention in recent decades in the cosmetic sector, even if, in some cases, modern science had not yet confirmed the traditional uses.[4] *C. coccineum* L is a characteristic example of a well-known plant but almost entirely ignored in ethnopharmacology, as ancient folk knowledge has been often dispersed in recent centuries.

MATERIALS AND METHODS

Plant Material

C. coccineum L plant was collected in March 2020 from the north of Mesan City south of Iraq. The plant was cleaned, washed with distilled water, dried at room temperature for two weeks. The plant was collected and crushed; then, they were kept in dark glass containers for further use.

Extraction

Extraction Alcoholic Extracted

A quantity of 10 g of *C. coccineum* L was used and soaked it in an alcoholic solution (100 mL of 70% ethanol). Ultrasonic separation of secondary metabolites from *C. coccineum* L for 48 hours at 45 degrees Celsius. Day after, the extracts were filtered three times using titron cloth. [5]

Polyphenol Isolated

The extraction of phenolic compounds from *C. coccineum* L by ultrasound was carried out using a water-ethanol mixture (30:70) at a temperature of (45°C), the amount of *C. coccineum* L (50 g), and the extraction time (48 hours).[6] Each 50 g of *C. coccineum* L was crushed and placed in a brown bottle 500 mL with 500 mL CH_3CH_2OH (70%) added. After the completion, the mixture was filtered with a cloth; the filtrate was collected and transferred into a separatory funnel with half the amount of chloroform. The extraction process was repeated five times to remove the inorganic materials. The resulting filtrate was mixed with its have volume of ethyl acetate; the separation process was repeated four times, and the resulting material was collected.

Isolation of Tannins from C. coccineum L

The whole *C. coccineum* L plant powder (50 gm) was mixed with (250 mL) of 10% NaOH. The mixture was then refluxed for 24 hours at 85°C. To separate suspended and non-dissolved

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matter, the mixture was cooled and purified. The filtrate was then treated with 10 mL (10%) of sulphuric acid before being purified and cleaned with dilute sodium bicarbonate to separate the acid and salts residues.[7]

Isolation of Flavonoids from Cynomorium coccineum L

The entire *C. coccineum* L plant powder (50 gm) was treated with 250 mL (80%) methanol at room temperature for 24 hours with continuous stirring After removing the precipitate from the methanolic extract, 25 mL of 1% lead acetate was applied to the filtrate. The filtrate was extracted with the Buchner funnel. The precipitate was then filtered and the filtrate was evaporated by adding (25 mL) acetone and (30 mL) concentrate hydrochloric acid.[8,9]

Antimicrobial Susceptibility Testing

The isolated compounds were dissolved in 1-g of 10% DMSO, then serially diluted two times in a liquid growth medium (MHB). The final concentrations had a range from 400 mg/mL to 0.78 mg/mL. Except for the 12th well, 100 L of each extract concentration was applied to the various wells of 96 well microtiter plates after shaking (growth control, without the extracted compounds (alcoholic extract, polyphenols, tannins, and flavonoids). Then, except for the 11th well (sterility monitoring, without bacteria), each well is inoculated with 100 L of a microbial inoculum (1106 CFU/mL) and incubated at 37ºC. At 620 nm, the MIC was determined. The plate was weighed before and after a 24 hours incubation at 37°C. The following equation was used to calculate the Bacterial Growth Inhibition.[10]

$$Percentage\ growth\ inhibition = \frac{OD\ of\ control}{OD\ of\ control - OD\ of\ the\ test}$$

RESULTS AND DISCUSSION

Alcoholic extract, polyphenols, tannins, and flavonoids were extracted from the plant of *C. coccineum L*. The yields were 1.0211 gm, 4.3 gm, 3.2 gm, 0.6 gm, respectively. The results were consistent with many previous studies that have shown that polyphenols compounds have a strong inhibitory effect on several pathogenic microorganisms such as bacteria, fungi, and yeasts due to the presence of several active compounds such as tannins, flavonoids, and polyphenols.[11] The inhibitory effect of flavonoid and polyphenol was also found to be greater than that of the plant's alcoholic extract and other isolated groups. This explains why this extract contains a high percentage of phenolic compounds.[12] Furthermore, the antibacterial action can be traced back to phenolic compounds that are known to have antibacterial activity, as phenolic compounds found in plant extracts trigger microscopic organisms' membranes and cell walls to be destroyed.[13] The presence of active compounds such as tannins, phenols, and flavonoids and the synergistic influence of chemical compounds active with each other can also contribute to antimicrobial activity.[14] The disparity in antibacterial agent effect between Gram-positive and Gram-negative bacteria is mostly due to differences in cell wall composition. Positive bacteria have a single layer in their cell wall, while negative bacteria have several layers identified by an external cell membrane.[15] The loss of inhibitory activity may also be due to a variation in form or bacterial strain, or the appearance of new bacterial mutations resulting from storage or repeated transfer of isolation bacteria, both of which may impair this inhibition (Figures 1 to 6).

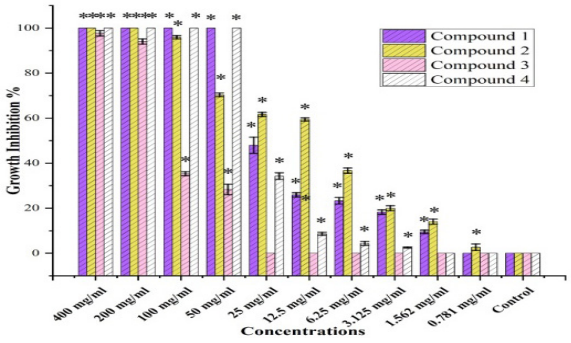

Figure 1: The effect of extracts of *C. coccineum L* of Klebsiella pneumoniae growth. the results show a significant difference at $p \leq 0.05$ between four compounds of plant extracts *C. coccineum L* and control (0 -+ 0) for all concentrations. The minimum inhibitory concentration at 50 to compound 1 and compound 4 compounds but complete growth inhibition at 100, 400 mg/mL, to compound 2 and compound 3, respectively.

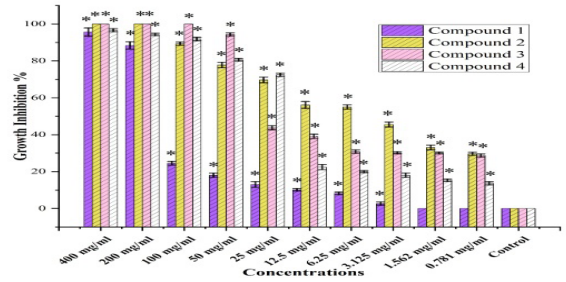

Figure 2: The effect of extracts of C. coccineum L on E. coli growth. The results show a significant difference at $p \leq 0.05$ between four compounds of plant extracts C. coccineum L and control (0 -+0) for all concentrations. The minimum inhibitory concentration at 100 to compound 2 and 200 mg/mL to compound 3, but complete growth inhibition at 400 mg/mL to compound 1 and compound 3.

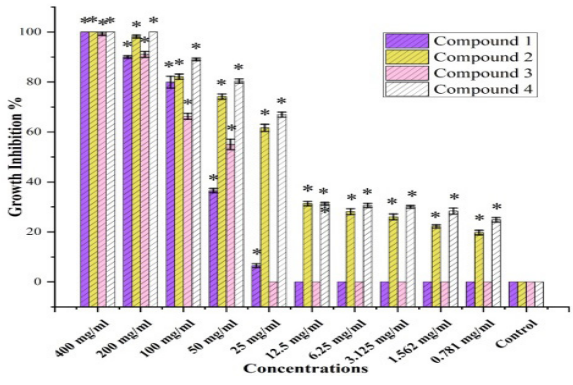

Figure 3: The effect of extracts of *C. coccineum L* on pseudomonas aeruginosa growth. The results show a significant difference at $p \leq 0.05$ between four compounds of plant extracts *C. coccineum L* and control (0 -+ 0) for all concentrations. The minimum inhibitory concentration at 200 mg/mL to compound 2 and compound 4, but complete growth inhibition at 400 mg/mL to compound 1 and compound 3.

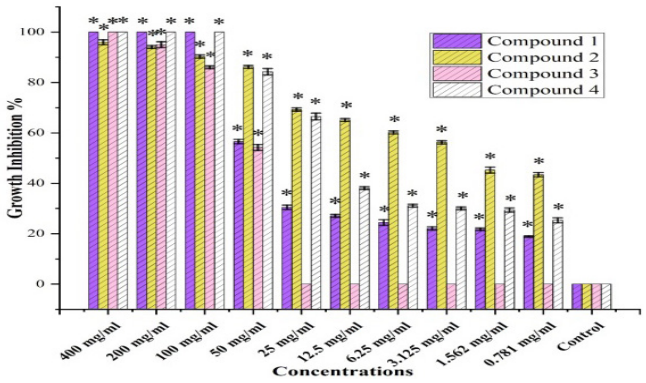

Figure 4: The effect of extracts of *C. coccineum L* on Staphylococcus aureus growth. the results show a significant difference at $p \leq 0.05$ between four compounds of plant extracts *C. coccineum L* and control (0-+0) for all concentrations. The minimum inhibitory concentration at 100 mg/mL to compound 1 and compound 4, but complete growth inhibition at 400 mg/mL to compound 3.

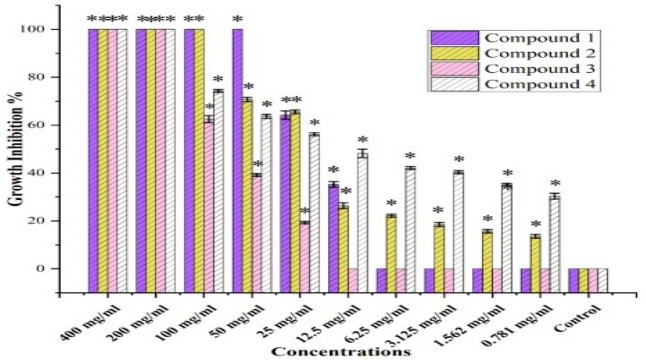

Figure 5: The effect of extracts of *C. coccineum L* on Staphylococcus saprophyticus growth. The results show a significant difference at $p \leq 0.05$ between four compounds of plant extracts *C. coccineum L* and control (0-+0) for all concentrations. The minimum inhibitory concentration at 50 mg/mL to compound 1 but complete growth inhibition at 100 mg/mL to compound 2, complete growth inhibition at 200 mg/mL to compound 3, and compound 4

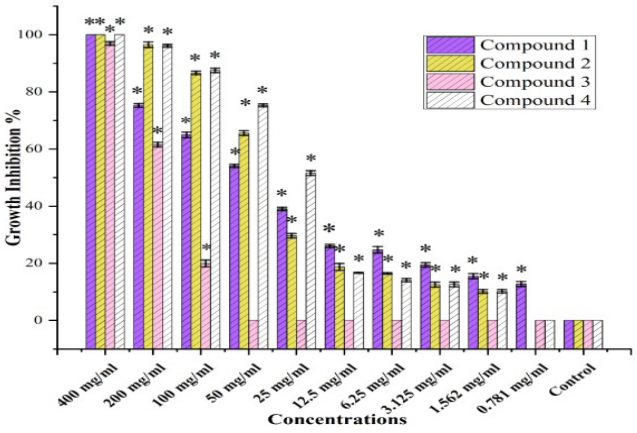

Figure 6: The effect of extracts of *C. coccineum L* of Streptococcus agalactiae growth. the results show a significant difference at $p \leq 0.05$ between four compounds of plant extracts *C. coccineum L* and control (0-+0) for all concentrations. The minimum inhibitory concentration at 400 mg/mL to compound 1, compound 2, compound 3, and compound 4.

Compound 1: Alcoholic extract, Compound 2: Polyphenol, Compound 3: Tannin, Compound 4: Flavonoid.

CONCLUSION

The present study revealed that the *C. coccineum L* extracts have the greatest phytochemicals (Polyphenols, Flavonoids, and Tannins). This study showed that isolated groups have a clear effect on the disease-causing bacteria. Thus, they can be used as an available and inexpensive treatment.

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